

14

UJUP¹⁴

7º ENCONTRO DE INVESTIGAÇÃO JOVEM DA U.PORTO

U.PORTO

CREDITS

LIVRO DE RESUMOS IJUP'14

7º ENCONTRO
DE INVESTIGAÇÃO
JOVEM DA U.PORTO

Universidade do Porto
Apoio Administrativo ID+i
t. 22 040 81 46
secidi@reit.up.pt

Design

Nelson Luís & Ricardo Gomes

Coordenação

Rui Mendonça

Impressão e acabamentos

Invulgar - artes gráfica

Tiragem

1000 exemplares

Depósito legal

340336/12

ISBN

978-989-746-033-3

NOTA

A Comissão Organizadora não se responsabiliza por erros ortográficos ou pela revisão gramatical dos resumos, sendo o conteúdo técnico-científico e a redação do trabalho da inteira responsabilidade dos respetivos autores.

SCIENTIFIC COMMITTEE

Albino Lima
Aurora Teixeira
Elisa Keating
Filipe Castro
Gonçalo Furtado
Graciela Machado
Helena Madureira
Jorge Moreira Gonçalves
Jorge Teixeira
Laura Oliveira
Manuel Simões
Marcela segundo
Maria Oliveira
Maria Paula Santos
Patricia Antunes
Patrícia Valentão
Paulo Aguiar
Pedro Gomes
Rita Faria
Rute Pedro

Secretariat

Paula Coelho

	WEDNESDAY, 12 th	THURSDAY, 13 th	FRIDAY, 14 th
08:40 - 09:00	REGISTRATION	REGISTRATION	REGISTRATION
09:00 - 10:30	PARALLEL ORAL SESSIONS I A1- Biomedicine I A2- Engineering I A3- Literature, Sociology & Political Sciences A4- Architecture & Arts I A5- Biological Sciences I	PARALLEL ORAL SESSIONS IV A1- Biomedicine IV A2- Economics & Management A4- Psychology & Education Sciences III A5- Biological Sciences IV	PARALLEL ORAL SESSIONS VIII A1- Biomedicine VIII A2- Architecture & Arts II A3- Biological Sciences VI A4- Psychology & Education Sciences V A5- Engineering IV A6- Astronomy
10:30 - 11:00	WELCOME SESSION	COFFEE BREAK & POSTER VIEWING	COFFEE BREAK & POSTER VIEWING
11:00 - 12:00	CONFERENCE Programa de aceleração de ideias do UPTEC Eng. ^a Clara Gonçalves Portugal 2020: Desafios e oportunidades para a Ciência e Tecnologia no quadro das Estratégias de Especialização Inteligente Prof. Mário Rui Silva	PARALLEL ORAL SESSIONS V A1- Biomedicine V A2- Public Health & Epidemiology I A3- Physics I A4- Psychology & Education Sciences IV A5- Biological Sciences V	
12:00 - 12:30	Exhibition opening <i>almost not: prints and other things</i> at Galeria dos Leões		
12:30 - 14:00	LUNCH BREAK	LUNCH BREAK	

PROGRAM

14:00 - 15:30

PARALLEL ORAL SESSIONS II

- A1- Biomedicine II
- A2- Chemistry I
- A3- Environment I
- A4- Psychology & Education Sciences I
- A5- Biological Sciences II
- A6- Math

PARALLEL ORAL SESSIONS VI

- A1- Biomedicine VI
- A2- Engineering II
- A3- Criminology, Economy & Law
- A4- Sport Sciences I
- A5- Agro Food I
- A6- Physics II

15:30 - 17:00

COFFEE BREAK & POSTER VIEWING

COFFEE BREAK & POSTER VIEWING

17:00 - 18:30

PARALLEL ORAL SESSIONS III

- A1- Biomedicine III
- A2- Chemistry II
- A3- Environment II
- A4- Psychology & Education Sciences II
- A5- Biological Sciences III
- A6- Geography & History

PARALLEL ORAL SESSIONS VII

- A1- Biomedicine VII
- A2- Engineering III
- A3- Public Health & Epidemiology II
- A4- Sport Sciences II
- A5- Agro Food II

TABLE OF CONTENTS

SCIENTIFIC COMMITTEE	5
PROGRAM	6
TABLE OF CONTENTS	8
FOREWORD	11
Parallel Oral Sessions I	13
A1- Biomedicine I	15
A2- Engineering I	21
A3- Literature, Sociology & Political Sciences	27
A4- Architecture & Arts I	35
A5- Biological Sciences I	43
Parallel Oral Sessions II	51
A1- Biomedicine II	53
A2- Chemistry I	61
A3- Environment I	69
A4- Psychology & Education Sciences I	77
A5- Biological Sciences II	85
A6- Math	93
Parallel Oral Sessions III	101
A1- Biomedicine III	103
A2- Chemistry II	111
A3- Environment II	119
A4- Psychology & Education Sciences II	127
A5- Biological Sciences III	135
A6- Geography & History	143
Parallel Oral Sessions IV	151
A1- Biomedicine IV	153
A2- Economics & Management	161
A4- Psychology & Education Sciences III	169
A5- Biological Sciences IV	177

Parallel Oral Sessions V	185
A1- Biomedicine V	187
A2- Public Health & Epidemiology I	195
A3- Physics I	203
A4- Psychology & Education Sciences IV	211
A5- Biological Sciences V	219
Parallel Oral Sessions VI	227
A1- Biomedicine VI	229
A2- Engineering II	237
A3- Criminology, Economy & Law	245
A4- Sport Sciences I	253
A5- Agro Food I	261
A6- Physics II	267
Parallel Oral Sessions VII	277
A1- Biomedicine VII	279
A2- Engineering III	287
A3- Public Health & Epidemiology II	295
A4- Sport Sciences II	303
A5- Agro Food II	311
Parallel Oral Sessions VIII	319
A1- Biomedicine VIII	321
A2- Architecture & Arts II	329
A3- Biological Sciences VI	337
A4- Psychology & Education Sciences V	345
A5- Engineering IV	353
A6- Astronomy	361
POSTERS	369
POSTERS Wednesday, 12th	371
POSTERS Thursday, 13th	453
POSTERS Friday, 14th	533

FOREWORD

Os méritos do IJUP são muitos e notórios. Trata-se de um evento que proporciona aos nossos estudantes uma primeira experiência de comunicação, divulgação e debate científico. Num ambiente similar ao de um congresso internacional, os estudantes têm o desafio de apresentarem publicamente os seus trabalhos científicos, o que exige não apenas clareza, assertividade e rigor mas também uma boa dose de criatividade.

A comunicação é inerente à atividade de qualquer cientista. No seu quotidiano profissional, os investigadores têm de comunicar constantemente com os restantes membros da comunidade científica. A partilha de conhecimento, a troca de experiências profissionais e o cruzamento de competências entre investigadores são, aliás, fundamentais ao avanço científico. Isto porque o progresso em ciência resulta, como sabemos, de um processo de acumulação de descobertas e resultados obtidos à escala global.

Por outro lado, numa sociedade onde a ciência e a tecnologia estão omnipresentes no dia a dia dos cidadãos, os investigadores devem manter a comunidade informada sobre o seu trabalho. Mais: dada a crescente especialização e complexidade da ciência, os cientistas têm a obrigação cívica de tornar acessíveis os resultados das suas investigações, bem como de analisar e debater as implicações que esses mesmos resultados possam ter na sociedade.

Dito isto, o IJUP enquadra-se claramente no objetivo de afirmar a U.Porto como uma instituição eminentemente vocacionada para a investigação científica. O evento é um estímulo à atividade científica dos nossos estudantes, tendo em vista não apenas uma formação académica mais completa mas também a preparação para carreiras profissionais em que a investigação e a inovação sejam preponderantes. Não tenho dúvidas de que o IJUP tem despertado ou consolidado a vocação científica de muitos jovens, criando terreno fértil para uma geração de estudantes mais propensa à produção de conhecimento e não apenas à sua apreensão.

É pois com muito orgulho que, desde 2008, a Universidade do Porto organiza o IJUP. Trata-se de um evento pioneiro a nível mundial, com o mérito acrescido de ser realizado essencialmente com trabalho voluntário, escassos meios e custos reduzidos. Acresce que, em cada edição, o IJUP conta com a adesão de muitas centenas de estudantes da Universidade do Porto, representando mais de uma vintena de disciplinas científicas.

Cumpre-me agradecer ao senhor vice-reitor Jorge Gonçalves, enquanto principal dinamizador do IJUP, bem como à sua equipa, a cujo esforço e dedicação se deve, em larga medida, o sucesso de todas estas edições do encontro.

Um agradecimento também às empresas e instituições que apoiam o 7.º IJUP. Neste particular, merece ser enaltecido o patrocínio do Santander Universidades.

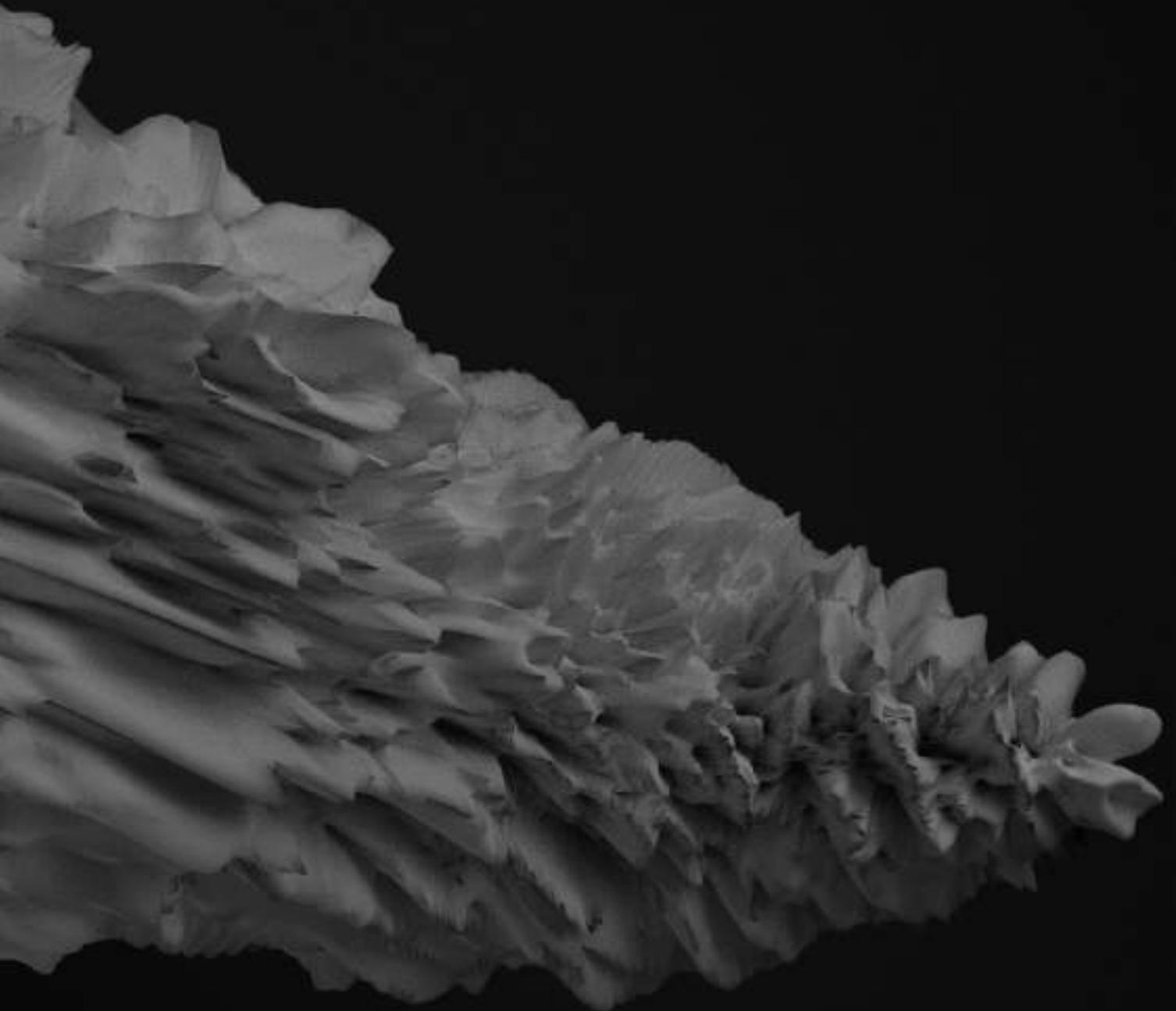
Agradeço ainda aos estudantes que participam no IJUP'14, esperando que mantenham a sua curiosidade científica, concretizem os seus projetos de investigação e contribuam assim para o desenvolvimento da ciência em Portugal.

José Carlos Marques dos Santos
Reitor da U.Porto





**PARALLEL
ORAL
SESSIONS**



A1

BIOMEDICINE I

I
PARALLEL
ORAL
SESSIONS



Opioid-induced hyperalgesia is mediated by a pain facilitatory area of the brain

A.R Costa¹, P. Carvalho¹, I. Tavares¹ & I. Martins¹

¹ Department of Experimental Biology, Faculty of Medicine of Oporto and IBMC, Porto, Portugal

Opiates are the most commonly used drugs for the treatment of moderate-to-severe postoperative and chronic pain. Chronic use of opioids can induce paradoxical hyperalgesia (opioid-induced hyperalgesia; **OIH**). OIH is characterized by hypersensitivity to innocuous or noxious stimuli during sustained opiate administration, and is reported both in clinical and pre-clinical settings. The molecular mechanisms of OIH are not fully understood. One of the mechanisms that contribute to OIH is the activation of brain areas involved in pain facilitation. Here we studied the involvement of an area located in the medulla oblongata, the dorsal reticular nucleus (**DRt**), which plays a unique and exclusive pain facilitatory role. For that, a model of OIH was used induced which consisted on the continuous infusion of morphine for 7 days and then we evaluated the effects of DRt blockade by lidocaine on the behavioral manifestations of OIH.

Male Wistar rats weighing 285-300g were anesthetized and implanted subcutaneously with osmotic pumps for the infusion of morphine (45 $\mu\text{g}\cdot\mu\text{l}^{-1}\cdot\text{h}^{-1}$) or saline (control group; n=6 per group). The animals were then placed on a stereotaxic frame for the implantation of a guide cannula into the left DRt. Seven days later, the cannula was used for the injection of lidocaine (0.5 μl ; 4% w/v). Pain behavior was tested by the von Frey and hot-plate tests, which test the development of mechanical allodynia and thermal hyperalgesia, respectively. The von Frey and hot-plate tests were performed before and at 2, 4 and 7 days after implantation of the osmotic pumps. Behavioral evaluation was performed before and 30 min after lidocaine injection. Upon completion of the experiments, the animals were anesthetized and injected with 0.5 μl of 0.6% Chicago sky blue dye through the guide cannula for the posterior histological verification of the injection site. Only animals with cannula placement correctly targeting the DRt were included in data analysis.

The continuous infusion of morphine, but not saline, induced a significant development of mechanical allodynia and thermal hyperalgesia at day 4 and 7. The administration of lidocaine at the DRt fully reversed mechanical allodynia and thermal hyperalgesia in morphine-infused animals. Lidocaine injection had no effect in saline-infused animals.

Our results indicate that OIH is mediated by a pain facilitatory area of the brain. It is now essential to determine the molecular pathways that mediated the pain facilitatory actions of the DRt during opioid-induced hyperalgesia in order to unravel the effects of chronic opioid exposure on pain facilitation.

Acknowledgements: FCT/COMPTE project PTDC/SAU-NSC/110954/2009 and IASP Early career Research Grant

Toxicological Assessment of Marine Cyanobacterial Extracts in Human Cell Lines – Proteomic and Gene Expression Approach

S. Freitas^{1,2}, A. Campos¹, R. Urbatzka¹ and R. Martins^{1,3}

¹ Interdisciplinary Centre for Marine and Environmental Research (CIIMAR), Portugal.

² Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

³ Centre of Health and Environment Research – CISA, Superior School of Health Technology of Porto, Polytechnic Institute of Porto, Portugal

Cyanobacteria are known to synthesize secondary metabolites that may have potential as drugs for the treatment of human diseases such as cancer [1]. At the Laboratory of Ecotoxicology Genomics and Evolution, LEGE – CIIMAR, studies have been performed concerning the bioactive potential of marine cyanobacteria isolated from the Portuguese coast. Recent results revealed significant cytotoxicity of extracts of the *Cyanobium* sp. strain LEGE06113 and the *Synechocystis salina* strain LEGE06155 on human cancer cell lines [2]. In this work we aimed to proceed to the characterization of the mechanisms involved in the reported cytotoxicity, by using a molecular and proteomic approach.

Cyanobacterial cultures have been performed, and crude extracts and fractions were obtained from lyophilized biomass. Cytotoxicity of strains was studied on the RKO human colon cancer cell line by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Real-time PCR was employed to analyze gene expression of genes CCNB1, CCNE, P21CIP, BAD, BCL-2 and two-dimensional gel electrophoresis (2D-GE) for protein expression.

The MTT assay confirmed cytotoxicity of an ethyl acetate fraction, real-time PCR results differences in CCNB1 (cell cycle) and BCL-2 (apoptosis) mRNA expression and proteomics revealed different protein patterns expressed in the treatment groups compared with control. Qualitative and quantitative differences were detected in many protein spots from the 2D-GE, according to the cyanobacterial strain and the identification of the altered proteins is ongoing.

Combining gene expression and proteomics, new insights into the molecular mechanisms leading to growth inhibition in human carcinoma cells were acquired and may be useful in the future to understand the underlying molecular mode of action – an important prerequisite for development of future cancer drugs.

References:

[1] Tan, L.T. (2010), Filamentous Tropical Marine Cyanobacteria: a Rich Source of Natural Products for Anticancer Drug Discovery, *Journal of Applied Phycology*, 22, 659-676.

[2] Costa M., Garcia M., Costa-Rodrigues J., Costa, MS., Ribeiro MJ., Fernandes MH., Barros P., Barreiro A., Vasconcelos V. and Martins R., (2014). Exploring Bioactive Properties of Marine Cyanobacteria Isolated from the Portuguese Coast: High Potential as a Source of Anticancer Compounds, *Marine Drugs*, 12(1), 98-114.

A mouse model for infection with Group B Streptococcus that mimics the human neonatal disease

Ana Puga^{1,2}, Ana Magalhães², Pedro Madureira^{1,2}, Adília Ribeiro^{1,2}, Margarida Correia-Neves^{3,4}, Augusto Faustino¹, Teresa Summavielle^{2,5}, Patrick Trieu-Cuot⁶, Elva Bonifácio Andrade^{1,2} and Paula Ferreira^{1,2}

¹ ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, University of Porto, Porto, Portugal.

² IBMC - Instituto de Biologia Molecular e Celular, University of Porto, Porto, Portugal.

³ Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal.

⁴ ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal.

⁵ ESTSP-Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, Vila Nova de Gaia, Portugal.

⁶ Institut Pasteur, Unité de Biologie des Bactéries Pathogènes à Gram-positif, CNRS URA 2172, 75724, Paris, France.

Bacterial meningitis is a serious life-threatening disease and a major cause of disability worldwide. Among the neonatal group, *Streptococcus agalactiae* or Group B Streptococcus (GBS) is the most common cause of bacterial meningitis. Efforts towards a better understanding of the pathogenesis and pathophysiology of GBS-induced meningitis are urgent. An animal model in which the induced disease closely resembles GBS infection in humans is still missing. Here, we developed a novel murine model of GBS-induced neonatal meningitis, addressing the natural course in human pathogenesis of bacterial vertical transmission from the lower genital tract to neonates. For that purpose, pregnant BALB/c female mice were infected intra-vaginally with a GBS strain, the BM110, which is greatly associated with human meningitis. Upon delivery, female vaginal tract colonization and bacterial transfer into the newborns were monitored at different time points. The obtained results showed that the progenitors were highly colonized at the delivery day and the bacterium was transmitted to their offspring. Indeed, GBS was found in the lungs, blood, liver and brain of pups, at different time points after birth. Moreover, approximately 40% of the infected offspring died in the first 4 days after birth. Histopathological analysis of the brain tissue sections from GBS-infected pups exhibited classical features of meningitis such as meningeal detachment, edema, venous congestion, and signs of hemorrhage, similarly to the ones observed in humans. As long-term sequelae are common among infants that survive to GBS infections, cognitive and motor performance as well as alterations in neurotransmission pattern was assessed in adult mice that survived to GBS infection. A decreased learning and memory performance, determined by Radial Maze Test, as well as decreased locomotor and exploratory abilities, conducted through Open Field Test, were observed in mice that survived to GBS infection when compared to age-matched controls. These behavior tests were highly associated with a significant alteration in the pattern of some neurotransmitters in different brain regions of mice that survived to GBS infection.

In conclusion, these results indicate that our mouse model shows the characteristics observed in humans and, therefore, could be a very relevant model to understand the pathophysiology of GBS-induced neonatal meningitis.

This work was supported by European Regional Development Fund (FEDER) through the Operational Competitiveness Program (COMPETE) under Project FCOMP – 01-0124-FEDER-015841 and by FCT, under Project PTDC/SAU –MIC/111387/2009.

Xenobiotics in Human Milk: Risks to Human Health

T. Castro, J.C.G. Esteves da Silva

CIQ(UP), Department of Chemistry and Biochemistry, Faculty of Science,
University of Porto, Portugal.

Currently there is an increased concern about the effect of environmental factors on disease development. Among these factors are the expositions to toxic chemical substances which increase the risks of cancers and neurological diseases, for example. In the last decades the development of new industrial substances to improve our quality of life leads to an exposure to certain xenobiotics which might trigger pathways of diseases development. Thus, it is very important to have knowledge of xenobiotics that have arisen in the human body and their interference on individual' health. Particularly, those unnatural substances that embryos, fetus and new born babies are being exposed.

This project has two main objectives: biographical research about the xenobiotics presents in human breast milk and the associated health risks for the baby during its development; collection and analysis of human milk samples for screening of the major xenobiotics families – this task is being coupled to a questionnaire to the anonymous milk donors to search for possible sources of xenobiotics exposition. Research is being focused on persistent organic pollutants (pesticides, industrial solvents and dioxins), toxic flame retardants, Teflon chemicals, personal care products, plasticizers and toxic metal ions.

This research is being focused on a town located in the north of Portugal (Viana do Castelo). The results obtained will allow the development of advertizing campaigns on environmental health issues towards selected vulnerable populations, namely pregnant women (and corresponding families) or couples that are thinking on having babies, explaining behaviors that should not be taken, or changed, to minimize the exposition towards xenobiotics families and health risk reduction.

In this communication the results of the bibliography review about xenobiotics in human breast milk will be presented as well as the preliminary results of the screening on real samples. Also, the current environmental health counseling strategy will be presented and discussed.

Modulation of the uptake of critical nutrients by breast cancer cells by lactate: impact on cell survival, proliferation and migration

M. Guedes^{1,2}, J. R. Araújo¹, A. Correia-Branco¹, F. Martel¹ and E. Keating^{1,3}

¹ Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Portugal.

² Faculty of Sciences, University of Porto, Portugal.

³ Center for Biotechnology and Fine Chemistry, School of Biotechnology, Portuguese Catholic University, Portugal.

Breast cancer is the most common cancer among women, both in developed and developing regions of the world. Although the survival is improving, it is expected that the burden of breast cancer will increase globally. Folate and glucose are critical nutrients for cancer cell proliferation and their cellular uptake and metabolism are good targets for anticancer therapies. Lactate, a by-product of cancer cell metabolism, may also serve as a nutrient for cancer cells and thus play a role in cancer progression.

In this context, the present work aimed to study the effect of lactate metabolites upon the transport of folic acid and glucose and upon the viability, proliferation and migration capacity of T47D cells (a model of human breast cancer cells).

First, we studied the uptake of deoxy-D-glucose (2-[1,2-³H(N)] (³H-DG)) by T47D cells. It was found to be: a) time-dependent, b) optimum at physiological pH, c) substantially inhibited by the facilitative glucose transporter (GLUT) inhibitor cytochalasin B and less inhibited by the sodium-dependent glucose co-transporter (SGLT1) inhibitor phloridzin, d) sodium-independent and e) slightly insulin-stimulated. Consequently, we conclude that ³H-DG uptake by T47D cells is mediated by members of the GLUT family (most probably GLUT1, given its characteristic expression in breast cancer cell lines, with a possible contribution of the insulin-responsive glucose transporters, GLUT4 and GLUT12) and it does not involve SGLTs.

The modulation of ³H-folic acid ([3',5',7,9-³H] sodium salt (³H-FA)) and ³H-DG uptake in T47D cells by lactate metabolites and the effect of these compounds upon the viability, proliferation and migration capacity of T47D cells were also evaluated.

Acute exposure (26 min) of T47D cells to lactic acid (LA) (but not to sodium lactate (SL)) caused a significant stimulatory effect on ³H-FA uptake. This effect was abolished in the presence of an inhibitor of the monocarboxylate transporter 1 (MCT1) (sodium propionate), indicating that the stimulatory effect of LA upon ³H-FA uptake by T47D cells involves MCT1. This effect was also observed in relation to ³H-DG uptake by T47D cells, although it was less expressive.

Chronic exposure (24 h) of T47D cells either to LA or to SL increased ³H-FA uptake, while not affecting ³H-DG uptake.

Chronic exposure of T47D cells either to LA or to SL significantly decreased cell viability, whereas chronic LA, but not SL, decreased cellular proliferation. Additionally, we observed that chronic exposure either to LA or to SL tended to stimulate T47D cell migratory capacity.

In conclusion, these results show a surprising effect of lactate on breast cancer cells, which deserves further research in the future to dissect the molecular mechanisms involved.

This work was supported by Fundação para a Ciência e Tecnologia (PTDC/SAU-OSM/102239/2008; SFRH/BPD/40170/2007).

A2

ENGINEERING I

I
PARALLEL
ORAL
SESSIONS



Sensitivity studies using analytical and numerical methodologies on variables influencing sheet metal Press Brake bending operations

Sara Miranda¹, Abel D. Santos^{1,2} and J. Bessa Pacheco^{1,2}

¹ Faculty of Engineering, University of Porto, Portugal.

² Institute of Mechanical Engineering and Industrial Management (INEGI), University of Porto, Portugal.

Press Brake bending operations belong to sheet metal forming processes and this kind of processes may have a simple geometric interpretation, in which a 2D analysis is usually performed. The bending operation of the sheet metal blank consists of a V shape forming by using a punch, with a certain nose radius, forcing the sheet plate against an open die, with a V section.

The forming result is a part with an angle obtained between the V legs (flanges), which is known as the bending angle. The operation to get the required V angle is called air bending or free bending [1].

The punch penetration inside the die, known as bending depth, is responsible for the bending angle. However the amount of penetration to reach the required bending angle depends both on the inside bending radius, with direct influence on the geometry for the angle evaluation, and on the amount of springback occurring after releasing the tools from the bent plate.

Different studies are presented for determination of inside bending radius, punch penetration and springback as a function of bending angle, using finite element analysis and analytical methodology. The sensitivity analysis is performed for different sheet thicknesses, different V legs and different materials (aluminium and steels). These results and presented methodology represent a contribution to a correct prediction of the inside bending radius and the expected springback, needed to obtain the punch penetration for the required final bending angle of a sheet component in Press Brake bending operations.

References:

[1] J. Bessa Pacheco, Abel D. Santos (2013), *A study on the Nose Radius influence in Press Brake Bending Operations by Finite Element Analysis*, Trans Tech Publications, Switzerland, Key Engineering Materials, Vols. 554-557, pp. 1432-1442.

Springback prediction in Sheet Metal Forming

Rui Amaral^{1,2} and Abel D. Santos^{1,2}

¹ Faculty of Engineering, University of Porto, Portugal.

² Institute of Mechanical Engineering and Industrial Management (INEGI), University of Porto, Portugal.

The increasing complexity of products on the market, combined with its steady reduction in development cycles, has made technological processes of sheet metal forming process commonly used in many areas of industry. With the trend towards the use of higher strength materials, which in turn should be lighter, created challenges to sheet metal forming processes. These new challenges created the need for development of numerical tools and mathematical models allowing a better modelling of this fabrication process and the prediction of springback, an important behaviour affecting the forming of sheet metal components.

The present paper aims to have a contribution in the development of such numerical tools, to predict the springback behaviour of magnesium AZ31 alloy, based on performed available experimental results, using the benchmark *Unconstrained Cylindrical Bending*, proposed at *Numisheet'2002 Congress* [1].

Numerical simulations were performed taking into account the behaviour of the materials under study and the springback phenomenon, responsible for dispersion of part shapes. The predictions were compared with experimental results in order to validate the numerical models featured (Fig 1).

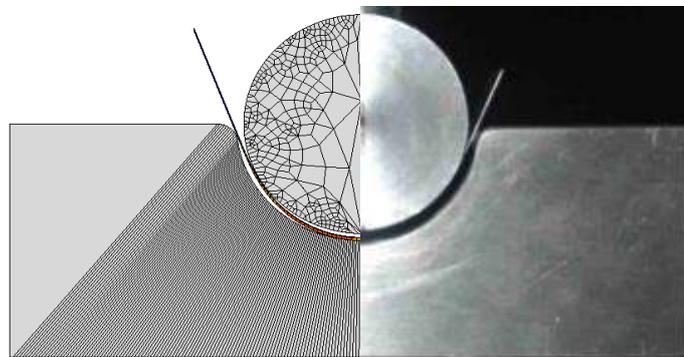


Figure 1: Comparison between numerical and experimental results.

References:

[1] Numisheet (2013), *Proceedings of the 5th International Conference and Workshop on Numerical Simulation of 3D Sheet Forming Processes*, Jeju Island, South Korea.

ELASTO-PLASTIC ANALYSIS OF THIN STRUCTURES USING A MESHLESS METHOD

H.M.S. Duarte¹, J. Belinha^{1,2}, L.M.J.S. Dinis^{1,2}, A.A. Fernandes^{1,2} and R.M. Natal Jorge^{1,2}

¹ IDMEC – Institute of Mechanical Engineering. Instituto de Engenharia Mecânica, Pólo FEUP, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

² FEUP – Faculty of Engineering of the University of Porto. Mechanical Engineering Department. Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

Considering a three-dimensional shell-like approach, a recently developed meshless method is extended to the elasto-plastic analysis of shells submitted to transversal loads. In this work it is used the Natural Neighbour Radial Point Interpolation Method (NNRPIM) [1,2], a recently developed advanced discretization meshless technique. In order to enforce the nodal connectivity, the NNRPIM uses the Natural Neighbour concept. This mathematical concept permits to construct the Voronoï diagram of the unstructured set of nodes discretizing the problem domain. With the Voronoï diagram it is possible to obtain the “influence-cells”, which are in fact influence-domains entirely nodal dependent. Additionally, the Voronoï diagram permits to determine a node-depending background integration mesh. This integration mesh is used in the numerical integration of the integro-differential equations ruling the studied physical phenomenon. The NNRPIM interpolation functions are constructed using the Radial Point Interpolators (RPI). The obtained interpolation functions possess the delta Kronecker property, simplifying the imposition of the natural and essential boundary conditions.

In order to obtain the variable field from the weak-form of Galerkin, the three-dimensional deformation theory is assumed using a shell-like formulation. In this work only isotropic elastoplastic bilinear materials and small deformations are assumed. The used non-linear solution algorithm is the Newton-Raphson initial stiffness method and the efficient “forward-Euler” procedure is used in order to return the stress to the yield surface [3]. In order to demonstrate the effectiveness of the method, elasto-plastic benchmark shell examples are analysed.

References:

- [1] Dinis L.M.J.S., Jorge R.M.N. and Belinha J. (2007). "Analysis of 3D solids using the natural neighbour radial point interpolation method". *Computer Methods in Applied Mechanics and Engineering*. Vol.196 (13-16) p.2009-2028.
- [2] Dinis L.M.J.S., Jorge R.M.N. and Belinha J. (2010). "A 3D Shell-Like approach using a Natural Neighbour Meshless Method: isotropic and orthotropic thin structures". *Composite Structures*. Vol.92 (5) p.1132-1142.
- [3] L.M.J.S. Dinis, R.M. Natal Jorge, and J. Belinha, (2009) "The Radial Natural Neighbour Interpolators Extended to Elastoplasticity." in "Progress on Meshless Methods" A.J.M. Ferreira, E.J. Kansa, G.E. Fasshauer and V.M.A. Leitão, Springer Netherlands p.175-198. ISBN: 978-1-4020-8821-6.

The IFC Standard as an Interoperability Agent in Data Exchange Between BIM Applications

S. Pinho¹

¹ Department of Civil Engineer, Faculty of Engineer, University of Porto, Portugal.

The philosophy around the BIM (Building Information Modeling) concept enables the joining of different project scopes in a single virtual model, which brings a growing need for constant information exchange between stakeholders in construction projects. A higher level of interoperability means higher levels of engagement among participants in the building project and therefore allows greater monitoring workflow, greater automation of processes and greater control of errors and failures throughout the life-cycle of the building. Thus, the implementation of BIM enables a significant increase of speed in project and building processes.

The IFC standard as an interoperability agent has been now implemented for over one and a half. This additional pressure now comes with the realization of the new IFC4 scheme as an ISO standardization, which represents a major breakthrough of the IFC specification.

Hereby, the aim of this study subject begin by investigate IFC entities that allow the structural definition of building elements defining their analytical functions, which is now supported by IFC4. The fast technological advances require us to be always one step ahead in all that concerns informatical systems. Therefore, in the IFC field, this thesis focused especially on simple concrete structural elements (like footings, columns, beams and slabs) whose multiplication allows the composition of regular building structures. Beyond identifying the classes needed to define the structural elements that make up a regular building, was also made a study of the current levels of interoperability for the transfer of such data and it is also shown a possibility of future application of the IFC model. This practical application of IFC classes demonstrates the great potential within the IFC specification, in what concerns the exchange of information between modeling programs and programs for calculation and structural analysis, in addition to being an agent for interoperability can also be seen as a BIM tool by this point of view, however, has been the target of criticism by various authors in regard to its applicability in the field of structures.

References:

[1] Pinho, S., (2013), *O Modelo IFC como Agente de Interoperabilidade: Aplicação ao Domínio das Estruturas*, Faculdade de Engenharia da Universidade do Porto.

A3

LITERATURE,
SOCIOLOGY &
POLITICAL
SCIENCES

I
PARALLEL
ORAL
SESSIONS



The “Imperial” Mcdonaldization: glocal crossings in a ancient coffee shop in Porto

Antônio A. O. Gonçalves¹

¹Department of Sociology, Faculty of Arts, University of Porto, Portugal.

The space that now houses the Imperial McDonald's in the city of Porto was before a sumptuous coffee shop on the Avenida dos Aliados. Founded in 1936, the Imperial coffee shop was built on the site of the extinct Central coffee shop. The project of the architects Ernesto Ernesto and Camilo Korrodi was constituted by two floors: one that gives access to the Avenida dos Aliados - main lobby - and one that is located in an underground floor that had once been a billiard hall. This communication had the propose to examine the *status quo* of this ancient Porto coffee shop having by horizon the mutual implications and syncretic crossings of the place with the surroundings and *vice versa*. To do so, aiming to intensify rotation of perspective in the analysis, three empirical plans were ascertained, namely: architecture, actors and products.

With this in mind, the methodological fundament was supported by ethnographic record, as in dense description proposed by Geertz (1978) ^[1]. In terms of research technique, direct observation (Lopes, 1997) ^[2] was constituted in a sophisticated mechanism of collecting and sensitive to the vicissitudes of empiricism. However, this prerogative does not advocate that the researcher should merely observe the observable, instead, due to the unpredictable nature of "the scene", the plurality of situations and problems it can thrive, analytical feature leads to very diverse procedures from the registration of wiggles to evoked symbols.

The conclusions reached from this research indicate the tangling of distinct cultural practices and regionalization of social interactions. With regard to space, the process of standardization and formal mischaracterization initiated by McDonald's from 1990 until November 1995, promoted the emergence of an architectural pattern massification of erudite. Moreover, the combination of scenarios, characters and social behaviors emerged therefrom creates a panorama of glocalization of reality (Lopes, 2008) ^[3]. In this way, unfolds a double process of resignification of space, people making use of material resources available - ambience, places, unoccupied tables, selection of items to feed themselves, sandwiches, soups, ice cream, coffees, beers - they reappropriate the environment. Moreover, McDonald's is aware of its consumer public, then insert some products of Portuguese culture, creating conditions for the first process. The myriad of possibilities of the uses and meanings of fast food is illustrated in recurrent cases of individuals with some degree of restraint in the conduct at the table, which alludes to the erudite culture. However to accomplish it in a context of mass culture, such as McDonald's, these agents consuming a more or less standardized menu of fast food chain but in a way that evokes the slow food therefore whether consciously or not they glocalize that reality supposedly homogeneous and Americanized.

References:

[1] Geertz, C. (1978), *A Interpretação das Culturas*, Jorge Zahar Editor, Rio de Janeiro.

[2] Lopes, J. T. (1997), *Tristes Escolas – Um Estudo sobre Práticas Culturais Estudantis no Espaço Escolar*, Afrontamento, Porto.

[3] Lopes, J. T. (2008), *Da Democratização à Democracia Cultural: uma reflexão sobre políticas culturais e espaço público*, Profedições, Porto.

Southern Europe's Welfare-State Model – arm in arm with ideology?

C. Lima Silva

Degree on Languages and Internation Relations, Faculty of Letras, University of Porto, Portugal

This paper is based on an essay by Pedro Adão e Silva (“O Modelo de Welfare da Europa do Sul. Reflexões sobre a utilidade do conceito.”), that analyses the characteristics and similarities of the Southern Europe's welfare models, whilst analysing the importance of the model approach and the necessity of developing particular methods for each case.

To investigate the different welfare models, I analysed several texts on the subject, using them alongside specific statistic data about economic and social indicators.

Throughout this text I consider the Welfare-State of the Southern Europe countries, analysing its specificities and comparing them using the tripartite model of Esping-Andersen, concluding if the Welfare-State typical of the Southern European countries constitute another model besides the ones already synthesised by Esping-Andersen and the pertinence of these kind of politics and social structures nowadays when a strong crisis has befallen not only over these countries (despite the particular impact of it over Southern Europe), but worldwide due to the economic globalization that increases the level of interdependence between nations.

During the research, I verified that the dynamics of the welfare model, used in such countries as Portugal or Spain, are largely different of the ones of the other models (these models are: the continental, bismarckian or corporate model; the liberal or anglo-saxonic model; and the social-democrat, beveridgian or scandinavian model). The importance of factors like family, the State or the work market is completely different of the one verified in countries using one of the three basic models, and lead the society to develop alternative strategies to achieve social well-being (for example: the welfare mix or the “welfare-society”) and adapting the politics of welfare to the social desposition. At the same time, I analysed the politic course of the portuguese State and the several governments since the end of the salazarist regime, observating their ideological positions and ideas and confronting them with the Welfare-State evolution. In between them I saw some differences, but a bigger level of adjustment.

All in all, I concluded that a new model should be considered alongside with the ones theorised by Esping-Andersen, the Latin Model. And this model, even though it may seem initially unfit, it complements the ideologies of the Southern European countries (specifically in the studied case, Portugal).

“O POETA DESTROI-VOS”: body and writing as resistance in Cesariny and Gullar

R. R. Lima¹, R. M. Martelo ²

¹ Student, Masters in Literary, Cultural and Interart Studies, Department of Portuguese and Romance Studies, Faculty of Letters, University of Porto, Portugal.

² PhD Professor, Masters in Literary, Cultural and Interart Studies, Department of Portuguese and Romance Studies, Faculty of Letters, University of Porto, Portugal.

Mário Cesariny and Ferreira Gullar have more in common than the Portuguese language (despite the differences between the European and the Brazilian uses of Portuguese): they have both lived in oppressive political contexts, experiencing similar situations of dictatorship and censorship in their countries (Portugal and Brazil, respectively). Besides, both the poets had to deal with limitations to their freedom of expression (and not only this kind of freedom). The aim of this study is to identify the strategies of discourse's subversion and the means of a contraculture affirmation adopted by each one of these authors.

In this perspective, the most representative poems have been selected from the anthology *Uma grande razão*, by Mário Cesariny [1] and from the books *Poema sujo* and *Dentro da noite veloz*, by Ferreira Gullar [2], and compared by the light of the studies of the lyric by Karlheinz Stierle [3], observing the conflict between the “social” and the “sentimental” identities.

These were the poems considered more operational for the comparison (Table 1):

Cesariny	Gullar
louvor e simplificação de Álvaro de Campos	Voltas para casa
autografia I	Poema Sujo (trechos)
a antonin artaud	Poema Sujo (trechos)
fidelidade	A vida bate/ Boato
passagem de emile henri	O açúcar
no país onde os homens são só até o joelho	Meu povo, meu poema

In these texts, there are apparent biographical marks, such as names of real people/places related with the poets' lives, that are not so simple as it would seem at a first sight. When the poets mentioned those names and circumstances, it was possible to read that they were not just telling us their memories, but questioning them and also their own identity (as real men and as a fictional entity, that is, the I lyric). The limits between these two categories showed to be very fragile, and the complexity of this relation seemed to be worsened by the limitations imposed to the ways of expression. Both Gullar and Cesariny were persecuted because of their works, but were capable of leaving a strong testimonial of resistance in poetical language and in life.

References:

[1] CESARINY, M. (2007), *Uma grande razão*, Assírio & Alvim, Lisboa.

[2] GULLAR, F. (2003), *Obra poética*, Quasi Edições, Famalicão.

[3] STIERLE, K. (1999) *Lenguaje y identidad del poema. El ejemplo de Hölderlin*, in Aseguinolaza, F. C., “Teorías sobre la lírica”, Arco/Libros, Madrid.

The influence of Guinevere's royal legitimacy over the chivalry's supremacy

E. Rabçal¹

¹Faculty of Arts, University of Porto, Portugal.

Arthurian literature is quite a vast area of research due to the variety and complexity of its themes and motifs. This literary *corpus* is composed of several narratives, in verse and in prose, telling the story of King Arthur and the Knights of the Round Table. The permanent presence of the main characters throughout the novels that are scattered over a vast number of manuscripts allows us to follow in detail the evolution of these characters, in order to better understand the role they play in the narrative.

This oral presentation will focus on queen Guinevere's actions, mainly those related to her royal legitimacy as it is told in the manuscript 752BNF and in the manuscript 2594ÖNB. Together they form the cyclic version of *Lancelot en prose*¹ and the Portuguese *Demanda do Santo Graal*². The research emphasizes the "Fausse Guenièvre" episode and Guinevere's final condemnation, at the end of the cycle, both of them extremely important for the characterization of the Queen. The comparative analysis of these two sections allows us to recognize the repetition of some narrative structures, such as the condemnation of the Queen, her release by Lancelot and her return to the throne by Arthur's side. Thus, it is perceptible how the process of legitimacy of the Queen, present in both episodes, contributes to consolidate one of the main goals of the Arthurian romance: to emphasize the undeniable importance of Chivalry, underlining the dependence of the Royalty on this social group.

The study and detailed analysis of these medieval narratives are very important tools to understand social dynamics and patterns through the Middle Ages, since they illustrate in a quite reliable way some aspects of medieval mentality. Depicting an idealization of society, the chivalry romances bring to the surface the inner ideology of a time when feudal alliances were the basis of collective stability³. They show how thin and delicate was the balance between the power of the King and the Knights in Western Medieval Europe⁴.

References:

[1] Mosès, François (1998), *Lancelot du Lac III. La Fausse Guenièvre*, Dir. Michel Zink, Collection Lettres Gothiques, Le Livre de Poche, Paris.

[2] Nunes, Irene Freire (2005), *A Demanda do Santo Graal*, Imprensa Nacional Casa da Moeda, Lisboa.

[3] Duby, Georges (1990), *A Idade Média, uma idade do Homem*, trad. Maria Assunção Santos, Teorema, Lisboa.

[4] Bloch, Marc (1998), *A sociedade feudal*, Trad. Liz Silva, Edições 70, Lisboa.

Almada, the invention of the poet

Zélia P. G. Moreira¹

¹Faculty of Arts, University of Porto, Portugal.

This brief paper has as its main concern to present one of the remarkable Portuguese writers of the last century, José de Almada Negreiros, highlighting some important qualities of his poetics.

A member of the *Orpheu's* group, Almada was a man that outlived his time, always reinventing himself as a poet, as a creator. It is that extreme desire to exceed himself over and over again that represents his journey or “itinerario universal” in his own words [1]. For that same reason we can never think about Almada and his oeuvre like two separate aspects, they are imbricated one another. It is this continuous process of work (equivalent to a continuous process of self-knowledge) that I will emphasize in this synthetic paper.

Many factors contribute to this endless act of creation; one is the continuous rewriting of his works. For Almada a text is never finished - this is why his literary system is a process, it is this permanent engendering of text, this incessant act of creation which is for him the definition of Poetry itself as he describes on the sixties [2] [3]. His method of writing we lead us to conclude that Almada is poetically very close to the German romanticism, not only by his extreme belief on the power of poetry but also by his fragmentary and aphoristic style.

Probably, the keyword to define Almada may be ingenuity. Already noticed and highly demonstrated by the critics [4], also inherited from romanticism, that concept plays even a wider role in Almada's poetics. My closing topic will focus on the implications of ingenuity on Almada's oeuvre as well as its impact on his posture and attitude along his maturity.

References:

[1] Negreiros, Almada (2004), *A Invenção do Dia Claro*, edição fac-similada, Lisboa, Assírio & Alvim, p. 38.

[2] Rubim, Gustavo (1998), “Palcos de Palavras, A cena da escrita na poesia de Almada Negreiros”, in Silva, Celina (coordenação), *Almada Negreiros. A Descoberta como Necessidade. Actas do Colóquio Internacional (Porto, 12-14 de Dezembro, 1996)*, Porto, Edição da Fundação Eng. António de Almeida, p. 333.

[3] Negreiros, Almada (2006), “Poesia é criação. Dez minutos antes da leitura por Alberto de Lacerda de poemas de outros poetas da nossa língua” in *Obra literária de José de Almada Negreiros/5, Manifestos e Conferências*, Lisboa, Assírio & Alvim.

[4] Silva, Celina (1994), *Almada Negreiros, A busca de uma poética da ingenuidade ou a (re)invenção da utopia*, Porto, Fundação Eng. António de Almeida.

Portrayal of the African people into Castro Alves' *Os Escravos*

C.C.E.Souza¹, J.A. Hansen².

¹ Program of Brazilian Literature, Department of Classical and Vernacular Letters, Faculty of Philosophy, Letters and Humanities, University of São Paulo, Brazil.

² Advisor, from the same Program.

Castro Alves is a poet of the Brazilian Romanticism, who dedicates an entire book to the abolitionist cause. The critics usually point to the emotional complexity of his portrayal of African slaves, defending that he was the first Brazilian poet to be interested in their suffering. This presentation intends to critically read this analysis of his poetry and propose another point of view.

Proposing something different demands a first movement of explaining briefly the standard approach of Castro Alves' poetry, demonstrating the weakest points of this analysis. This demonstration will be done through the lecture of some verses from the poems "A Visão dos Mortos" and "O Navio Negroiro", from *Os Escravos*. These verses will also be applied to demonstrate the relevance of our proposal.

This critical reading of Castro Alves' abolitionism has demonstrated two complementary results. 1. Slavery, to the poet, isn't an important social issue because of the suffering of lots of human beings who did nothing to deserve that. It's important, on his point of view, inasmuch as it causes a backwardness of Brazilian nation. And 2. The African slave does not have a fully human complexity in Castro Alves' poetry, because all the actions of them and all the barbaric situations that they need to go through are important just as it creates the feeling of pity and indignation into the reader's mind. The African subjects are not portrayed because the lyrical speaker is interested in their suffering, but because it's interested in making the reader feel pity of their suffering.

To present all the whole analysis – or, more appropriately, these analyses – in only ten minutes would be impossible if we intended to analyze all the standard critics. Therefore, we've selected as *corpus* the text "Poesia e Oratória em Castro Alves", as presented in the book *A Formação da Literatura Brasileira: Momentos Decisivos*, written by Antonio Candido. The nationalistic character of this text – one of the most important to Castro Alves' study – allows us to reflect about the reasons why the nationalist/paternalist character of Castro Alves' abolitionism has not been a target to the contemporary investigations of his poetry.

This research has been made under Professor João Adolfo Hansen's guidance and would not be possible without the discussions with Professor Cilaine Alves Cunha, Professor Jaime Ginzburg and with colleagues, such as Renato Razzino. This research was financially supported by FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo.

References:

[1] Alves, C. (2004), *Obra Completa*, Editora Nova Aguilar, Rio de Janeiro.

[2] Mello e Sousa, A.C. (1975) *A Formação da Literatura Brasileira: Momentos Decisivos v.2* Editora Itatiaia, São Paulo.

A4

ARCHITECTURE
& ARTS I

I
PARALLEL
ORAL
SESSIONS



Invisible shapes: the influence of serif shapes in the legibility of long printed texts

C. Ferreira¹

¹ Department of Design, Faculty of Fine Arts, University of Porto, Portugal.

Serif typefaces are generically accepted as more adequate for long printed texts. Typographers like Gerard Unger [1] have supported the use of serifs on Cognitive Psychology studies. However many of these studies had their methodology based on obsolete theories. In the last few years investigators like Kevin Larson and Sofie Beier [2-4] have been adapting the typographic legibility tests to the current reading model, known as Parallel Letter Recognition. In 2013 Matthew Carter and Kevin Larson presented new hypothesis at the ATypI conference [5]: when developing the Microsoft font *Sitka*, the tests suggested that wedge serifs and reduced terminals could improve the global legibility of the font.

The goal of this study is to investigate if serifs can really help the reading process when their shapes are defined by legibility tests, instead of historical or aesthetical motivations. For that, an unpublished font family with serif variations will be tested. We expect to corroborate the hypothesis raised by Carter and Larson. If so this study may provide objective data that can help the process of choosing fonts for editorial projects with long printed texts.

References:

- [1] Unger, G. (2007), *While you're reading*, English Translation, Mark Batty Publisher, New York, pp. 166-167
- [2] Larson, K. (2004), *The Science of Word Recognition: or how I learned to stop worrying and love the bouma*, Microsoft, available from: <http://www.microsoft.com/typography/ctfonts/WordRecognition.aspx> [Accessed 18 December 2013]
- [3] Beier, S. (2009), *Typeface Legibility: Towards defining familiarity*, (Doctor of Philosophy), The Royal College of Art.
- [4] Beier, S. and Larson, K (2010), *Design Improvements for Frequently Misrecognized Letters*, Information Design Journal, 18 (2), 118-137.
- [5] Carter, M. and Larson, K. (2013), *Designing with Science*, (Oral Presentation), 57th annual conference of Association Typographique Internationale, Amsterdam, 9–13 October 2013, Abstract available from: <http://www.atypi.org/past-conferences/atypi-amsterdam-2013/amsterdam-programme/activity?a=297> [Accessed 18 December 2013]

Aquatipia as stratigraphic painting

Ana Margarida Rocha¹, Graciela Machado², Teresa Almeida³

¹ Painting Department, Faculty of Fine Arts, University of Porto, Portugal.

² i2ADS - Institute of Research in Art, Design and Society, Department of Drawing, Faculty of Fine Arts, University of Porto, Portugal.

³ Research Unit VICARTE "Glass and Ceramic for the Arts", i2ADS - Institute of Research in Art, Design and Society, Department of Fine Arts, Faculty of Fine Arts, University of Porto.

The possibility of monitoring the appearance of the Earth surface is now available to everyone, through applications that provide satellite imagery[1], as we follow the actions of sedimentation, erosion, drought. These phenomena transformation and passing of time, result in graphic structures and materic compositions that are repeated, regardless of scale.

This artistic work seeks to recreate these actions of sedimentation and erosion, adding and removing material, drawing a parallel between the action of nature on their surfaces and the actions of the artist on his substrates. It is about trying to decompose the time and space in the landscape, in a map of actions and forms.

Based on traditional media of painting, drawing and printmaking, its limits are tested by a combination of processes and procedures. An experimental work program is developed within the exploration of expressive potential of painting through a mediated process of image transfer, mainly based on *aquatipia*. Having as origin the traditional marbling techniques[2] applied in crafts[3], such procedures test the accumulating layers of oil paint, transferred from the surface of water, and combined it with the use of masks, its application to substrates with specific qualities of transparency, translucency, brightness, and plasticity, such as polypropylene, polyester and glass. Special attention is given to this substrate, considering a potential for combining bidimensional and tridimensional image transfer making techniques such as sandblasting, handblasting, acid etching [4] and slumping[5].

References:

[1] Cosgrove, D. (2001). *Apollo's Eye: A Cartographic Genealogy of the Earth in the Western Imagination*. Baltimore: The Johns Hopkins University Press.

[2] Wolfe, R. (1990). *Marbled Paper: Its History, Techniques, and Patterns*. Pennsylvania: University of Pennsylvania Press.

[3] Greenhalgh, P. (2002). *The Persistence of Craft: The Applied Arts Today*: Piscataway: Rutgers University Press.

[4] Schmuck, J. (2009). *The Joy of Coldworking*. Clemmons: Four Corners International.

[5] Beveridge, P; Doménech, I.; Pascual, E. (2005). *Warm Glass: A Complete Guide To Kiln-forming Techniques Fusing, Slumping, Casting*. New York: Lark Books.

Images of glass: glass as a médium for printing

I. Trabulo

Drawing Department, Faculty of Fine Arts, University of Porto, Portugal.

This study seeks technical means throughout an investigating process of the methods on how to print over and within vitreous surfaces .

Opting to work two types of printing in the field of printmaking : screen printing and direct printing .

Thus , collecting and testing transfer papers , looking for ways of producing paints that can be appropriate for this type of techniques , linking two areas within fine arts that are quite distinct and far apart: stained glass and engraving.

The great interest concerning this research is the exploration between how glass and printing techniques work together , finding ways to transfer picture decals direct from objects , through the techniques of printing on the glass surface.

Thus trying to discover the possibilities of screen printing for this purpose , such as the direct printing through the use of inking roller directly over the surface of the object.

Assuming the human body as an object, being the matrix used for the production of images, with markings that are obtained through the application of pressure. To produce these prints it is required to have touched something, in other words the information is transferred from one surface to another.

Since the main purpose of this study, is to turn theses markings, lasting and permanent, marks which are caused by us on a day- to-day basis almost unconsciously, on their characteristic ephemerality, images like, dermatoglyphics , fingerprints , etc., permanently engraving them in the vitreous surfaces.

References:

Petrie, K. (2006), *Glass and Print*, A & C Black Publishers.



Between Architecture and Plastic Arts

Mariana Martins Matos

Faculty of Architecture, University of Porto, Portugal.
Master's Degree Thesis

Between Architecture and Plastic Arts is about the constant relation between these two fields, the intensity of how they have been mixed in the last few years, the architect's freedom to create and his common sense.

In the last few decades, Architecture has become more and more mixed with Plastic Arts, adopting some of its ways of expression and concepts. Architecturally speaking, «*occupying a space means to transform the city*» what turns any architectural piece a «*testimony of ubarnity*»¹. Having this responsibility, what attitude is the architect supposed to have: of a technician or of an artist? Does the architect have the same creative freedom as a plastic artist? How far can he go in the arts field, what are the architecture limits in the arts? When does the architect cross the line between creative freedom and the bad sense? Where does architecture stops being architecture and becomes another art?

In order to find answers, some examples of hybrid pieces of architecture and arts will be studied. By *hybrid pieces* I mean architectural buildings that get very close to art pieces - due to some of their aesthetic characteristics - and art pieces that get very close to architecture - because of their scale and possibility of physically experimenting it. This study is meant to understand what characteristics can Architecture have in common with Plastic Arts - like rules and concepts - its creative limits and the architect's freedom as an artist - which he is. Besides this, it is important to think how Architecture can use these plastic arts' experiences to its own benefit.

The work is divided in two main parts. In the first one, important concepts, like *Art* and *Architecture*, will be defined. After proving that Architecture is an art, some of its specificities will be distinguished from the plastic arts' and the importance of architect's role as an artist will be clarified. Still in this part, but in a later chapter, the complicities between architecture and arts along History will be studied.

In the second part, some hybrid works will be analyzed and compared in a critical way, in order to put the already mentioned concepts and some theories in practice. Not only will the works be analyzed, but their artists' justifications and some criticism that have been made to them.

Then, in the last chapter, final considerations will be made. Here, the questions raised along the work are clarified, in order to determine the architect's freedom in the creative act. These conclusions are not supposed to be determinant and universal but, certainly, a contribute to the discussion of this present issue, and an instrument to help on the definition of a self attitude as a thinker and as a creator, in a future architecture path.

References:

[1] Siza, A. (2009), *01 Textos*, Civilização Editora, Porto.

Order and Chaos in Architecture

M. Kovalchuk¹

¹ Faculty of Architecture, University of Porto, Portugal.

During my studies in Architecture, I faced many uncertainties about process and creation of an Architectural Project. This way, I would like to take an opportunity presented by my thesis, to develop an investigation and a theory based on the dichotomy between two opposite, yet completely related concepts: order and chaos.

The main goal of my work is to understand how order and chaos can function in contemporary architecture, how can they apply to the project process and, on the other hand, how they reflect on spatial perception of a human being.

My investigation begins with studying and understanding the evolution of etymology of order and chaos throughout history in different contexts: scientific, philosophical, and architectural. According to a point of view of Geometry, order and chaos are the basic components of the composition of architectural and urban structures. Yet, from the point of view of spatial perception, other definitions can be applied as those related to Phenomenology.

As I proceed my work, I discover that order, positivist *per se*, has been the main paradigm of universal and also architectural thinking: “There where is born the order, is born well-being” as Le Corbusier summarized [1]. Though known by its negativist overtone, in the last century, the concept of chaos has been taking on a new character. According to recent discoveries in Science such as Complexity theories, Chaos theory and Catastrophe theory have been the major influence on our view of Universe and Contemporary Architecture as well [2]. I attempt to explain how these advances have also influenced Technology and Computation as nowadays it is impossible to speak about Architecture without mentioning Technology and especially Parametric Design.

It is noticeable how some contemporary architects and architecture theorists took these advances into account and tried to apply them to their practice. The final section of my thesis will concentrate on comparison between selected contemporary architects in order to discuss these new aspects and abilities of contemporary architecture, many of its flaws and problems.

My work is still in progress so I would like to enrich it by further investigation as well as communicating with people involved in Architecture and other different areas.

References:

[1] Le Corbusier (1927), *Towards an Architecture*, Getty Research Institute, Los Angeles, p.312.

[2] Jencks, C. (1995), *The Architecture of the Jumping Universe*, Academy Editions, p.107-158

Robotic Construction of Free Form Walls using Standard Cork Bricks

M. Oliveira¹, J. Campos¹, J. Azevedo¹, N. Maia², G. Veiga³, A. P. Moreira², J. P. Xavier¹, R. Póvoas¹ and J. P. Sousa¹

¹ Faculty of Architecture, University of Porto, Portugal.

² Faculty of Engineering, University of Porto, Portugal.

³ INESC-TEC, Portugal.

With the beginning of the XXI century, the use of CAD/CAM technologies in architecture has challenged the standardization paradigm, which has ruled the building construction industry since the industrial revolution. Relevant works like the Frank Gehry's Guggenheim Museum in Bilbao or the Herzog&Meuron's Olympic Stadium in Beijing, demonstrates the growing interest in the exploration of formal and building solutions that would be very difficult to attain without these digital technologies.

In this context, this paper describes the IJUP project developed by a group of students and researchers from FAUP and FEUP. To investigate that condition, the team proposed to explore the use of the most advanced and flexible manufacturing technology available today – the industrial robotic arm – in the construction of free form walls using regular components like bricks. Given that these kinds of materials are standard and are manually assembled on site, masonry construction is traditionally limited to the materialization of simple forms. Thus, the problem consisted in: how to design and build complex geometries in architecture based on using standard building components?

Drawing from the pioneering work of Gramazio & Kohler developed at the ETH Zurich, the team selected cork as the material to be investigated under this robotic construction approach. As the first research of this kind in Portugal, this work implied a interdisciplinary collaboration between architecture and engineering to define communication protocols between the design information and the manufacturing instructions. From the development of parametric models of free form walls made by cork bricks, the team implemented a workflow, where all the unique spatial coordinates of each building element are extracted to inform their material assembly by the robotic arm. The production of a physical mock-up (Figure 1) proves the possibility to expand the applications of cork in architecture towards the production of non-standard building forms, without having to change the current industrial set-ups in the factory.



Figure 1 – The robotic construction of a free form wall made out of standard cork bricks.

Nanotechnology: Some Applications in Architecture and Design

A. Malheiro

Faculty of Architecture, University of Porto, Portugal.

Nanotechnology is the manipulation of structured systems at atomic level, and could be used as a process for a new materiality and spatiality in architecture.

This work has as object the evaluation of the relation between Architecture and Nanotechnology. To understand the potential of Nanotechnology, I study applications in architectural project, construction and building recuperation, as like in design, related with the production of “nanotechnological” products.

The first, of three parts, exposes the “*history and concepts of Nanotechnology*”, in a context of exploration by architects and designers. In this sense, it was presented a consideration about the history of materials, concepts, pioneer’s, methods and nanotechnology applications. This part allows me to understand the importance of the material evolution as the importance of a multidisciplinary approach of Nano science/Nanotechnology.

The second part, focus the relation of “*Nanotechnology with Architecture and Design*”. This part exposes the historical relation, after the 50’s, among architecture/design and technology/science; the arise of new practices of architecture and design. In this sense, is demonstrated a new material formulation only possible by the technological developments, that results in the availability of nanomaterial’s with architecture and design application. At last, it was developed a critic reflection about the risks, social impact and environment concerns of materials at nano scale. With this knowledge I could understand the role of a new technology in the development of new architecture paradigms.

The third part exposes the “*application cases*”. Such comprehend the recognition of the progressive utilization of nanotechnological products and the pedagogical changes. In this sense, I try to evidence the future influence of new nanotechnological processes on who designs, to prepare architects and designers for a new “vision” on drawing and constructive process. Afterward, were presented considerations about the critic thought and the construction of “*intelligent*” environments, namely by the use of nano-sensors. To conclude, three groups of nanomaterial’s, complemented as possible by exemplificative cases, are used as representative of possible Nanotechnology applications. The last part was fundamental for a clear interpretation of what already exists and what can be done in the future of architecture and design practice.

The study reflects the potential of nanotechnology, fomenting new project and construction paradigms, having a direct expression in surfaces and new architectonic tools. Despite, the nanotechnology paradigm, arouse problems and deliberation by architects and designers at the development of products that must favor ecological features, economical accessibility and “*intelligence*”.

A5

**BIOLOGICAL
SCIENCES I**

**I
PARALLEL
ORAL
SESSIONS**



Cannibalism in the brown shrimp *Crangon Crangon*

J. Reis^{1,2}, C. Moreira¹ and J. Campos¹

¹ CIMAR/ CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Portugal.

² Department of Biology, Faculty of Sciences, University of Porto, Portugal

The brown shrimp *Crangon crangon* (Linnaeus, 1758) is a very common crustacean in the European shallow coast from Norway to Morocco and in the Mediterranean and Black Seas. Due to its high abundance the species is a very important component in the ecosystem's trophic web, both as prey and predator. The brown shrimp is considered an opportunistic predator, feeding on the most available prey species within a list of more than 20 organisms [1]. However, cannibalism is very common in this species and might account to up to 20% of its diet [2]. In fact, cannibalism might be a result of the species' opportunistic behavior. Nevertheless, no information exists on size selectivity i.e. till which size proportion can a cannibal-shrimp feed on a conspecific.

The objective of the present project was then to study the size relationship between the potential cannibal-shrimp and its "prey-shrimp". For this an experimental design was set with two observation aquaria, each divided in 32 compartments by an acrylic structure. The temperature (13 to 15°C) and water salinity (26 to 30) were controlled on a daily basis. The photoperiod during the observations was 12/12h. Shrimps of known size were divided into 16 sets of two groups: a single "cannibal-shrimp" and five "prey-shrimps" of the same size but smaller than the "cannibal", each group placed separately in one compartment. The predator was kept in starvation during 48h before the observations. After this 48h period, the "cannibal" was transferred into the preys' compartment along with an extra dead shrimp with the size of the other preys. After a 24 hour period, the animals were separated, measured in case of moult, and the sex of the "cannibal" was identified. The number of the remaining "prey" shrimps was also registered.

Over 90% of the observed shrimps showed cannibal behavior with no significant difference between sexes: 97% and 94% of the females and males respectively. Results suggest that when cannibalism occurs the dead animal is preferred because only about 5% of the shrimps that showed cannibalism did not eat the dead prey. This confirms the opportunistic feeding behavior of the species. Nevertheless live preys were eaten in a significant number of cases probably motivated by the starving condition of the 'cannibal'. In fact the starving condition seems to be a trigger for cannibalism since predation upon live shrimps in the control trials was observed only twice, suggesting that well fed animals prey much less frequently on conspecifics. Future research on the theme should compare cannibalism in the presence of alternative prey.

[1] Campos, J., Van der Veer H.W. (2008) *Autecology of Crangon crangon (L.) with an emphasis on latitudinal trends*. Oceanography and Marine Biology: An Annual Review 46, 65-104.

[2] Pihl, L., Rosenberg, R. (1984) *Food selection and consumption of the shrimp Crangon crangon in some shallow marine areas in western Sweden*. Mar Ecol Prog Ser 15, 159-168

***Solanum nigrum* L.'s Glutathione-S-transferase plays a role in the detoxification of paracetamol**

Rui Silva², Ana Gomes¹, Ana Nascimento¹, Carla Gonçalves¹, Fábio Rangel¹, Filipe Oliveira¹, Helena Brigas¹, Henrique Duarte², Lídia Bírolo¹, Luís Esteves¹, Mafalda Faria¹, Marta Cortesão¹, Miguel Rocha¹, Patrícia Costa¹, Rodrigo Guimarães¹, Rui Ribeiro¹, Sara Campos², Sílvia Chambel¹, Steeve Lima¹, Susana Maia¹, Valerie Pantano¹, Jorge Teixeira³

¹ Cellular and Molecular Biology MSc, Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal.

² Bioengineering MSc, Faculdade de Engenharia, Universidade do Porto, Portugal

³ BioISI - Biosystems & Integrative Sciences Institute, Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal.

Glutathione-S-transferases (GSTs) are some of the main enzymes responsible for cellular detoxification of xenobiotic compounds. GSTs conjugate the activated or naturally reactive xenobiotic with reduced glutathione (GSH) resulting in a less reactive and more hydrophilic compound that can be compartmentalized in vacuoles or cell walls, no longer interfering with normal cellular metabolism [1]. *Solanum nigrum* L. has a great potential in the phytoremediation of polluted areas as it accumulates in its above-ground tissues high quantities of metals, such as cadmium, and also some organic pollutants [2-5]. Thus, the objective of this work was to study the possible involvement of GST on the metabolization of two new emerging organic pollutants: paracetamol and amoxicillin. In this way, hydroponic cultures were established with Hoagland's solution (H - control), and two test-conditions were established: 300 mg/L paracetamol (H+OP1) and 300 mg/L amoxicillin (H+OP2) - both externally supplied to the plantlets for a week. Then, total plantlet GST activity was spectrophotometrically determined [6-7] ($n \geq 4$). It was observed that GST increased its activity by 2.5 fold in plants exposed to paracetamol, but not to amoxicillin ($p < 0,001$), thus concluding that GST played a defensive role in the detoxification of paracetamol. Nevertheless, further studies are required to explore GST's role in the metabolization of other drugs and chemical compounds found in water and soils, resulting from anthropogenic activities.

References

- [1] Pilon-Smits, E. (2005), Annual, Review of Plant Biology, 56, pp. 15-39.
- [2] Marques, A.P.G.C., Oliveira, R.S., Rangel, A.O.S.S. and Castro, P.M.L. (2006), Chemosphere, 65, pp. 1256-1263.
- [3] Rezek, J., Macek, T., Mackova, M., and Triska, J. (2007), Chemosphere, 69, pp. 1221-1227.
- [4] Sun, Y., Zhou, Q. and Diao, C. (2008), Bioresource technology, 99, pp.1103-1110.
- [5] Teixeira, J., de Sousa, A., Azenha, M., Moreira, J.T., Fidalgo, F., Silva, A.F., Faria, J.L. and Silva, A.M.T. (2011), Chemosphere, 85, pp. 744-750.
- [6] Schröder, P., Fischer, C., Debus, R. and Wenzel, A. (2002), Environmental Science and Pollution Research. 10 (4), pp. 225-234.
- [7] Schröder, P., Daubner, D., Maier, H., Neustifter, J. and Debus, R. (2008), Bioresource technology. 99, pp. 7183-7191.

Signalling role of avian barred plumage: a test with common waxbills

C. Marques^{1,2}, H. Batalha² and G. Cardoso²

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² CIBIO, Research Center in Biodiversity and Genetic Resources, University of Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

The class Aves shows exquisite types and combinations of pigmentation patterns in the plumage [1]. Visual plumage patterns, such as bars and spots, are thought to evolve as camouflage, because pigmentation patterns are less conspicuous against heterogeneous backgrounds compared to plain surfaces [2]. This would allow birds to blend with their habitat, therefore, escaping predation. However, it may be that sexual selection also paid a role in plumage pattern's evolution. Barred plumage consists of alternating bars of lighter and darker pigmentation, forming a pattern of bars transversal to the feathers' axis. Recently, it has been suggested that this pattern could function as camouflage and also as signaling, because regularity of the barred pattern could indicate whether the plumage is in good condition [3].

Here, we ask if the regularity of barred plumage could be a social communication signal, using the common waxbill *Estrilda astrild* (Fig.1), a highly gregarious species. If barred plumage is used as a social signal of quality, we predict that its regularity should be higher in males and adults than in females and juveniles, as typical of sexual ornaments and other signals.

In a dataset of 635 individuals, we measured regularity of barred plumage in the back of the body. The method was based on analysis of standardized digital photographs, using software that aligns the pigmentation bars and quantifies deviations to an ideal regular pattern [4]. As predicted, there was higher regularity and contrast in adult males than females or juveniles. These results are consistent with barred plumage having a signaling function, perhaps indicating individual quality, and support the hypothesis that barred plumage patterns may fulfill double function of signaling and camouflage.

References:

- [1] Gluckman, T.-L. (2014), Pathways to elaboration of sexual dimorphism in bird plumage patterns, *Biological Journal of the Linnean Society*, in press (doi: 10.1111/bij.12030)
- [2] Bradbury, J. W. and Vehrencamp, S. L. (1998), *Principles of animal communication*. Sinauer, Sunderland.
- [3] Gluckman, T.-L. and Cardoso, G. C. (2010), The dual function of barred plumage in birds: camouflage and communication, *Journal of Evolutionary Biology* 23:2501-2506
- [4] Gluckman, T.-L. and Cardoso, G. C. (2009), A method to quantify the regularity of barred plumage patterns, *Behavioural Ecology and Sociobiology* 63:1837–1844

The evolution of the eye developmental genes in the barn owl (*Tyto alba*)

V. Maia¹, R. Borges^{1,2}, A. Antunes^{1,2}

¹ Department of Biology, Faculty of Science, University of Porto, Portugal.

² CIMAR/CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal.

The barn owl presents adaptations to night vision that are unique within birds. In fact, the owls constitute an outlier regarding the visual traits, exhibiting a notorious binocular visual for improved nocturnal activity. The post-adaptation to low light environments is an evolutionary hypothesis that we can assume for the barn owl lineage, but a deeper analysis of the molecular signatures that potentially have been acting during this period is necessary. In this study we characterized the eye developmental genes of the barn owl, as well the molecular signatures they have experienced. The main objective is to pinpoint which of these genes potentiated the adaptations to night vision in this lineage.

Here we proceed to extract eye-related developmental genes of the mammalian group, using prior information from Gene Ontology database. The genes were posteriorly identified in the *Tyto alba* genome using tBLASTn searches. Comparing the number of eye developmental genes in mammals we concluded that *Tyto alba* has only about 50% of those genes. Further research is required to understand if such massive gene loss represents a simplification of the barn owl visual system. To accomplish such goal we will implement a phylogenetic approach to complement the analyses and obtain further insight on the molecular basis of the nocturnal vision adaptation in a such exceptional group of birds-kind.

The role of morphological complexity driving the invertebrate biodiversity of intertidal macroalgae

A.C. Torres ^{1,2,3}, P. Veiga ^{2,3}, M. Rubal ^{2,3}, I. Sousa-Pinto ^{2,3}

¹ Institut of Biomedical Sciences Abel Salazar, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313 Porto

² Laboratory of Coastal Biodiversity, Centre of Marine and Environmental Research (CIIMAR),
University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

³ Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4150-181
Porto, Portugal

Oceans harbor very heterogeneous habitat and thus, a high diversity of organisms. Habitat heterogeneity increases biodiversity offering a wide range of different microhabitats and niches, allowing the coexistence of species [1]. The number and size of the microhabitats will interfere with biotic relationships between organisms [2], food availability, niche sharing [3,4] and frequency of individual size of organisms that inhabit certain place [5]. In coastal areas vegetated habitats harbor higher organism diversity than those without macrophytes. However, different species of macrophytes can show different levels of morphological complexity. In this study intertidal macroalgae were used as model organism to test the role of morphological complexity driving the biodiversity of their associated invertebrate assemblages. For this aim, 6 different macroalgae were considered and their morphological complexity was analysed measuring their area and perimeter fractal dimensions, as well as their biomass.

Studied macroalgae showed different values of biomass, area and perimeter fractal dimensions. Moreover, invertebrate assemblages were significantly different among the studied macroalgae. Rank correlation analyses showed significant relationship between biomass values and diversity of all the studied taxa. Similarly fractal area values showed significant relationship with diversity of all studied taxa except for Polychaeta, while fractal perimeter values only showed significant relationship with the diversity of crustacean and total assemblage. Multivariate correlation methods showed a significant correlation among biomass and fractal area with the studied taxa except for Polychaeta assemblage that was only correlated with biomass. However, the proportion of variability explained by these multivariate correlations was low.

This study showed that morphological complexity plays a minor but significant role driving the invertebrate assemblage diversity and structure. However, the relative importance of the different complexity measures (i.e. biomass, fractal area and perimeter) was variable among taxa.

References:

- [1] Levin SA (1992). The problem of pattern and scale in ecology. *Ecology* 73: 1943-1967
- [2] Finke DL, Denno RF (2003). Intra-guild predation relaxes natural enemy impacts on herbivore populations. *Ecol. Entomol.* 28: 67-73
- [3] May RM (1972). Will large complex systems be stable? *Nature* 238: 413-414
- [4] McCoy ED, Bell SS (1991). Habitat structure: the evolution and diversification of a complex topic. Chapman and Hall, pp. 3-27
- [5] Schmid PE, Tokeshi M, Schmid-Araya JM (2002). Scaling in stream communities. *Proc. R. Soc. Lond. Ser. B* 269: 2587-2594

Phylogeography of mtDNA haplogroup L2

M. Silva^{1,2}, **F. Alshamali**^{1,3}, **L. Pereira**^{1,4}, **P. Soares**¹

¹ Institute of Molecular Pathology and Immunology of the University of Porto, Portugal.

² Department of Biology, Faculty of Sciences, University of Porto, Portugal.

³ General Department of Forensic Sciences & Criminology, Dubai Police GHQ, Dubai, United Arab Emirates.

⁴ Department of Pathology and Oncology, Faculty of Medicine, University of Porto, Portugal.

Despite its probable Western African origin, mitochondrial DNA (mtDNA) haplogroup L2 is very frequent across Western, Eastern and Southern Africa. However, its expansion into the East is not yet properly understood. It is assumed that it occurred during the Bantu migrations, in part due to its high frequency in South African Bantu-speakers, but evidence from hypervariable region I (HVRI) of mtDNA suggests that it might have occurred earlier [1]. However, HVRI offers little phylogenetic resolution and a more precise molecular clock should be employed to produce more reliable results. Moreover, the majority of L2 sequences available in the literature are from Western Africa (and African-Americans), which might have biased previous results.

In this sense, the present study aims to reconstruct the phylogeny of L2 haplogroup, by sequencing 52 mitogenomes representative of the haplogroup diversity across Africa, with focus on East Africa. These will be combined with more than 500 sequences available in online databases. Phylogenetic reconstruction will be performed using parsimony, maximum likelihood and Bayesian inference, employing different molecular clocks (including a whole-genome clock corrected for purifying selection and a synonymous clock) [2].

The resolved L2 phylogeny provides a high resolution picture of the structure of the mtDNA L2 haplogroup. East African L2 lineages most probably arrived in the region during the early Holocene, when climate conditions improved and gene flow between East and Central Africa was probably frequent, as similarly suggested by a high resolution analysis of haplogroup L3 [3]. However lineages in Southern Africa (including within the Bantu Eastern route) differ from the ones in East Africa and cluster directly with Central/West Africa lineages suggesting an independent migration during the Bantu period.

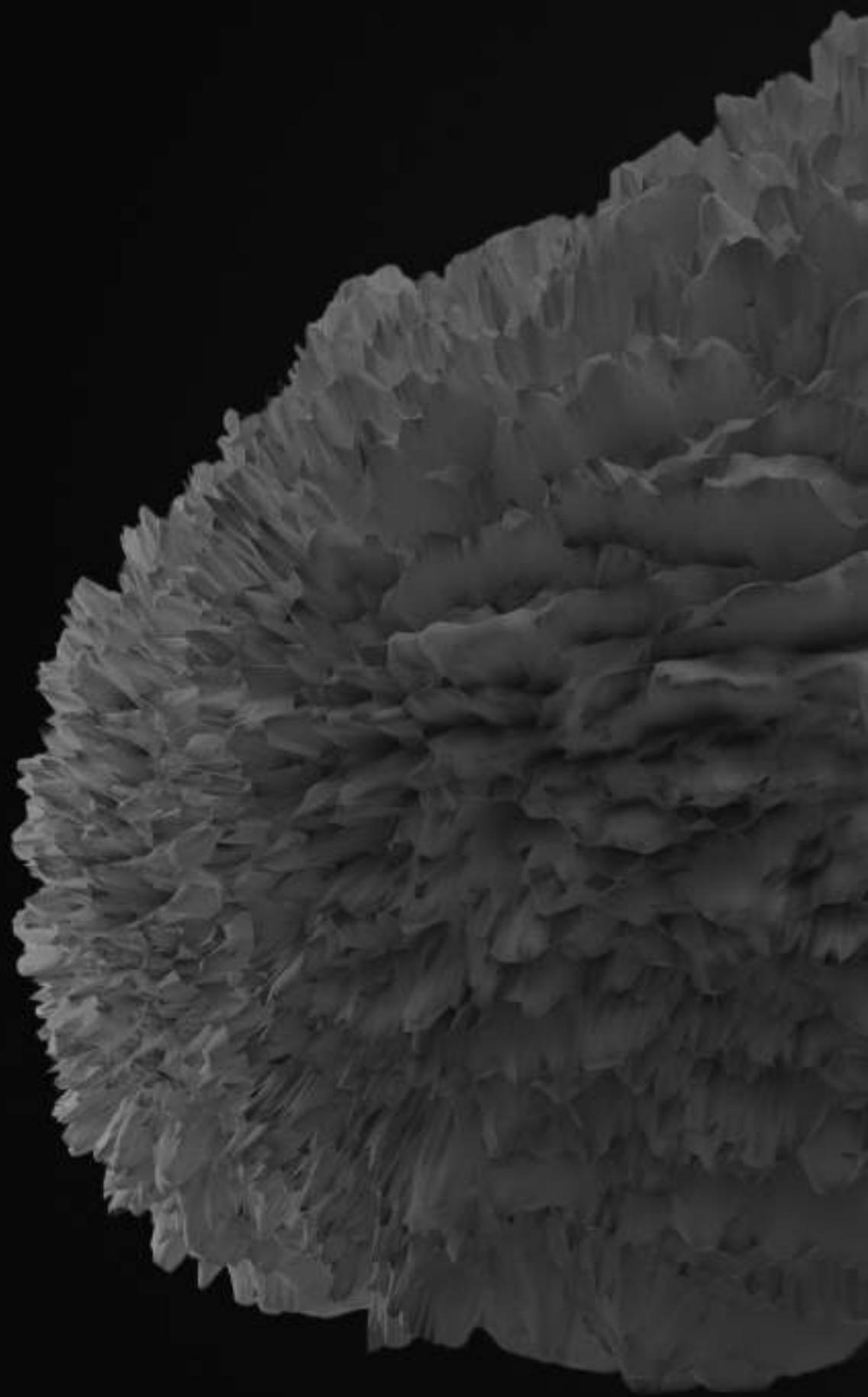
These preliminary results suggest a more complex migration pattern of L2 than a simplistic and unrealistic single migration model. Haplogroup L2 offers insights into some of the major demographic occurrences in the African continent.

References:

[1] Soares, P., Rito, T., Pereira, L. and Richards, M. (in press), *A genetic perspective on African prehistory*, in Jones, S.C. and Stewart, B. "Africa from MIS 6-2: Population Dynamics and Paleoenvironments", Vertebrate Paleobiology and Paleoanthropology Book Series, Springer.

[2] Soares, P., Ermini, L., Thomson, N., Mormina, M., Rito, T., Röhl, A., Salas, A., Oppenheimer, S., Macaulay, V. and Richards, M.B. (2009), *Correcting for purifying selection: an improved human mitochondrial molecular clock*. American Journal of Human Genetics, 84(6), 740–59.

[3] Soares, P., Alshamali, F., Pereira, J.B., Fernandes, V., Silva, N.M., Afonso, C., Costa, M.D., Musilová, E., Macaulay, V., Richards, M.B., Černý, V. and Pereira, L. (2012), *The Expansion of mtDNA Haplogroup L3 within and out of Africa*. Molecular Biology and Evolution, 29(3), 915–927.





**PARALLEL
ORAL
SESSIONS**



A1

BIOMEDICINE II

II
PARALLEL
ORAL
SESSIONS

Molecular mechanisms underlying the beneficial effects of Neuregulin-1 in the treatment of Pulmonary Arterial Hypertension

D. Santos-Ribeiro¹, P. Mendes-Ferreira¹, B. S. Alves¹, R. Adão¹, C. Maia-Rocha¹, A. Leite-Moreira¹, C. Brás-Silva¹

¹ Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto, Portugal.

Pulmonary arterial hypertension (PAH) is a syndrome based on diverse etiologies, characterized by persistent increase in pulmonary vascular resistance and afterload of the right ventricle (RV), leading to failure and death. Neuregulin (NRG)-1 is associated to several physiological processes regulating cardiac development, as well as cardiac and vascular homeostasis [1]. Following the notion that NRG-1 has protective effects in the cardiovascular system the question arise whether pharmacological NRG-1/ErbB activation has any therapeutic potential in PAH and ventricular dysfunction. Thus, we aim to explore the effects of NRG-1 treatment in PAH and its impact in myocardial function, in an animal model of monocrotaline (MCT)-induced PAH. In this work, we studied molecular mechanisms underlying the beneficial effects of NRG-1 treatment of PAH.

Male Wistar rats randomly received MCT or vehicle. After 14 days, animals were arbitrarily assigned to receive NRG-1 treatment or vehicle. The study resulted in 4 groups: CTRL, CTRL+NRG-1, MCT and MCT+NRG-1. Echocardiographic, hemodynamic studies and sample collection were performed 21 to 24 days after MCT administration.

In this study we show that NRG-1 treatment is able to restore PAH-induced severe abnormalities in cardiac function and structure. Molecular studies revealed that NRG-1/ErbB system components expression in MCT animals are changed, as demonstrated with increased levels of NRG-1 and decreased levels of ErbB4 receptors that were reversed by NRG-1 treatment. We also found increased levels of ErbB2 receptors, ADAM-17, ADAM-19, and increased eNOS expression in the RV of MCT and MCT+NRG-1 animals. NRG-1 treatment reversed changes in glucose transporters and in markers of apoptosis, as well as decreased the expression of IL6 and TNF- α found in MCT group. Moreover, we found that the increased expression of BNP, ET-1 and HIF is attenuated or reversed with NRG-1 therapy.

Concluding, we show that NRG-1 treatment might decrease PAH, restore cardiopulmonary function and attenuate or reverses the expression of markers of cardiac overload, hypertrophy and hypoxia. These beneficial effects of NRG-1 are associated with the modulation of different signaling pathways, namely apoptotic, metabolic, survival/ proliferation, and inflammation pathways.

[1] Mendes-Ferreira, P., De Keulenaer, G. W., Leite-Moreira, A. F., & Brás-Silva, C. (2013). *Therapeutic potential of neuregulin-1 in cardiovascular disease*. *Drug discovery today*, 18(17-18), 836–42.

Importance of oxygen and nitrogen reactive species on the control of *Leishmania infantum* infection

F. Marques¹, S. Vale-Costa¹, S. Cortes³, A. Tomás^{1,2}, L. Campino³ and M. S. Gomes^{1,2}

¹ Iron and Innate Immunity Group, Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal.

² Department of Molecular Biology, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal.

³ Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal.

Leishmania is a genus of parasitic protozoa of man, dogs and rodents. Sandflies are intermediate hosts for *Leishmania* and a mean of transmission between mammals by feeding on infected blood. There are several species of *Leishmania*, which can be divided according to the clinical symptoms they induce. *Leishmania infantum* is the most prevalent visceralizing species in the Old World, including Portugal, and is responsible for thousands of deaths worldwide every year [1]. In humans, the parasite invades immune cells, namely macrophages, which should be responsible for killing the parasite [2]. The parasite can survive and proliferate inside macrophages by avoiding the triggering of its microbicidal mechanisms and/or resisting their action.

In this work, the importance of two enzymes that are part of the innate immune cells' microbicidal mechanisms was studied on a mammalian model of *L. infantum* infection. Mice lacking NADPH Oxidase or Nitric Oxide Synthase 2, responsible for oxygen and/or nitrogen reactive species production respectively [3], were infected with three strains of *L. infantum* and the progression of parasitic burden was followed. The effect of the reactive species on the proliferation of *Leishmania* was further evaluated by exposing cultures of parasites to *in vitro* generated oxygen and nitrogen oxidants.

In vivo results showed that the increased susceptibility induced in mice by the absence of each enzyme was different between strains of *L. infantum*. *In vitro* susceptibility to reactive oxygen and nitrogen species was also different between strains and partially correlated to *in vivo* results. Interestingly, the only dermatropic strain tested behaved like cutaneous leishmaniasis-causing species in that it was more susceptible to nitrogen reactive species [4].

In summary, the present work contributes to the enlargement of the knowledge about the progression of *L. infantum* on the mammalian host, particularly regarding the importance of oxidative microbicidal mechanisms.

References:

- [1] Alvar, J., *et al.* (2012), *Leishmaniasis worldwide and global estimates of its incidence*, PLoS One, 7 (5), e35671
- [2] Liese, J., *et al.* (2008), *The innate immune response against Leishmania parasites*, Immunobiology, 213 (3-4), 377-387
- [3] Van Assche, T., *et al.* (2011), *Leishmania-macrophage interactions: insights into the redox biology*, Free Radic Biol Med, 51 (2), 337-51
- [4] Horta, M., *et al.* (2012), *Reactive oxygen species and nitric oxide in cutaneous leishmaniasis*, Journal of parasitology research, 2012 203818

Modulation of right ventricle function by Neuregulin-1 - therapeutic implications in pulmonary hypertension

B. S. Alves¹, D. Santos-Ribeiro¹, R. Adão¹, C. Maia-Rocha¹, P. Mendes-Ferreira¹, A. Leite-Moreira¹, C. Brás-Silva¹

¹ Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto, Portugal.

Neuregulin (NRG)-1 is implicated in the preservation of left ventricular (LV) performance in pathophysiological conditions [1]. Nevertheless, the role of NRG-1 in right ventricular (RV) failure secondary to pulmonary arterial hypertension (PAH) remains unknown. The main goal of this study was to investigate the effects of NRG-1 treatment in PAH and its repercussion in myocardial function, in an animal model of monocrotaline (MCT)-induced PAH. In order to distinguish indirect from direct myocardial effects, we also used a rat model of RV hypertrophy without PAH (banding of the pulmonary artery - PAB) [2].

Seven-week-old male Wistar rats randomly received a subcutaneous injection of MCT (60 mg/kg) or an equal volume of vehicle. Additionally, another set of rats were subjected to PAB or sham operation. Fourteen days after surgery or MCT injection, rats were arbitrarily assigned to receive therapy with NRG-1 (40µg/Kg/day) or vehicle. The study resulted in 8 experimental groups: CTRL (n=24), CTRL+NRG-1 (n=13), MCT (n=21), MCT+NRG-1 (n=24), SHAM (n=7), SHAM+NRG-1 (n=5), BAND (n=5) and BAND+NRG-1 (n=5). Echocardiographic, hemodynamic studies and sample collection were performed 21 to 24 days after MCT administration or PAB surgery.

MCT animals developed PAH, demonstrated by impaired pulmonary flow, increased RV systolic pressures and decreased cardiac output. The increase in cardiomyocyte passive tension, observed in the MCT group, were both reduced in the MCT+NRG-1 group. Administration of MCT resulted in RV hypertrophy, both at the whole heart and at the cardiomyocyte level, simultaneous with increased fibrosis. MCT animals treating with NRG-1 decreased overall hypertrophy and fibrosis. Moreover, NRG-1 treatment also improved ventricular function and reverted RV morphohistological changes in animals submitted to PAB.

In conclusion, we show that NRG-1 treatment is able to restore PAH-induced severe abnormalities in cardiopulmonary function, having also cardiac-specific effects in myocardium. These results suggest that the NRG-1 pathway has a relevant role in the pathophysiology of PAH and right ventricular dysfunction, representing a potential therapeutic target in these conditions.

[1] Odiete, O., Hill, M.F. and Sawyer, D.B. (2012), *Neuregulin in cardiovascular development and disease*, Circulation Research, 111(10), 1376-85.

[2] Schafer, S., Ellinghaus, P. and Janssen, W. (2009), *Chronic inhibition of phosphodiesterase 5 does not prevent pressure-overload-induced right-ventricular remodeling*, Cardiovascular Research, 82(1), 30-9.

Cholesterol and sphingomyelin are myelin-associated lipid inhibitors that modulate axonal regeneration following injury

FM. Mar ^{1,2}, T. Silva^{1,2}, MM. Morgado^{2,4}, LG. Rodrigues³, D. Caiola³, A. Marques², V. Sousa², J. Coentro^{2,3}, Sá Miranda³, Sousa MM^{1,2} and Brites P²

¹ Institute of Biomedical Sciences Abel Salazar-ICBAS, University of Porto, Portugal

² Group of Nerve Regeneration, Institute for Molecular and Cellular Biology-IBMC, University of Porto, Portugal.

³ Lysosome and Peroxisome Biology groups, Institute for Molecular and Cellular Biology-IBMC, University of Porto, Portugal.

⁴ Faculty of Engineering, University of Porto, Portugal.

Lack of axonal regeneration following spinal cord injury (SCI) has been mainly ascribed to the inhibitory environment of the injury site i.e. to chondroitin sulphate proteoglycans (CSPG) and myelin associated inhibitors (MAIs) [1]. Here, we used shiverer mice (shi) to assess axonal regeneration following SCI in the presence of MAIs and CSPG, but in the absence of compact myelin [2]. Although in vitro shi neurons displayed a similar intrinsic neurite outgrowth to WT neurons, in vivo, shi fibers had increased regenerative ability, suggesting that the WT spinal cord contains additional inhibitors besides MAIs and CSPG. Our data shows that besides myelin protein, myelin lipids are highly inhibitory for neurite outgrowth and demonstrates that this inhibitory effect is released in the shi spinal cord given its decreased lipid content. Specifically, we identified cholesterol and sphingomyelin as novel MAIs with activity in multiple neuron types. We further demonstrated the inhibitory action of cholesterol and sphingomyelin in vivo, by showing that delivery of 2-hydroxypropyl- β -cyclodextrin (HP β CD), a drug that reduces the levels of these lipids [3, 4], leads to increased axonal regeneration following SCI. In summary, our work shows that myelin lipids are important modulators of axonal regeneration that should be considered together with protein MAIs as critical targets in strategies aiming at improving axonal growth following injury. In this respect, our study provides the initial preclinical data needed to evaluate the possible use of HP β CD in clinical trials with SCI patients.

References:

[1] Lee JK, Zheng B. *Role of myelin-associated inhibitors in axonal repair after spinal cord injury*. Exp Neurol 2012;235:33-42.

[2] Chernoff GF. *Shiverer: an autosomal recessive mutant mouse with myelin deficiency*. J Hered 1981;72:128.

[3] Aqul A, Liu B, Ramirez CM, Pieper AA, Estill SJ, Burns DK, et al. *Unesterified cholesterol accumulation in late endosomes/lysosomes causes neurodegeneration and is prevented by driving cholesterol export from this compartment*. The Journal of neuroscience : the official journal of the Society for Neuroscience 2011;31:9404-13.

[4] Davidson CD, Ali NF, Micsenyi MC, Stephney G, Renault S, Dobrenis K, et al. *Chronic cyclodextrin treatment of murine Niemann-Pick C disease ameliorates neuronal cholesterol and glycosphingolipid storage and disease progression*. PloS one 2009;4:e6951.

Effects of high-fat diet, energy restriction, exercise and atorvastatin treatment on angiogenic factors expression in the aged rat myocardium

D. Fernandes¹, I. Tomada¹, A.M. Gouveia^{1,2}, A. Navarrete Santos³, A. Simm³, H. Almeida¹, D. Neves¹

¹ Department of Experimental Biology, Faculty of Medicine, and IBMC of Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal.

² Faculty of Nutrition and Food Science, Universidade do Porto, Portugal.

³ University Clinic for Cardiac & Thoracic Surgery, Centre for Medical Basic Research, Halle Germany

Aging and high-fat food regular consumption directly induce endothelial dysfunction and constitute risk factors for cardiovascular diseases (CVD). These conditions compromise heart function, which is partly due to angiogenesis impairment. Hence, we aimed to characterize the cellular organization of the heart and the expression pattern of vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang1), angiopoietin-2 (Ang2), hepatocyte growth factor (HGF) and receptors VEGFR1, VEGFR2, Tie1, Tie2 and c-Met, in aged Sprague-Dawley rats (18 months) under high fat diet (HFD) and energy restriction (ER). The superimposed effects of regular exercise (Ex) and atorvastatin treatment (S) to ER were also studied.

The morphological characterization of tissue was performed by immunofluorescence labelling of the endothelium and the smooth muscle (SM), followed by morphometric assessment of SM layer thickness. Dual-immunolabelling of VEGF/VEGFR2, Ang1 or Ang2/Tie2, and qRT-PCR study of vascular growth factors and receptors expression was carried out. Levels of advanced glycation end-products (AGE) were also analysed in this study, in particular total fluorescent AGE, and carboxymethyllysine, imizadolone, argpyrimidine (ArgPyr), and pentosidine by dot-blot.

The results showed that HFD treated rats present the higher heart's SM content and that ER reduced these effects. VEGF was localized in the SM and VEGFR2, Ang1, Ang2 and Tie2 were mainly expressed in the endothelium. qRT-PCR results showed a significant increase in the VEGF/VEGFR2 expression ratio in the rats treated with ER+S and ER+Ex, comparatively to rats previously fed with HFD and further submitted to ER (HFD/ER). Lower expression levels of Ang1/Tie2 were also found in HFD/ER, ER, and ER+S animals, relative to controls. In line, lower levels of Ang1/Ang2 ratio were observed in most of the experimental groups submitted to ER (ER, HFD/ER, HFD/ER+S+Ex, ER+S, ER+S+Ex, ER+Ex) comparatively to age-matched controls. This last finding was unexpected since it suggests that ER, Ex and S increase vascular instability and probably angiogenic capacity. In what concerns AGEs, a significant increase in ArgPyr levels was observed with aging. We also found that pentosidine levels increase in HFD animals, which was reversed by ER, Ex and S treatments.

Briefly, the presented data suggest that regular consumption of HFD favors structural damage, imbalance of angiogenic factors expression and AGEs deposition in heart tissue, and that energy restriction could counteract these modifications.

The anticarcinogenic effect of the dietary compound kaempferol in a human breast cancer cell line is dependent on inhibition of glucose cellular uptake

C. Azevedo¹, A. Correia-Branco¹, J. Araújo¹, J. T. Guimarães^{1,2}, E. Keating^{1,3}, F. Martel¹

¹ Department of Biochemistry (U-38 FCT), Faculty of Medicine, University of Porto, Portugal

² Department of Clinical Pathology, São João Hospital Center, Porto, Portugal

³ Center for Biotechnology and Fine Chemistry, School of Biotechnology, Portuguese Catholic University

Cancer cells present an altered metabolism, with an increased rate of glucose uptake and lactate production instead of oxidative metabolism (the Warburg effect) [1]. Dietary polyphenols are known to possess cancer preventive and anticancer effects [2]. Recently, our group verified that the polyphenols quercetin and epigallocatechin-3-gallate inhibit glucose uptake by the MCF-7 breast cancer cell line [3]. So, we decided to investigate if other polyphenols could also interfere with glucose uptake by these cells.

Uptake of ³H-deoxy-D-glucose (³H-DG) by MCF-7 cells was time-dependent and saturable ($K_m=6.5\pm 0.5$ mM and $V_{max}=63.6\pm 2.3$ nmol/mg prot). Acutely (26 min), myricetin (10-100 μ M), chrysin (100 μ M), genistein (10-100 μ M), resveratrol (10-100 μ M), kaempferol (10-100 μ M) and xanthohumol (10-100 μ M) inhibited ³H-DG uptake. By contrast, (+) catechin and (-) epicatechin slightly (by 10-15%) increased it. Kaempferol was found to be the most potent inhibitor of ³H-DG uptake by MCF-7 cells, with an IC_{50} of 4.0 (1.6-9.8) μ M. Kaempferol (100 μ M) behaved as a mixed-type inhibitor (it simultaneously increased the K_m (to 15.6 ± 2.4 mM) and the V_{max} (to 106.9 ± 10.6 nmol/mg prot)). Chronically (24h), kaempferol was also able to inhibit ³H-DG uptake (IC_{50} of 13.6 (2.8-66.9) μ M). This effect was associated with a 40% decrease in GLUT1 mRNA levels. Exposure of cells to kaempferol induced an increase in extracellular lactate levels over time (to $731\pm 32\%$ of control after a 24-h exposure), suggesting that this flavanol inhibits MCT1-mediated lactate cellular uptake. Additionally, kaempferol (100 μ M) revealed antiproliferative (sulforhodamine B (SRB) and ³H-thymidine incorporation assays) and cytotoxic (extracellular lactate dehydrogenase activity determination) properties. These effects of kaempferol, obtained at 5.56 mM extracellular glucose, were mimicked by low extracellular (1 mM) glucose conditions and were reversed by high extracellular (20 mM) glucose conditions.

In conclusion, kaempferol potently inhibits glucose uptake by MCF7 cells, apparently by affecting GLUT1-mediated glucose uptake. This effect may contribute to the antiproliferative and cytotoxic effect of this dietary compound in these cells.

References:

- [1] Cairns, R. *et al.* (2011) *Regulation of cancer cell metabolism*, Nature Reviews Cancer 11, pp. 85-95.
- [2] Crozier, A. *et al.* (2009) *Dietary phenolics: chemistry, bioavailability and effects on health*, The Royal Society of Chemistry 26, pp. 1001-1043.
- [3] Moreira, L. *et al.* (2013) *Quercetin and Epigallocatechin gallate seriously impair glucose uptake and metabolism by MCF7 cells and compromise both cell survival and proliferation*, Experimental Cell Research 319, pp. 1784-1795.

Decellularizing colorectal tumours: the role of macrophages and extracellular matrix

ML Pinto^{1,2}, E Rios^{3,7}, A Silva¹, AT Pinto^{1,4}, AP Cardoso^{1,4}, D Nascimento¹, P Pinto do Ó^{1,4}, F Carneiro^{3,5,6}, MB Barbosa^{1,2}, MJ Oliveira^{1,3,6}

¹INEB- Instituto de Engenharia Biomédica, UPorto; ²ICBAS- Instituto Ciências Biomédicas Abel Salazar; ³Departamento de Patologia e Oncologia, Faculdade Medicina, UPorto; ⁴FEUP- Faculdade Engenharia, UPorto; ⁵IPATIMUP- Instituto de Patologia e Imunologia Molecular da UPorto; ⁶Serviço de Anatomia Patológica, Centro Hospitalar de São João

Tumours are highly complex microecosystems composed of cancer cells, extracellular matrix (ECM) components and other cell types. The molecular crosstalk established between cancer cells and the surrounding environment is crucial for tumour progression [1]. Macrophages have been described as key elements in this process, preventing the establishment and spreading of cancer cells – M1 macrophages – or supporting tumour growth and progression – M2 macrophages [2]. Knowing that macrophages are highly plastic cells, it is possible that tumours explore this characteristic in their benefit. It is therefore important to unravel how the colorectal tumour microenvironment, namely ECM components, affects macrophage differentiation. In this work, we are particularly interested in creating a 3D-organotypic model that will allow us to elucidate how the ECM contributes to macrophage differentiation and polarization.

We started by optimizing the decellularization protocol and accessed its efficiency as well as tissue morphology and architecture by light microscopy and Scanning electron microscopy (SEM). The effects of tissue decellularization on DNA and Glycosaminoglycans (GAGs) contents were evaluated. These matrices were then repopulated with freshly isolated monocytes and allowed to differentiate for 7 or 14 days.

DNA quantification and DAPI staining confirmed the efficiency of the decellularization method. SEM analysis allowed the visualization of the ECM fiber meshwork while staining with Hematoxylin-Eosin and Masson's Trichrome revealed that decellularized fragments retain the histological features of the tissues. Decellularization reduced significantly the GAGS content in normal and tumours but other ECM components, such as laminin and fibronectin, are retained. The obtained results clearly evidenced that decellularized matrices are biocompatible and that monocytes were able to colonize them, differentiating into macrophages within the fiber network. A preliminary analysis of their differentiation profile revealed that, while macrophages repopulating normal tumour matrices display an intense CD163 staining, a M2 surface marker, the ones in tumour matrices are mostly negative.

With this strategy, we were able to develop an innovative model that allows studying of the intricate interplay established at the tumour microecosystem. In the future, we expect to help elucidating the role of tumour ECM components on macrophage differentiation and polarization, contributing to the design of novel therapeutic strategies targeting macrophages.

References:

- [1] Mareel M, Oliveira MJ, Madani I. (2009), Cancer invasion and metastasis: interacting ecosystems. *Virchows Arch.* 454(6):599-622.
- [2] Condeelis J and Pollard JW. (1986). Macrophages: Obligate Partners for Tumor Cell Migration, Invasion, and Metastasis. *Cell*, Volume 124, Issue 2, 263-266.



A2

CHEMISTRY I

II
PARALLEL
ORAL
SESSIONS

Preparation of gold nanostars and functionalization with the pentapeptide CALNN

D. Pereira¹, P. Quaresma¹, and E. Pereira

¹ Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Portugal.

Nanoparticles can be made of different metals or mixtures of metals and may present different shapes and sizes, which translate into different optical properties, depending on the syntheses and the final application.

These materials may lodge in its surface a great number and diversity of biomolecules owing it to its high superficial area, capacity of change coating agent and interactions between coating agent and biomolecule yielding bionanoconjugates.

In this work, we have synthesized gold nanostars and studied the exchange of capping agent from the original PVP to the pentapeptide CALNN. These gold nanostars will be further used to prepare bionanoconjugates with tyrosinase, and to evaluate the enzymatic performance of the nanobioconjugates compared to the free enzyme.

For the syntheses of nanostars, initially seeds of gold of ≈ 15 nm were prepared by the Turkevitch method [1]. Then, the seeds the citrate capping agent was exchanged by polyvinylpyrrolidone (PVP). The amount of capping agent's molecules for nm^2 of particle area was calculated according to the method of Liz-Marzán [2], resulting in approximately 60 molecules. The polymer's calculated quantity was dissolved in 5 ml of ultra-pure water and added dropwise to a previously prepared solution under vigorous agitation for 2 hours, moderately stirring continuously for more 12 hours.

For the formation of nanostars, the seeds were washed and resuspended in ethanol. Initially 3 g of PVP were dissolved in 30 mL of dimethylformamide with agitation at which 164 μL of HAuCl_4 (50mM) were added. After waiting for 2 minutes the seeds were added to a volume of 1200 μL of ethanol with stirring for 2 hours. The resulting nanostars were washed and resuspended in ultra-pure water. The synthesized nanostars had an average diameter of 86 ± 14 nm according to Transmission Electron Microscopy (TEM) image.

For the coating agents exchange, from a polymer to a peptide, the nanostar's approximate concentration was calculated and the peptide's (CALNN) volume necessary for the nanoparticles functionalization determined, adding a 5 fold excess. The solution was kept at 4 $^\circ\text{C}$ for 7 days. To check the exchange of capping agent, the stability of nanostars between pH 3 and 7 was assessed by UV/vis. The results show that the CALNN nanostars aggregate between pH 3 and 5, whereas the original PVP-nanostars do not, thus confirming the successful exchange of the capping agent. Further characterization of the CALNN-nanostars will be done by X-Ray Photoelectron Spectroscopy (XPS) and Raman spectroscopy.

References:

[1] Turkevich, J., P.C. Stevenson and J. Hillier (1951), *A study of the nucleation and growth processes in the syntheses of colloidal gold. Discussions of the Faraday Society*, (11) pp.55-75

[2] Barbosa, S., et al. (2010), *Tuning size and sensing properties in colloidal gold nanostars*. *Langmuir*, 26 (18), 14943-50

Functionalized clays for catalytic esterification reactions

J. Novais, S. M. Silva, A. F. Peixoto and A. C. Freire

¹ REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Portugal.

Currently, there is a strong demand to improve chemical reactions efficiency and decrease or even eliminate byproducts. Catalysts are widely used in a great variety of chemical processes and are very important to achieve sustainability and profitability in the chemical industry.[1] Homogenous acid catalysts are important in several organic reactions such as fatty acid esterifications, but have some limitations, such as the difficulty to be separated from the reaction media and their reusability. The alternative is the use acid heterogeneous catalysts that have the advantage of being reusable and easier to recover. Clays are good alternatives within the heterogeneous catalysts because they are cheap, versatile and easily available from natural sources and can be straightforwardly functionalized to achieve maximum catalytic activity.[2-3] The objective of this work was to use acid-functionalized clays in the esterification of stearic acid. For this purpose three commercial clays were used as starting materials, Halloysite nanotubes (HNTs), Cloisite Na⁺ (clois-Na) and Montmorillonite (K10). The clays were functionalized with different organosilanes with the suitable reactive groups to allow the introduction of sulphonic groups into the clays surface. All the resulting materials were characterized by FTIR-ATR, XRD and XPS and the stearic acid esterification reactions were monitored by GC to determine the substrate conversion.

According to the catalytic data the best catalyst was the HNTs functionalized with phenyl-trietoxisilane and then activated with ClSO₃H with a conversion of 99%, after 1h of esterification reaction. The catalyst was reused up to 4 cycles without losing the catalytic activity. The results showed that clays acted as good catalysts for stearic acid esterification, after functionalization with sulphonic groups, a consequence of their acidity, surface area and porosity.

Acknowledgements: This work was funded by Fundação para a Ciência e a Tecnologia (FCT) and FEDER through grant no. PEst-C/EQB/LA0006/2011 and through Operation NORTE-07-0124-FEDER-000067 – NANOCHEMISTRY funded by FEDER and CCDRN. AFP thanks FCT for her grant (SFRH/BPD/72126/2010).

References:

- [1] Prakash, B. J., Bhat, Y. and Reddy, C. R. (2011), *Clays as Sustainable Catalysts for Organic Transformations*, in Luque, R., "Green Chemistry", Nova publishers, Spain, 63-114.
- [2] Dasgupta, S. and Torok, B. (2008), *Application of clay catalysts in organic synthesis: A review*, Organic preparations and procedures International, vol. 40, 1-65.
- [3] Nagendrappa, G. (2002), *Organic Synthesis using clay catalysts: Clays for "Green Chemistry"*, Resonance, 64-67.

Degradation of UV-filters 4-methyl-benzylidene camphor, benzyl salicylate and phenyl salicylate in chlorinated waters.

M.M. de Oliveira e Sá, M.S. Miranda, J.C.G. Esteves da Silva

CIQ(UP), Department of Chemistry and Biochemistry, Faculty of Science,
University of Porto, Portugal.

UV-filters belong to the personal care products category and have been found in several water supplies, waste waters and natural aquatic environmental around the world [1,2]. PPCPs have been also found in swimming pool water due their use by swimmers [1,2]. Moreover, chlorine used in the water disinfection process reacts with these compounds generating chlorinated byproducts. Chlorination reactions of some UV-filters like 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid, 2-ethylhexyl-4-methoxycinnamate and 4-tert-butyl-4'-methoxydibenzoylmethane are already known [1,2]. Recently, some UV-filters, such as 4-methyl-benzylidene camphor (4-MBC), benzyl salicylate (BzS) and phenyl salicylate (PS), were detected in human placental tissues [3]. These data raised concerns about human health risks of UV-filters and their disinfection by-products.

In a ongoing project [1,2], the degradation of UV-filters 4-MBC, BzS and PS in chlorinated waters is being studied. Besides the stability of UV-filters in chlorinated water some of the major products and their toxicology are being characterized.

UV-filters degradation experiments were typically performed using 1 mg/L of each UV-filter, 10 mg/L of free chlorine at pH=7. HPLC was used to monitor UV-filter degradation and byproducts formation. Usually UV-filters degradation begins immediately after contact with free chlorine solution as well as stable by-products formation. This communication will present and discuss the kinetics of UV-filters degradation in chlorine disinfected water and some of the major by-products identified.

References:

- [1] Santos, A.J.M., Miranda, M.S. and Esteves da Silva, J.C.G. (2012) *The degradation products of UV filters in aqueous and chlorinated aqueous solutions*, Water Research, 46, 3167-3176
- [2] Santos, A.J.M., Crista, D.M.A., Miranda, M.S., Almeida, I.F., Sousa e Silva, J.P., Costa, P.C., Amaral, M.H., Lobão, P.A.L., Sousa Lobo, J.M. and Esteves da Silva, J.C.G. (2013) *Degradation of UV filters 2-ethylhexyl-4-methoxycinnamate and 4-tert-butyl-4'-methoxydibenzoylmethane in chlorinated water*, Environmental Chemistry, 10, 127-134
- [3] Jiménez-Díaz, I., Molina-Molina, J.M., Zafra-Gómez, A., *et al.* (2013), *Simultaneous determination of the UV-filters benzyl salicylate, phenyl salicylate, octyl salicylate, homosalate, 3- (4-methylbenzylidene) camphor and 3-benzylidene camphor in human placental tissue by LC – MS/MS. Assessment of their in vitro endocrine activity*, Journal of Chromatography B, 936, 80-87

Molecular Design of Potential Chk1-targeting Anti-Cancer Drugs

P. Araújo, L. Pinto da Silva and J.C.G. Esteves da Silva

Centro de Investigação em Química (CIQ-UP), Department of Chemistry and Biochemistry, Faculty of Sciences of University of Porto, Portugal.

Checkpoint kinase 1 (Chk1) is an enzyme of major importance in G2/M checkpoint. Checkpoints have the ability to halt the progression of the cell cycle if the conditions are not perfect to its occurrence. When there is DNA damage, G1 checkpoint elongates this phase giving the needed time to DNA repair mechanisms to act or, if the damage is too severe, trigger the apoptotic process. Protein p53 plays an essential role in G1 checkpoint. Due to the essential role of this protein in cell survival, mutations in it can be found in a large amount of human tumours. In p53-deficient tumour cells G1 checkpoint is inactivated, increasing the G2/M checkpoint relevance in the protection of DNA integrity. The inactivation or downregulation of Chk1 in p53-deficient tumour cells originates catastrophic mitotic events that lead to apoptosis, due to the absence of G1 and G2/M checkpoints. As a result, Chk1 inhibition emerged as a potential enhancement of classical cancer treatment methods as radio or chemotherapy.

Huang et al. (2013) [1] held a structure based design and optimization of Chk1 inhibitors after obtaining a hit compound using Automated Ligand Identification (ALIS) reaching significant biochemical improvements. These authors obtained two promising compounds, 39 (C39) and 40 (C40).

We have studied the Chk1 inhibition by the two compounds aforementioned in a computational approach [2]. This method allowed us to infer what residues played the most relevant role in this inhibitor-enzyme interaction. Using this knowledge we designed several Chk1 inhibitors with the objective to obtain a molecule with improved inhibition potential, starting from C39 (Figure 1). This communication will present and discuss the most relevant results.

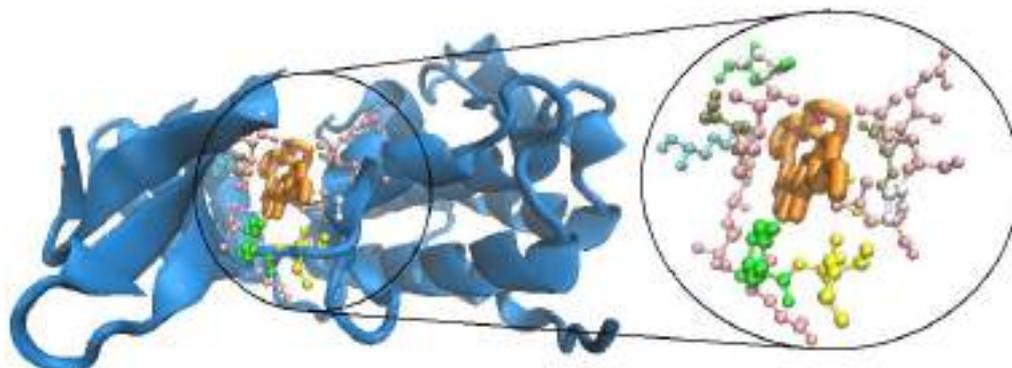


Figure 1: Representation of Compound 39 in the active site of Checkpoint kinase 1

References:

- [1] Huang, X. et al. (2013), Structure-based design and optimization of 2-aminothiazole-4-carboxamide as a new class of CHK1 inhibitors, *Bioorg. Med. Chem. Lett.* 23, 2590-2594.
- [2] Araújo, P., Pinto da Silva, L., Esteves da Silva, J.C.G. (2014), Comparative theoretical study of the binding of potential cancer-treatment drugs to Checkpoint kinase 1, *Chem. Phys. Lett.* 591, 273-276

Computational Study of Electronic and Structural Properties of Chemical Reactions Inside Carbon Nanotubes

C. Figueiredo¹ and A. L. Magalhães¹

¹ REQUIMTE/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Chemical species, under spatial and chemical confinement, may show dramatic changes in their physical and chemical properties when compared to gas phase conditions.

This work aims to characterize the structural features and energetics of simple chemical reactions when confined inside different single walled carbon nanotubes (SWCNTs). We hope it can contribute to the planning of new and cleaner chemical processes with applications in scientific research, drug design and industrial processes.

Using the known and relatively simple S_N2 Menshutkin reaction ($\text{H}_3\text{N} + \text{H}_3\text{CCl} \rightarrow \text{H}_3\text{NCH}_3^+ + \text{Cl}^-$), where reactants are neutral and products are charged, we are able to study the effects caused by the SWCNT confinement medium over the reaction path. The electronic properties of SWCNT are known to be fully determined by their (n,m) chiral indices, which essentially define the role-up direction of a graphene sheet[1]. In this work, SWCNT with different chiral indices are planned to host the referred chemical reaction.

Quantum mechanical calculations based on Density Functional Theory (DFT) are used to characterize different stages of the chemical reaction. We have started with B3PW91 functional and 6-31++g(d,p) function basis set for the reaction system and 3-21g basis set for the SWCNT. In the future other combinations will be tested.

The preliminary results show the enhancement of exothermicity of the reaction and the lowering of the corresponding activation barrier for the (8,0) SWCNT when compared with gas phase, which is in accordance with previously published results for this particular reaction [2,3].

References:

- [1] Reich, S.; Thomsen C. and Maultzsch, J. (2004), *Carbon Nanotubes*, Wiley, Berlin.
- [2] Halls, M. D. and Schlegel, H. B. (2002), *Chemistry Inside Carbon Nanotubes: the Menshutkin SN2 Reaction*, *Journal of Physical Chemistry B*, 106, 1921-1925.
- [3] Castejon, H. and Wiberg, K. B. (1999), *Solvent Effects on Methyl Transfer Reactions. 1. The Menshutkin Reaction*, *Journal American Chemical Society*, 121, 2139-214.

QM/MM studies on the catalytic mechanism of human renin

A.R. Calixto, N.F. Brás, P.A. Fernandes and M.J. Ramos

REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

The main goal of the present work is to describe, with computational methods, the atomistic details of the catalytic mechanism of human renin to allow future studies on its inhibition. Renin belongs to Renin-Angiotensin system which represents the major regulator of arterial blood pressure. Due to its high specificity for only one substrate (angiotensinogen) and its rate-determining function, renin was identified as an ideal target for new antihypertensive drugs [1-2].

In order to describe the catalytic mechanism of human renin, the initial system (renin + angiotensinogen) was divided in two layers that were studied at different theoretical levels (Density Functional Theory (DFT) and Molecular Mechanics (MM)). The geometries were optimized with the ONIOM methodology, at the B3LYP/6-31G(d):Amber level. The energies of the stationary points were calculated using a significant increase in the number of atoms in the DFT layer and a large basis set (6-311++G(2d,2p)).

Our results suggest that the angiotensinogen hydrolysis by renin occurs by an acid/base mechanism. It begins with the formation of a stable gem-diol intermediate, followed by the scissile bond nitrogen protonation and it ends with the completely cleavage of the peptide bond. We observed that the formation of the gem-diol intermediate is rate limiting, with a barrier of 20 kcal.mol⁻¹.

The obtained results also allow us to conclude that a structural water molecule, which is common in the most aspartic proteases, seems to be indifferent to the rearrangement of the active site during the renin catalytic mechanism. With this work it was also possible to conclude that the catalysis of a mutated substrate (Leu10Phe) appears to be quite similar to the catalysis of the natural substrate. The present data were also compared with the results obtained by our group to mouse submandibular renin [3].

This work provides important clues about renin catalytic mechanism which will enable further studies on its inhibition and, consequently, on the development of new antihypertensive drugs.

References:

- [1] Benzençon O., et al. (2009), *Design and Preparation of potent, nonpeptidic, bioavailable renin inhibitors*, Journal of Medicinal Chemistry, 52: 3689-3702.
- [2] Rahuel J., et al. (2000), *Structure-based drug design: the discovery of novel nonpeptide orally active inhibitors of human renin*, Chemistry and Biology, 7: 493-504
- [3] Brás, N.F. et al. (2012), *The catalytic mechanism of mouse renin studies with QM/MM calculation*, Phys. Chem. Chem. Phys., 14, 12605-12613.



A3

ENVIRONMENT I

II
PARALLEL
ORAL
SESSIONS

Oxytetracycline degradation using an homogeneous photo-Fenton process mediated by ferricarboxylate complexes

D. Queirós¹, J. Pereira², A. Reis³, O. Nunes³, R. Boaventura², M. Borges^{4,5} and V. Vilar²

¹Department of Geosciences, Environment and Spatial Planning, Faculty of Sciences, University of Porto, Portugal.

²LSRE - Laboratory of Separation and Reaction Engineering. Faculty of Engineering, University of Porto, Portugal.

³LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Portugal.

⁴Department of Biology, Faculty of Sciences, University of Porto, Portugal; ⁵CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal.

In recent years, research has been focused on alternative methods for the elimination of micropollutants, such as antibiotics, present in different effluents, since conventional wastewater treatment methods fail to remove them [1]. Advanced Oxidation Processes (AOPs), such as photo-Fenton (PF), can be a good solution. This study aimed to assess the efficiency of the PF reaction in the degradation of the antibiotic Oxytetracycline (OTC), using two photoactive Fe (III) complexes, Fe (III)-oxalate (FeOx) and Fe (III)-citrate (FeCit). These iron complexes present a higher solubility which allows working at higher pH values and can use a higher fraction of the solar radiation spectrum than the Fe (III)-hydroxy species [2]. Experiments were done with 20 mg L⁻¹ OTC in distilled water at pH 5.0, 2.0 mg Fe (III) L⁻¹ and 1:3 (FeOx) or 1:1 (FeCit) molar ratio. The aqueous solutions were recirculated through a tubular photoreactor with a CPC (compound parabolic collector) irradiated by simulated solar radiation (Suntest XLS+). Parameters tested were: temperature (10, 25 and 35 °C) and UV irradiance (25, 37 and 44 W_{UV} m⁻²), as well as the presence of some anions (Cl⁻, HCO₃⁻, SO₄²⁻ and NO₃⁻) and humic acids (5 mg C L⁻¹) in solution. Finally, the process efficiency was tested in the treatment of an effluent from a trout farm spiked with OTC, and under real conditions of solar radiation at pilot-plant scale. HPLC-DAD, TOC and *Escherichia coli* DSM 1103 growth inhibition were used to evaluate antibiotic degradation efficiency, mineralization, and antibacterial activity, respectively. Higher OTC degradation rates for both Fe (III)-complexes were achieved at higher temperatures and at lower UV irradiances. Furthermore, the presence of anions showed no major interference in the FeOx process, unlike humic acids, which inhibited OTC degradation. As regards the trout farm effluent spiked with OTC, higher degradation kinetic rates were obtained using FeOx, although slightly higher mineralization was achieved with FeCit. Process efficiency of FeOx at pilot-scale under real conditions of solar radiation was similar to that obtained at lab-scale, with total loss of antibacterial activity and a high percentage of remaining DOC as low-molecular-weight carboxylate anions.

Acknowledgments: Project AQUAPHOTOBIO - Solar Photocatalytic Degradation of Antibiotics: chemical, ecotoxicological and biodegradability assessment - PP-IJUP-2011-180, for financing.

References:

[1] Homem, V. and Santos, L. (2011), *Degradation and removal methods of antibiotics from aqueous matrices - A review*. Journal of Environmental Management 92, 2304-2347.

[2] Rodríguez, E., Núñez, B. and Beltrán, F. (2009), *Effects of some carboxylic acids on the Fe(III)/UVA photocatalytic oxidation of muconic acid in water*. Applied Catalysis B: Environmental 89, 214-222.

Innovative Technologies for the Treatment of Winery Wastewaters: Combination of Biological and Advanced Oxidation Processes

A. Ferreira¹, P. Soares¹, F. Moreira¹, R. Boaventura¹ and V. Vilar¹

¹ LSRE - Laboratory of Separation and Reaction Engineering - Associate Laboratory LSRE/LCM, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

Portugal is one of the main producers of wine in the world, with consequent production of large volumes of winery wastewaters. The treatment of this type of effluent is complex due to the high load of organic matter, presence of recalcitrant compounds and seasonal character, fluctuating widely in volume and composition [1]. Advanced oxidation processes (AOPs), which are based on the attack of powerful reactive chemical species, such as hydroxyl radicals ($\cdot\text{OH}$), can be a reliable process for the elimination of recalcitrant organic compounds. This work aims to establish a winery wastewater treatment strategy comprising two steps: (i) an initial biological oxidation performed in an immobilized biological reactor (IBR) and (ii) further oxidation using photo-Fenton (PF) or photoelectron-Fenton (PEF) processes, executed in two distinct lab-scale photoreactors: a sunlight simulator for PF trials and a UVA lamp connected to an electrochemical cell for H_2O_2 generation for PEF assays, both equipped with tubular photoreactors and compound parabolic collectors (CPCs). PF and PEF experiments were conducted using the following operational conditions: pH 2.8, $T = 30\text{ }^\circ\text{C}$, three different initial iron concentrations and three different strategies to supply H_2O_2 .

The winery wastewater exhibited a strong lilac colour and intense odour, a high organic content (dissolved organic carbon (DOC) of 5.7 g C/L and chemical oxygen demand (COD) of 16.0 g O_2 /L); high biodegradability (biochemical oxygen demand over a 5-day period (BOD_5) of 10.7 g O_2 /L; BOD_5/COD ratio of 0.67 and 98.6% of biodegradability after 28 days according to Zahn-Wallens test); acidic pH (3.7), low nitrogen content (18 mg N/L) and considerable total polyphenols content (54 mg caffeic acid/L). A 10-day biological oxidation process promoted high DOC decay (97.2%) and moderate polyphenols removal (65.4%), achieving a final COD value of ca. 373 mg O_2 /L, which is above the discharge limit into natural waters imposed by the Portuguese legislation (decree law n^o 236/98), 150 mg O_2 /L. A further oxidation, using a PF or a PEF process, was applied and optimum conditions were determined: $[\text{Fe}^{2+}]_0 = 70\text{ mg/L}$ and H_2O_2 range of 50-100 mg/L for PF and $[\text{Fe}^{2+}]_0 = 35\text{ mg/L}$ and an applied current density of 25 mA/cm^2 for PEF. Under optimum PF and PEF conditions, COD values of 55 and 146 mg O_2 /L were reached after 120 and 240 min, respectively. The combination of biological and chemical oxidation processes proved to be an efficient strategy for the remediation of winery wastewaters.

References:

[1] Petruccioli, M., Duarte, J. and Federici, F. (2000), *High-rate aerobic treatment of winery wastewater using bioreactors with free and immobilized activated sludge*, Journal of Bioscience and Bioengineering, 90 (0), 381-386.

Acknowledgments: U.Porto /Santander Totta "Projetos Pluridisciplinares 2011" Ref. PP-IJUP2011-46

Evaluation of the toxicity of textile effluents and its removal by cork adsorption

A.M. Castro¹, I. Lopes², M.N. Vieira¹ and R. Pereira^{1,2}

¹ Department of Biology, Faculty of Science, University of Porto, Portugal.

² Department of Biology, CESAM, University of Aveiro, Portugal

Textile industry effluent, a byproduct from one of the most important economic activities of the country, are of great environmental concern due to their complex chemical content, toxicity and low biodegradability [1]. For these reasons these effluents demand a proper treatment before being discharged in the environment. Traditional methods present many limitations such as high costs, addition of more chemical compounds and limited efficiency in the removal of hazardous substances [2]. Is therefore necessary to find a more efficient and cost effective solution. In this context, this study aims to explore the efficiency of a byproduct from the cork industry (other important national economic activity), granulated cork, in the removal of organic compounds from a textile effluent, and in the reduction of their toxicity.

Due to the complexity of these effluents their risk assessment only through chemical analyses is insufficient, demanding the combination with ecotoxicological assays [1]. For this reason, the ecotoxicity of a textile effluent from an industry located in the center of the country, both before and after secondary treatment, was assessed for three trophic levels of the aquatic ecosystem, studying the influence of the samples in the bioluminescence of *Vibrio fischeri*, in the survival of *Daphnia magna*, in the feed rate of *D. magna* and in the growth of *Pseudokirchinella subcapitata* and *Lemna minor*. The results showed that raw effluent was toxic and that the treatment performed at the textile company reduced significantly its toxicity, although a certain level of sub-lethal toxicity remained.

Adsorption assays evaluated the performance of the adsorbents in the removal of color, conductivity, chemical oxygen demand (COD) and toxicity of textile effluents. Two adsorbents were used: granulated cork 1 (G1), with 0,5-3 mm diameter and granulated cork 2 (G2), with a 3-5 mm diameter. The granulated cork (G1 e G2) adsorption assays were unsatisfactory in the removal of chemical parameters, such as color, conductivity and COD, however they eliminated the toxicity of the raw effluent for *Daphnia magna*. So far no solutions have been found for the complete remediation of textile industry effluents, however in this work we found promising results to proceed testing the adsorption efficiency of cork by products in parallel with ecotoxicity testing.

References:

- [1] Tigini, V., Giansanti, P., Mangiavillano, A., Pannocchia, A., and Varese, G. C. (2011). *Evaluation of toxicity, genotoxicity and environmental risk of simulated textile and tannery wastewaters with a battery of biotests*. *Ecotoxicology and environmental safety*, 74(4), 866–73.
- [2] Santos, A. B., Cervantes, F. J., e Van Lier, J. B. (2007). *Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology*. *Bioresource technology*, 98(12), 2369–85.

Correlation between the habitats productivity and species richness (amphibians and reptiles) in Portugal, through remote sensed data

S. Alves¹, A. C. Teodoro^{1,2}, N. Sillero² and L. Duarte^{1,2}

¹ Department of Geosciences, Environment and Land Planning, Faculty of Sciences, University of Porto, Portugal.

² Geo-Space Sciences Research Centre, Faculty of Sciences, University of Porto

Several biogeographic theories propose that the species richness depends on the structure and ecosystems diversity. The habitat productivity, a surrogate for these variables, can be evaluated through satellite imagery, namely using vegetation indexes (e.g. NDVI). We analyzed the correlation between species richness (from the Portuguese Atlas of Amphibians and Reptiles) and NDVI (from Landsat, MODIS and Vegetation images). The species richness database contains more than 80000 records, collected from bibliographic sources (at 1 or 10 km of spatial resolution) and fieldwork sampling stations (recorded with GPS devices). Several study areas were chosen for Landsat images (three subsets), and all Portugal for MODIS and Vegetation images. The Landsat subareas had different climatic and habitat characteristics, located in the north, center and south of Portugal. Different species richness datasets were used depending on the image spatial resolution: data with metric resolution were used for Landsat, and with 1km resolution, for MODIS and Vegetation images. The NDVI indexes and all the images were calculated/processed in an open source software (Quantum GIS). Several plug-ins were applied in order to automatize several procedures. We did not find any correlation between the species richness of amphibians and reptiles (not even after separating both groups by species of Atlantic and Mediterranean affinity) and the NDVI calculated with Landsat, MODIS and Vegetation images. Our results may fail to find a relationship because as the species richness is not correlated with only one variable (NDVI), and thus other environmental variables must be considered.

Keywords: NDVI, Amphibians and Reptiles, MODIS, Landsat. Vegetation, Correlation analysis

The environmental impacts from the Portuguese fish consumption patterns: assessing cod, hake, sardine, horse mackerel, chub mackerel and salmon

A. Cavadas¹, B. Neto², A. Teixeira³, S. Rodrigues³

¹ Faculty of Engineering, University of Porto, Portugal.

² CEMUC, Faculty of Engineering, University of Porto, Portugal.

³ Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

The Portuguese population is one of the largest seafood consumers worldwide. The consumption patterns are responsible for multiple impacts on the environment.

In order to investigate these potential impacts, 60% of the total mass of seafood consumed in Portugal was assessed through the Life Cycle Assessment methodology. The species assessed include Atlantic cod (salted, dried and frozen), European hake (fresh and frozen), European pilchard (sardine) (fresh and frozen), Atlantic horse mackerel (fresh and frozen), Atlantic chub mackerel and Atlantic salmon (fresh and frozen). Each specie was assessed along each life cycle stage, i.e.: capture (or farming), processing, transport and wholesaling.

The impacts of the abovementioned (six in total) main consumed species were modeled using *SimaPro 7.3.3* and assessed using *CML 2 baseline 2001* methodology. Abiotic Depletion Potential (ADP), Acidification Potential (AP), Eutrophication Potential (EP), Global Warming Potential (GWP 100), Ozone Layer Depletion Potential (ODP), Human Toxicity Potential (HTP 100), Freshwater Aquatic Eco-toxicity Potential (FAETP 100), Marine Aquatic Eco-toxicity Potential (MAETP 100), Terrestrial Eco-toxicity (TETP 100) and Photochemical Oxidant Formation Potential (POFP) are the impact categories assessed.

Results show that the production and combustion of diesel together with the use of cooling agents are the operational inputs that contribute mostly to the environmental impact categories identified. Small pelagics, like sardines, chub and horse mackerel have lower impact. This is mainly due to the higher fuel efficiency of the fishing technique used (i.e. artisanal purse seine fleet) and the absence of use of cooling agents for fish refrigeration.

Regarding the consumption patterns, cod and hake account for about one third of the total seafood mass consumed in Portugal and contribute to over than 80% of the impact for all individual categories analysed (exception is verified for FAETP and TETP). Hake has the largest contribution to all environmental impact categories, ranging from approximately 40% for HTP to over than 70% for ODP. Small pelagics (sardine, horse and chub mackerel) account for 23% of the seafood consumed and have the lowest environmental impact contributions, ranging from 5% for HTP to 8% for ADP and AP. Salmon accounts only for 4% of the total seafood consumed and it is the main responsible for the impacts in FAETP (90%) and TETP (100%) while its potential impacts in other categories can be larger as 17% (as for POFP).

In conclusion it is possible to observe that small pelagics captured locally and/or fish captured by fuel efficient fleet can constitute the main driver towards a lower environmental burden associated with seafood consumption.

Antarctic Dry Valleys: geochemical soil properties and microbial communities

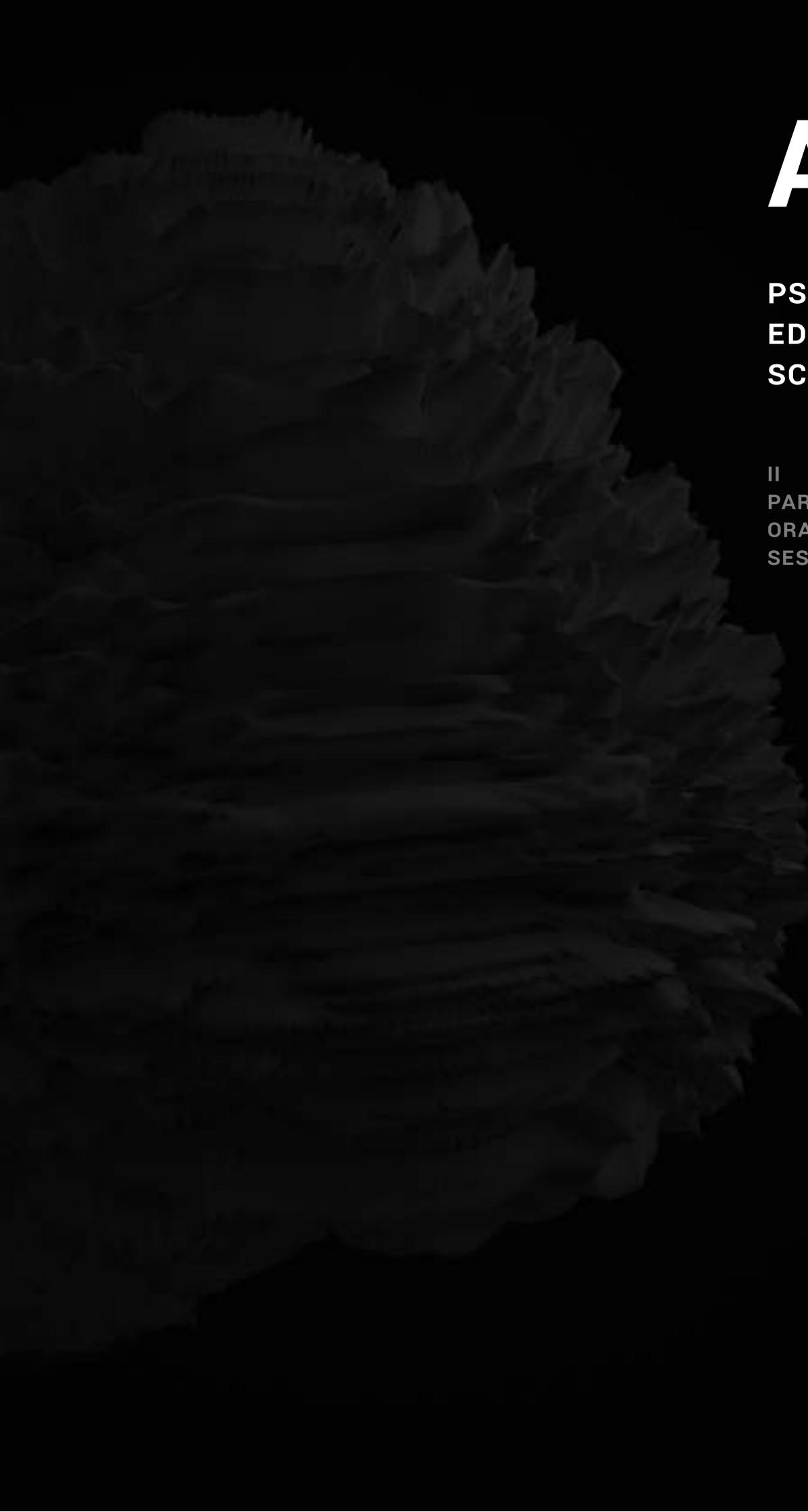
J. Séneca¹, M. Monteiro¹, H. Ribeiro¹, L. Charles², C. Magalhães¹, S. C. Cary^{2,3}

¹Centre of Marine and Environmental Research, University of Porto, Portugal, Rua dos Bragas, n° 289, 4050-123 Porto, Portugal

²Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand.

³College of Earth, Ocean and Environment, University of Delaware, Lewes, Delaware, USA.

The Dry Valleys of Eastern Antarctica are vast, ice-free regions believed to be the coldest, driest desert on Earth. The severity of such conditions shaped unique microbial communities, which are believed to drive all processes in the system; however fundamental questions on their diversity, abundance, activity, and their contribution to biogeochemical cycling and ecological processes remain unanswered. In order to answer these questions, several field expeditions to the Dry Valleys' region of southern Victoria Land, Antarctica, have been performed on behalf of an ongoing New Zealand-based international program focused in evaluating the biocomplexity of the Antarctic Dry Valleys (ICTAR—www.ictar.aq). In this presentation we describe the past expedition to Antarctica in January 2013 to the Victoria Valley, under the NITROEXTREM project (Propolar), integrated in the ICTAR international program. The 2013 Antarctic field campaign covered a sampling area of approximately 300 km² in Victoria Valley and surrounding areas. One main field camp and three sub camps were set up to cover a total of 86 random sampling locations during three weeks of field campaign, with the main goal of building a model to link biodiversity with landscape and environmental factors. Samples collected within the 86 random tiles, along with the characteristics of the soil where they were collected, were used for different scientific proposes, like invertebrate, geochemical, cosmo-dating, microbial, and vegetation analysis by integrating interdisciplinary complementary studies. In this study we present the geochemical characteristics (pH, conductivity, water availability) of these cold desert soils and the activity and abundance of microbial communities, in order to have a preliminary idea of what environmental variables may rule the distribution of the microorganisms present in these extreme environments. Results revealed that soil geochemical characteristics of Dry Valleys varied significantly among the studied area (Victoria Valey), which is reflected in the high gradient observed in terms of microbial abundance and activity. Our results suggested that the high degrees of geochemical heterogeneity that characterize these environments are the main driving forces that ruled microbial population dynamics in Antarctica Dry Valleys. We believe that this project will provide critical findings to understand the dynamics of the microbial communities in the permanently cold environments, and a better understanding on how these microbial communities respond to changes in polar ecosystems, as global climate changes.



A4

**PSYCHOLOGY &
EDUCATION
SCIENCES I**

**II
PARALLEL
ORAL
SESSIONS**

Measuring the direct and indirect effect of the content area in the psychometric quality multiple-choice items– A case study in clinical anatomy

J. Pais¹, A. Silva³, B. Guimarães¹, E. Coelho¹, A. Povo³, I. Lourinho¹, M.A. Ferreira^{1,3} and M. Severo^{1,2}

¹ Center for Medical Education, Faculty of Medicine of the University of Porto, Porto, Portugal

² Department of Clinical Epidemiology, Predictive Medicine and Public Health, University of Porto Medical School, Porto, Portugal

³ Institute of Anatomy, Faculty of Medicine of the University of Porto, Porto, Portugal

Introduction: A study had showed a high prevalence of low psychometric quality multiple-choice items in some clinical anatomy content areas [1].

The aim of this study was to understand if the association between the content area and the psychometric quality is explained by the item writing flaws of the MCQ.

Methods: 800 MCQ from 8 different exams of Clinical Anatomy were classified according to the area they referred (Abdomen, Pelvis and Perineum, Upper Limb, Lower Limb, Neck, Thorax, Head and Imagiology), and as standard or flawed. A flawed item was one that violates at least one of the 31 principles cited in a review article [2]. The classification was made by 4 judges (2 teachers and 2 students) independently. The association between the content area and the presence of flawed questions was assessed using the Pearson chi-square test.

Results: The mean difficulty index ($p=0.002$) ranged from 52% (Imagiology) to 66% (Pelvis and Perineum) and the mean biserial coefficient ($p=0.002$) ranged from 0.30 (Thorax) to 0.39 (Upper Limb).. There is also a relationship between the content area of the questions and the presence of flawed questions ($p=0,001$). After adjusting for the examination, item-writing flaws and content areas, the content area explained approximately 2.1% ($p=0.004$) and 1.8% ($p<0.001$) of the total variance for difficulty and discrimination index.

Discussion: The content area showed a relation with both difficulty and discrimination index even after adjusting for item-writing flaws, however explained small part of the items quality.

References:

- [1] Severo, M. and M.A. Tavares (2010), *Meta-evaluation in clinical anatomy: a practical application of item response theory in multiple choice examination*, in *Anat Sci Educ*, 2010. **3**(1): p. 17-24.
- [2] Haladyna, T. M., Downing, S. M., and Rodriguez, M. C. (2002), *A review of multiple-choice item-writing guidelines for classroom assessment*, *Applied Measurement in Education*, 15 (3), pp. 309-333.

LGB Identity and Fear of Intimacy: an exploratory study.

João Teixeira Duarte¹, Maria Emília Costa¹

¹ Faculty of Psychology and Social Sciences, University of Porto, Portugal.

This study grounds itself on Mohr and Fassinger's multidimensional model of the LGB identity, along with Meyer's minority stress model and Costa's integrative proposal of the development of intimacy, having aimed to explore the existence of relationships between the two dimensions underlying the concept of fear of intimacy – fear of losing the self and fear of losing the other – and the several dimensions that compose the LGB sexual identity. This study also explores the possible existence of connections between fear of intimacy and self-identification within the straight-gay spectrum, as well as connections between the first construct and the number of sexual partners in the last six months.

Thus, the Fear of Intimacy Components Questionnaire (Sobral & Costa, sub) and the Lesbian, Gay and Bisexual Identity Scale (Kendra & Mohr, 2008) were used in a sample of 163 homosexual males. The results were analysed through SPSS 22, having hinted that self-identification acquires a main role in the healthy development of the homosexual identity, contributing not only to a better adaptation in an intrapersonal level but also in the dynamics between the individual and their developmental contexts.

Relationships between fear of intimacy and the several dimensions of LGB identity surfaced, pointing towards repercussions of the models of self and other within the development of these individuals' sexual identity, namely the way they perceive themselves and other male homosexuals in their community.

The Influence of the Performance Management and Evaluation in the Behavior of Workers

T. Cardoso¹, C. Brandão¹

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

The main goal of this study is to explore how the recent process of performance management and evaluation in the Portuguese public sector (i.e.SIADAP) is associated with the adoption of impression management behaviors. Thus, we propose to answer the following research questions: (1) How does SIADAP influences the behaviors of workers?; and (2) How does the behaviors of workers influences the appraisers?

In order to achieve the proposed objective and to answer to the research questions, we adopted a qualitative, descriptive and exploratory approach. The semi-structured interview was the technique of information selected. The interviews were held at the participants' workplace. All interviews were audio taped, with the participants' authorization, and then transcribed. The information was analyzed using the content analysis, with support of NVivo10.0.

The results of this investigation suggest that the SIADAP is associated with workers impression management behaviors, specifically, the behavior of ingratiation, exemplification, intimidation and supplication. As reasons for the adoption of these behaviors were considered two factors, in particular the goal relevance of the impressions one creates and the discrepancy between the appraisee current and desired images. Also, we found that all participants believe that the appraisee's behaviors influence the appraisers (specifically at the level of the appraisal), with some appraisees considering this may be associated with the appraiser being impressionable or when belonging to the female gender . On the other hand, the appraisers modify their behaviors in two specific ways which are, appraising the subordinate in a way that reflects the existing relationship with that subordinate and resorting more frequently to the workers who adopt impression management behaviors.

This study also showed that when appraisees think about the impact of the performance management and evaluation system on their behavior, they focus not only on what happens before the evaluations but also (and primarily) on what happens after been appraised.

City of Professions, Project *Grow and Show Up*. Early intervention for the academic and professional success.

Carlos Daniel Moreira Barros¹

¹ Faculty of Psychology and Education Sciences of the University of Porto

This paper describes a single intervention in schools and pre-prepared by teachers responsible for the pre-school classes (ages between 3-5 years) and was based, in my perspective, on an adaptation of media language and tasks to be performed based on the Bronfenbrenner bioecological model of development[1-2], Piaget's Cognitive Theory[3], the third stage (Initiative Vs Guilt) of the child's psychological development according to Erik Erikson Psychosocial Theory[4] and the Gottfredson's theory of circumscription and compromise[5]. As such, we intend to discuss the nature and effectiveness of this early intervention especially for pre-school which was intended to enhance a first approach to the world of the professions, the exploitation of social stereotypes and gender, as well as reflective exploration of vocational constructs.

The results of this study indicate the relevance of including the family in the middle school intervention, as well as better preparation by teachers responsible for the pre-school classes for this problematic.

Keywords: career counseling, early intervention, preschool.

References:

- [1] Bronfenbrenner, U. (1979). *The ecology of human development: Experiments by nature and design*. Cambridge, Massachusetts: Harvard University Press.
- [2] Bronfenbrenner, U. (2005). *Making human beings human. Biological perspectives on human development*. London: Sage Publications.
- [3] Terra, Márcia Regina. *Human development in Piaget's theory*. Retrieved June 26, 2013, available at <http://www.unicamp.br/iel/site/alunos/publicacoes/>
- [4] Erikson, E. H. (1987). *Childhood and Society*. 2nd ed. Rio de Janeiro: Zahar editors
- [5] Gottfredson, L. (1996). Gottfredson's theory of circumscription and compromise. In D. Brown, L. Brooks & Assoc. (Eds.), *Career choice and development* (pp.179-232). San Fransisco, CA: Jossey-Bass.

The impact of literacy on mirror discrimination

B. Coelho¹, E. Madureira¹, F. Lima¹, S. L. Castro¹, T. Fernandes^{1,2}

¹Faculty of Psychology and Educational Sciences, Universidade do Porto, Portugal, ²Faculty of Psychology, Universidade de Lisboa, Portugal

Literacy is a highly demanding visual task that requires developing fine-grained knowledge of letters and orthographic patterns. Literacy acquisition induces the reorganization of evolutionary older systems, affecting specific properties of visual object recognition [1-3]. Learning a script with mirrored letters (i.e. b-d and p-q) requires the suppression of the *mirror invariance* property of the visual system, which triggers *mirror discrimination*, which generalizes to visual categories outside the written domain [2,3]. Noteworthy, mirror images can be reflected across the external axis (EA) or across the object axis (OA). Whereas both adults and children present difficulties in discriminating mirror images across the OA, it seem that only children, especially preschoolers, present difficulties in discriminating mirror images across the EA [4].

This study was aimed at investigating thoroughly the impact of literacy acquisition on object orientation processing, disentangling the role of maturation and of learning to read. Three groups of children - *first-graders* (literate children at the end of 1stgrade), *older preschoolers* (preliterate children matched in age with 1stgraders), and *younger preschoolers* (preliterate children matched in kindergarten-level with older preschoolers) performed a visual search task on pictures of familiar objects presented among distractors differing only by orientation-contrasts. First-graders were better able to discriminate any orientation contrast than preschoolers. More importantly, errors were non-uniformly distributed across orientation-contrast in the three groups, with mirror errors occurring more often than plane-rotation errors. Whereas the three groups did not differ on number of mirror errors across the OA, first-graders presented less mirror errors across the EA than the two preschool groups. The regressions analyses showed that literacy-related abilities, but neither IQ nor age, strongly predicted the decrease of mirror errors of the EA-types. We thus demonstrated that literacy specifically impacts on discriminating mirror reflections across the EA, which generalizes to familiar nonlinguistic objects. While mirror discrimination continues to be harder, even for older and literate participants, the training on mirror discrimination by literacy impacts on visual object recognition. This research can thus give clues for understanding associated deficits and developing interventions programs in reading development disorders.

References:

- [1]Dehaene, S. & Cohen, L. (2011). The unique role of visual word form area in reading. *Trends in Cognitive Sciences*, 15(6), 254-262. doi: 10.1016/j.tics.2011.04.003
- [2]Kolinsky, R., Verhaeghe, A., Fernandes, T., Mengarda, E. J., Grimm-Cabral, L., & Morais, J. (2011). Enantiomorphy through the looking-glass: Literacy effects on mirror-image discrimination. *Journal of Experimental Psychology. General*, 140(2), 210–238. doi: 10.1037/A0022168
- [3]Fernandes, T., & Kolinsky, R. (2013). From hand to eye: the role of literacy, familiarity, graspability, and vision-for-action on enantiomorphy. *Acta Psychologica*, 142(1), 51-61. doi: 10.1016/j.actpsy.2012.11.008
- [4]Gregory, E., Landau, B., & McCloskey, M. (2011). Representation of object orientation in children: Evidence from mirror-image confusions. *Visual Cognition*, 19(8), 1035-1062. doi: 10.1080/13506285.2011.610764

Peer Instruction in the appropriation of concepts in a structuring CU for the Teaching of Engineering

C. Cabreira¹, A. Santos¹, A. Mouraz², D. Urbano³

¹ Student of Educational Sciences, Faculty of Psychology and Educational Sciences, University of Porto, Portugal

³ Researcher of CIEE, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

⁴ Researcher, Faculty of Engineering, University of Porto, Portugal

The Bologna Process is the justification for the restructuring of Higher education which brings qualitative changes in pedagogical practise. Peer Instruction has been indicated as a strategy which is able to promote a more significant and longer lasting learning for the students involved. In this case study, which was centred around a curricular unit (CU) which is the structure for the respective courses, the effects of the use of this pedagogical resource when associated with the synchronous assessment of the students' knowledge.

This work follows a Case study and the object of analysis was a Curricular Unit of a theoretical nature from the Faculdade de Engenharia da Universidade do Porto (Porto University Engineering Faculty) where recourse was had to peer instruction. Taking into account that this is a study focused on a Curricular unit and in order to appreciate the diversity of the elements characterising the matter in question, recourse was had to two data collection methods allowing qualitative (interviews) and quantitative (responses obtained from the students in moments of synchronous response to multiple choice questions) data to be obtained. The data was treat in accordance with its nature with recourse to two forms of treatment: the quantitative data was subject to statistical analysis while the qualitative data – the content of the interviews – were analysed using the NVIVO 10 program.

From the data submitted it can be seen that the students significantly improved their responses after a peer discussion. This result is in line with the conclusions of earlier studies reported in the review of the literature (Cummings, & Roberts, 2008; Crouch & Mazur, 2001) [1-2]. When the immediate results of peer instruction are compared with the results of the final examinations of the same students it is found that they obtained worse results than those resulting from the peer discussions although they were consistently better than those resulting from the initial assessment. The time factor can be put forward as the logical explanation for this difference.

In conclusion it can be affirmed that the study of this curricular unit validated the use of peer instruction but the methodology needs to be more systematic to become part of the strategies regularly used by students.

References:

[1] Cummings, K. & Roberts, S. (2008). "A study of Peer Instruction Methods with School Physics Students". C. Henderson, M. Sabella & L. Hsu (ed). *Physics Education Research Conference* (pp. 103-106). sl. American Institute of Physics.

[2] Crouch, C. & Mazur, E. (2001). "Peer Instruction: Ten years of experience and results". *American Journal of Physics*, 69 (9), 970-977.



A5

**BIOLOGICAL
SCIENCES II**

**II
PARALLEL
ORAL
SESSIONS**

Characterization of cholinesterase activity in three biofouling invertebrate species of NE Atlantic

M. Freitas¹, I. Cunha², and J. Almeida²

¹ Faculty of Sciences, University of Porto, Portugal.

² CIIMAR/CIMAR- Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal.

Carboxylesterase/cholinesterases (CoEs/ChEs) are a family of enzymes specialized in the hydrolysis of carboxyl esters. ChEs are CoEs specifically adapted to the hydrolysis of acetylthiocholine. The majority of animals have two ChEs, although nematodes have more than 4 and certain groups of Diptera and some teleost fish, have only one [1]. In vertebrates, the two ChEs are acetylcholinesterase (AChE; EC 3.1.1.7) and pseudocholinesterase (PChE; Ec 3.1.1.8) [2]. *Mytilus galloprovincialis*, *Pollicipes pollicipes* and *Balanus perforatus* are some of the most common invertebrate species of biofouling communities in NE Atlantic. In the last years, it has been suggested that AChE may be involved in the settlement of marine organisms, and AChE inhibitors were found to inhibit larval settlement [3]. With the intent of developing a rapid *in vitro* antifouling screening assay for ChEs inhibition, it became necessary to characterize the different forms of ChEs present. Thus, this work aims to characterize the different forms of ChEs present in selected tissues of 3 species according to their preference for specific alkylthiocholine substrates: acetylthiocholine (AcSCh), butyrylthiocholine (BuSCh), propionylthiocholine (PrSCh) and acetyl- β -methylthiocholine (A β SCh). ChEs activity was assayed according to the Ellman's method.

B. perforatus presented the highest ChE activity for all substrates tested and *P. pollicipes* presented the lowest. In *M. galloprovincialis* ChEs activity has the highest activity when AcSCh was used as substrate, while in *P. pollicipes* this was observed with AbSCh and in *B. perforatus* with both AcSCh and AbSCh that presented a similar activity. This may indicate the presence of other esterases activity in mussel, since AbSCh is specific for AChE, and very few on none in *B. perforatus*. PrSCh presented an activity pattern similar to A β SCh and AcSCh although values were lower in all species. The main difference was on *P. pollicipes* that did not reach a plateau with this substrate, even at the highest concentrations. The substrate whose the pattern differed the most was BuSCh. Values were the lowest for all species and, higher levels of activity were observed at lower concentration in *M. galloprovincialis* and *P. pollicipes*, while in *B. perforatus* the values increased along the concentration range, not reaching a plateau within the concentration range tested. In *M. galloprovincialis*, all the substrates except BuSCh presented inhibition of activity by substrate at higher concentrations.

References:

- [1] Johnson G, More SW (2012). *The carboxylesterase/cholinesterase gene family in invertebrate deuterostomes*. Comp Biochem Physiol 7D: 83-93.
- [2] Karczmar AG (2010). *Cholinesterases (ChEs) and the cholinergic system in ontogenesis and phylogenesis, and non-classical roles of cholinesterases – A review*. Chem Biol Interact 187: 34-43
- [3] Turk T, Frangez R, Sepcic K (2007). *Mechanisms of toxicity of 3-alkylpyridinium polymers from marine sponge Reniera sarai*. Mar Drugs 5: 157-167

Effect of dietary L-tryptophan supplementation on growth performance and feed utilization in *Dicentrarchus labrax* reared at two densities

S. Moutinho¹, H. Peres², C. Castro², F. Coutinho², E. Matos³, T. Aires³, A. Oliva-Teles^{1,2} and A. Pérez-Jiménez²

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal.

³ Sorgal, Sociedade de Óleos e Rações S.A., Ovar, Portugal.

Tryptophan is an essential amino acid for protein accretion and fish growth, besides being responsible for serotonin and melatonin synthesis, neurotransmitters that have relevant biological functions, namely in stress mitigation. The present study aimed to evaluate the effect of dietary tryptophan supplementation in the mitigation of negative effects induced by crowding stress in European sea bass (*Dicentrarchus labrax*). For that purpose 12 groups of fish (8.0 g initial weight) were randomly distributed into two rearing systems: high density (HD - stressful condition; 60 fish per tank) and low density (LD - non-stressful condition; 30 fish per tank), in 60 L water content tanks. Triplicate groups of fish within each system were hand-fed, to satiation, during 6 weeks, with the following experimental diets (45% protein, 18% lipids): control (non-supplemented) or L-tryptophan supplemented at 2.5, 5 and 10 fold the L-tryptophan level in the control diet. Dietary tryptophan levels were 0.42, 1.06, 2.12 and 4.25% DM for the control, 2.5T, 5T and 10T diets, respectively. Results indicate that rearing density did not influence growth and feed efficiency (Table 1). Performance of fish fed diet 10T was considerably worse than in all other groups, suggesting a toxic effect of high dietary tryptophan levels.

Acknowledgments: This study was supported by the project PP-IJUP2012-SOJA DE PORTUGAL-12.

Table 1. Growth performance and feed efficiency of sea bass reared at two densities (HD and LD) and fed on the experimental diets.

		FBW (g)	Weight gain (g kg ABW ^{†-1} day ⁻¹)	DGI (%) ¹	Feed intake (g kg ABW ^{†-1} day ⁻¹)	FER ²	Survival (%)
HD	Control	24.4 ±0.6	23.6 ±0.4	2.10 ±0.06	27.5 ±0.3	0.86 ±0.02	100.0 ±0.0
	2.5T	23.6 ±1.0	22.9 ±0.7	2.02 ±0.09	28.2 ±0.5	0.81 ±0.02	100.0 ±0.0
	5T	21.3 ±0.5	21.1 ±0.4	1.79 ±0.05	25.6 ±0.2	0.82 ±0.01	98.9 ±1.1
	10T	14.8 ±0.8	13.7 ±1.2	1.05 ±0.11	24.3 ±0.9	0.57 ±0.07	99.4 ±0.6
LD	Control	25.6 ±1.2	24.3 ±0.8	2.20 ±0.10	29.4 ±0.7	0.83 ±0.05	98.3 ±1.7
	2.5T	24.5 ±1.4	23.5 ±1.0	2.10 ±0.13	29.3 ±0.1	0.80 ±0.04	98.3 ±1.7
	5T	22.9 ±0.7	22.4 ±0.6	1.95 ±0.07	28.2 ±0.4	0.79 ±0.02	100.0 ±0.0
	10T	14.1 ±0.2	12.8 ±0.3	0.96 ±0.03	25.7 ±0.3	0.50 ±0.02	96.7 ±3.3
Diet (C-2.5T-5T-10T)		c-bc-b-a	c-bc-b-a	c-bc-b-a	c-c-b-a	b-b-b-a	n.s.
Density		n.s.	n.s.	n.s.	*	n.s.	n.s.
Diet × Density		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Values are means ± S.E. (n=3). Two-way ANOVA was performed. Letters indicate influence (P<0.001) of the experimental diets. Asterisks indicate influence (P<0.01) of density. [†]Average body weight: (initial body weight + final body weight)/2. DGI: ((FBW^{1/3} - IBW^{1/3})/ time in days) × 100. FER: (wet weight gain/dry feed intake).

Carob seed germ meal as a partial fish meal substitute in diets for meagre (*Argyrosomus regius*): growth and health status

C. Barroso¹, B. Costas², H. Peres², E. Matos³, A. Couto^{1,2}, A. Oliva-Teles^{1,2} and P. Enes²

¹ Faculty of Science, University of Porto, Portugal.

² Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal.

³ Sorgal, Sociedade de Óleos e Rações S.A., Ovar, Portugal.

Considering the importance of aquaculture in the world food sector, it is widely recognized that this industry should become sustainable from every angle. For instance, measures should be directed towards an eco-friendly production for a sustainable development. Therefore, replacement of fish meal (FM) by alternative protein sources in aquafeeds and species diversification are necessary if further growth in aquaculture production is to be pursued [1,2]. Ingredients based on vegetable crops are the most promising alternative feed ingredients, particularly if they are produced locally, contributing thus to the sustainability and cost-effectiveness of fish-farming [2]. The present proposal aims to contribute to this endeavour by focusing in two important issues in the Mediterranean aquaculture panorama. Insights in the culture potential of meagre (*Argyrosomus regius*) will be provided, as well as evaluation of nutritional potential of carob seed germ meal (CG). This is an emergent protein source obtained from carob seed (*Ceratonia siliqua*), a locally produced plant, and that presents high protein content (45-50%) and a balanced amino acid profile.

Four experimental diets were formulated to be isonitrogenous (53% protein) and isolipidic (18% lipids). A FM-based diet was used as control and in the other diets incorporated CG at 7.5% (diet CG7.5), 15% (diet CG15) and 22.5% (diet CG22.5). Fish were fed to apparent visual satiety during 8 weeks. The replacement of FM by CG had no effect on the growth performance of meagre juveniles. Fish weight increased from an initial value of 35g to 149.9g, 150.8g, 137.6g and 137.3g at the end of the trial, for fish fed the control, CG7.5, CG15 and CG22.5 diets, respectively. Feed intake was also similar among experimental groups. In contrast, feed efficiency (FE) and protein efficiency ratio (PER) were significantly affected by diet composition. FE and PER were higher with diet CG7.5 than in the control, while fish fed the CG22.5 diet showed the lowest values. No differences were found for haematocrit, haemoglobin and total red and white cell counts among fish fed the experimental diets after 8 weeks of feeding. Results from this study suggest that it is possible to partially replace FM by GC up to 7.5%, without compromising meagre growth and health status. However, high dietary inclusion of CG negatively affect fed utilization. Digestive enzymatic activity, gut and liver histology, plasma biochemical and immune parameters are currently being analysed and may further contribute to uncover the potential of CG as novel ingredient in practical diets for meagre while maintaining good welfare.

[1] Monfort, M.C., 2010. *Present market situation and prospects of meagre (Argyrosomus regius), as an emerging species in Mediterranean aquaculture. Studies and Reviews. General Fisheries Commission for the Mediterranean*. No. 89. Rome, FAO. 2010, pp. 1-4.

[2] Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., et al. (2007), *Expanding the utilization of sustainable plant products in aquafeeds: a review*. *Aquaculture Research*, 38, 551-579.

Supported by IJUP/SOJA DE PORTUGAL (PP_IJUP2012_SOJA DE PORTUGAL 20).

Preliminary results about the assessment of the bycatch in the beam trawl fishery on the Portuguese North coast

J. Oliveira¹

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

The beam trawl fishery is inserted in the category of bottom trawl wherein the net, always towed by one vessel, moves over the bottom and in contact with him [1]. Bycatch is the part of a catch of a fishing unit taken incidentally in addition to the target species towards which fishing effort is directed [2]. This represents a significant problem in the European fisheries, constituting a waste of resources to society and is an environmental impact issue.

The main goal of this assessment was to contribute to the characterization of the bycatch by monitoring the fishing operations onboard.

From July 8, 2013 to November 25, 2013, 172 observations on board of a beam trawler based at Póvoa de Varzim fishing harbor were made. For all cruises information was taken about the fishing operation (fishing time, travel time, number of tows) and about catch, bycatch and discards composition.

As first results, we obtained a target and bycatch species list, as well as some of its proportion related to total catch. Main bycatch species were *Polybius henslowii* (96,27%), *Callionymus lyra* (2,56%) and *Lepidotrigla cavillone* (1,17%). Comparing to other studies made in the North region of Portugal, *Trisopterus luscus* is reported as one of the most representative bycatch species [3] but our data did not show that, and on the other hand, in the South of the country, the brown-shrimp represents one of the major discards [4].

References:

- [1] Magalhães, L.V. (2009), *Contribuição para o Conhecimento da Biologia de Polybius henslowii*, Dissertação de Mestrado.
- [2] FAO and the FMFH Partners (2013), *Glossary of Fisheries & Aquaculture*.
- [3] Ceia, F. R., Viegas, M. C., Afonso-Dias, M. (2004), *Caracterização da pescaria de camarão-brancolegítimo (Palaemon serratus) com arte de arrasto de vara na região Norte de Portugal*, Relat. Cient. Téc. IPIMAR, Série digital (<http://ipimar-iniap.ipimar.pt>), 22, 19pp.
- [4] Gamito, R., Cabral, H. (2003), *Mortality of brown-shrimp discards from the beam trawl fishery in the Tagus estuary, Portugal*, Fisheries Research, 63, 423-427.

Biomonitoring of coastal contamination using a biomarker approach: the use of gooseneck barnacle as a sentinel species

A. S. Ramos¹, S. C. Antunes^{1,2}, B. Nunes^{2,3}

¹Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre s/n, Edifício FC4, 4169-007 Porto - Portugal

²CESAM-Centro de Estudos do Ambiente e do Mar, Campus Universitário de Santiago, Universidade de Aveiro, 3810-193 Aveiro, Portugal

³Departamento de Biologia, Campus Universitário de Santiago, Universidade de Aveiro, 3810-193 Aveiro, Portugal

Anthropogenic contamination of the aquatic environment is an emergent issue, caused by several human activities, including agriculture, industries, and domestic wastewaters, becoming an alarming situation for marine species. This makes the impact evaluation of contaminant mixture in biota an urgent need. Ecotoxicological tests, more specifically biomarker-based assays, are a valid method for that assessment, being useful tools for the evaluation of impact in biota of contaminant mixtures under realistic conditions. Biomarkers allow the identification, estimation, comparative assessment and management of the risks posed by pollutants in the environment and can be associated to sentinel species for a more realistic assessment of biological effects caused by xenobiotics.

This work aimed to validate the use of *Pollicipes pollicipes* (gooseneck barnacle) as a sentinel species for the assessment of anthropogenic contamination in aquatic ecosystems. For that purpose, two biomonitoring studies were conducted, each with one year of duration.

In the first part of the study, the barnacle collection was made once per season, in 3 different beaches (Aguda, Lavadores and Matosinhos beaches) in the North coast of Portugal. The biomonitoring program involved the evaluation of biochemical markers with distinct biological meanings, i) neurotoxicity, by measuring the activity of neurotransmitter enzyme cholinesterase (ChE), ii) oxidative stress/phase II detoxification, by the quantifying the enzymatic activity of glutathione-S-transferases (GSTs) and iii) peroxidative damage, by assessing levels of thiobarbituric acid reactive substances (TBARS), in cirrus and muscle tissue. The obtained results showed a significant variation between sampling sites, and a seasonal variation in the biomarkers.

In the second part of the study, organisms were collected every month, at Lavadores beach. Biochemical markers were similarly determined ChE activity, GSTs activity and TBARS levels in cirrus, muscle and also in hemolymph tissues. The hemolymph evaluation was introduced to validate its use as a non-invasive biomarker, since it is a non-destructive technique that allows repeated individual evaluation over time in the same organism, allowing a compilation of a historical data. The obtained results showed again a significant seasonal variation.

High-resolution diving behaviour revealed by satellite tagging of blue sharks

C. Vila Pouca^{1,2}, N. Queiroz¹

¹ Research Center in Biodiversity and Genetic Resources (CIBIO-UP), Vairão, Portugal.

² Department of Biology, Faculty of Sciences, University of Porto, Portugal.

Recent advances in satellite tagging technologies have provided increased resolution in studying the movements, patterns of activity and behaviour of individual animals in relation to different environmental features. Such accurate records of long term vertical movements of large predators at fine temporal resolutions have enabled the identification of variable behavioural patterns amongst species, such as diel temporal shifts in behaviour. In addition, by investigating diving behaviour in relation to changing oceanographic variables and to potential foraging success, we are beginning to understand why such behaviours occur at particular times and places. The characteristics of dive profiles, including dive shape, have often been useful to address such questions. For most species, dives can be classified into predefined dive types based on their two dimensional shape, and these types, or the dive characteristics within a type, may reflect activities such as foraging, travelling or resting. Concerning marine fish, two main dive types, V- and U-shaped, have been largely identified. Overall it is thought that V-shaped dives are associated with transiting/prey searching behaviour, while U-shaped profiles are related to foraging on aggregated prey. Here we analysed high resolution data (10s) on depth and temperature experienced by satellite tagged blue sharks *Prionace glauca* in the eastern Atlantic Ocean and obtained a detailed description of their vertical movements. A total of 6479 dives by seven blue sharks were recorded. Five dive classes, previously described for other species, were found to be commonly performed by blue sharks. U and V-shaped dives were the most frequently performed by blue sharks (ca. 70% of the total number of dives) with all other dive types each representing <5% of the total. We tested the spatial occurrence of U and V dives in blue sharks and found they performed more U dives in high productivity regions, identified by persistent thermal fronts, suggesting a foraging function for this class of dives (Fig. 1). By contrast, energetically demanding V shaped dives were usually observed over more oligotrophic waters, and are therefore more likely associated with exploratory behaviours. The results suggest that fine scale dive profiles can be used to assess habitat use in pelagic predators such as sharks and to identify areas at high risk from fisheries. Analysing dive profiles of different apex species might aid in the recognition of key marine habitats, such as foraging areas, that represent potential marine protected areas.

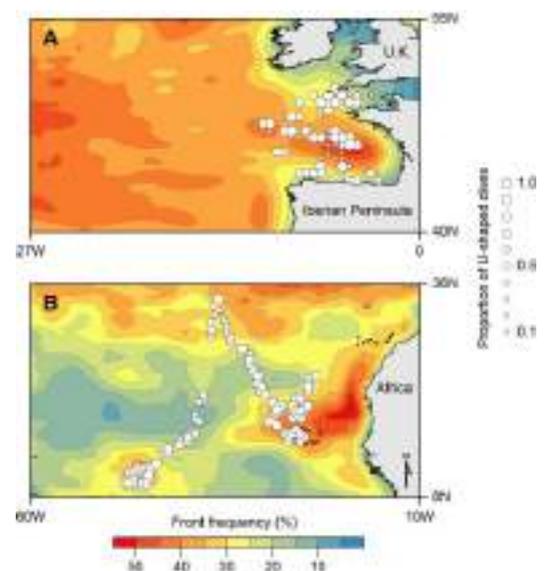


Figure 1. Proportion of U-shaped dives of six blue sharks overlaid on frequency of thermal fronts.

A6

MATH

**II
PARALLEL
ORAL
SESSIONS**

An example of a summation error and ways of dealing with it.

Dulce Pereira da Silva¹, Tiago André Moreira Gonçalves¹, J. Rocha e Silva^{1,2}

¹ Department of Mine Engineering, Faculty of Engineering, University of Porto, Portugal.

² ENEAS: Faculty of Engineering, University of Porto, Portugal

In numerical analysis, the Kahan summation algorithm (also known as compensated summation), compared to the candid approach, significantly reduces the numerical error in the total obtained, by adding a sequence of finite precision floating point numbers. This is quite easily seen in the summations performed over a spreadsheet, as it was demonstrated in a lab work done in Numerical Analysis, FEUP (EMG0012). The students were asked to produce proposals for more precise summation algorithms and to justify their choices. This is the result of two of them.

A simply sum of a sequence of n numbers has a worst-case error that grows proportional to n , and a root mean square error that grows as \sqrt{n} for random inputs (the round off errors follow a random walk). Keeping a separate running compensation (a variable to accumulate small errors) enables us to surpass the problem. With compensated summation, the worst-case error bound is independent of n , so a large number of values can be summed with an error that only depends on the floating-point precision. Similar, earlier techniques are presented, for example, the Bresenham's line algorithm - for the analogous geometry problem - that keeps track of the accumulated error in integer operations and the Delta-sigma modulation (that integrates, not just sums the error).

References

- [1] McNamee, John Michael (2004). *A comparison of methods for accurate summation*, ACM SIGSAM Bulletin Homepage archive, Volume 38 Issue 1, March 2004, pages 1-7.
- [2] Hingham, Nicholas (2000). *The accuracy of floating point summation*, <http://www.cs.berkeley.edu/~demmel/AccurateSummation.pdf>
- [3] Demmel, Ames, *et al* (2006). *Error bounds from extra-precise iterative refinement*, Journal ACM Transactions on Mathematical Software (TOMS), Volume 32, Issue 2, June 2006.
- [4] *IEEE Standard 754 for Binary Floating-Point Arithmetic*
- [5] W Kahan (1981). *Why do we need a floating-point arithmetic standard?* Unpublished note, February, 1981 - cs.nyu.edu
- [6] Goldberg, David (1991). *What every computer scientist should know about floating-point arithmetic*, Computing Surveys, Association for Computing Machinery, Inc.
- [7] Bailey, *et al* (2008). *Techniques for the automatic debugging of scientific floating-point programs*, perso.univ-perp.fr

Testing universality in complex systems

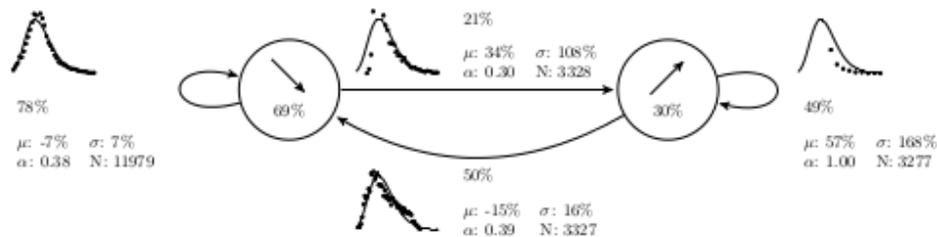
Renato Fernandes¹, Ricardo Cruz¹, Alberto Pinto¹

¹ Department of Mathematics, Faculty of Science, University of Porto, Portugal.

BHP is a nonparametric distribution that has been found to be a good model for explaining a wide range of phenomena, including the fluctuations of order parameters in theoretical examples such as the Sneppen model [1], auto-ignition fire models [2], self-organized models and percolation models [1], width power in steady state systems [1], the Wolf's sunspot numbers [3], and also in river heights and flow, from the Danube river [4] to the Mississippi [5]. Rivers, being intermittent dynamical systems, are interesting case studies of the universality of BHP.

The universal nonparametric BHP pdf is the pdf of the fluctuations of the total magnetization, in the strong coupling (low temperature) regime for a two-dimensional spin model (2dXY), using the spin wave approximation, and is named after the authors. [1]

We continue work by Gonçalves et al [6] in modeling the BHP in fluctuation of the Paiva river. Without interference of regulators such as dams or glaciers, is the closest to a natural flow one might expect. Looking at embedding of various dimensions of the relative differences of the laminar regimes of the river, we analyse how many past days of flows are enough for an accurate prediction of the following level.



References:

- [1] S.T. Bramwell, P.C.W. Holdsworth, and J.F. Pinton, Universality of rare fluctuations in turbulence and critical phenomena, *Nature* 396 (1998), pp. 552– 554.
- [2] P. Sinha-Ray, L. Borda de Água, and H.J. Jensen, *Threshold dynamics, multifractality and universal fluctuations in the SOC forest fire: Facets of an auto-ignition model*, *Phys. D* 157 (2001), pp. 186– 196.
- [3] R. Gonçalves, A. Pinto and N. Stollenwerk, Cycles and universality in sunspot numbers fluctuations, *Astrophys. J.* 691 (2009), pp. 1583– 1586.
- [4] S.T. Bramwell, T. Fennell, P.C.W. Holdsworth and B. Portelli, Universal fluctuations of the danube water level: A link with turbulence, criticality and company growth, *Europhys. Lett.* 57 (2002), p. 310.
- [5] K. Dahlstedt and H.J. Jensen, Fluctuation spectrum and size scaling of river flow and level, *Phys. A* 348 (2005), pp. 596– 610.
- [6] R. Gonçalves, H. Ferreira, A. Pinto and N. Stollenwerk. Universality in nonlinear prediction of complex systems. *Journal of Difference Equations and Applications*, Vol. 15, 11-12 (2009), pp. 1067– 1076.

The matrix perturbation theory and the influence of the seminal paper of Von Neumann and Goldstine (1947).

K. Matos¹, J. Rocha e Silva^{1,2}

¹ Department of Mine Engineering, Faculty of Engineering, University of Porto, Portugal.

² ENEAS – Faculty of Engineering; University of Porto, Portugal

In 1947, just when modern computers were being invented, John von Neumann and Herman Goldstine wrote a paper^{[1] [2]} - *J. von Neumann and H. H. Goldstine, Numerical inverting of matrices of higher order, Bull. Amer. Math. Soc., 53 (1947), pp. 1021–1099* - to illustrate the mathematical analyses that they believed would be needed to use the new machines effectively and to guide the development of still faster computers. Their foresight and the congruence of historical events made their work the birth certificate of Numerical Analysis. Von Neumann once remarked that to found a mathematical theory one had to prove the first theorem, which he and Goldstine did for the accuracy of mechanized Gaussian elimination. But their paper was about much more than that: Von Neumann and Goldstine described what they surmised would be the significant questions once computers became available for automatic calculus and they suggested enduring ways to answer them.

During Numerical Analysis classes (EMG0012), after the theoretical presentation of the matrix perturbation theory^[3], the students were asked, as a complementary work, to read the paper by Von Neumann and Goldstine (1947) After understanding it in its historical context^[4] they should apply it (namely chapter 7th), to fully understand the matrix perturbation theory. This is the result of one of those complimentary lab works.

Acknowledgements

We are grateful to Professor Graciano de Oliveira for all his work on the progress of Linear Algebra and for all the good scientific talks about the subject.

References

[1] Von Neumann, John, H. H. Goldstine, *Numerical inverting of matrices of high order*, Bull. Amer. Math. Soc. 53 (1947), 1021-1099.

[2] Goldstine, Herman *The Computer from Pascal to von Neumann*, Princeton University Press, 1980,

[3] Golub, G, van Loan, C. (1983). *Matrix Computations*, The John Hopkins University Press.

[4] Turing, M. (1947) *Roundig off errors in Matrix Processes*. National Physical Laboratory, Teddington, Middlesex.

[5] Bunch, James R. (1987). *The Weak and Strong Stability of Algorithms in Numerical Linear Algebra**, Linear Algebra and Its Applications, Volumes 88–89, April 1987, Pages 49–66

[6] Datta ,Biswa Nath (1994), *Linear and Numerical Linear Algebra in Control Theory: Some Research Problems*, Linear Algebra and Its Applications Volumes 197-198, January–February 1994, Pages 755–790.

[7] Higham, Nicholas J. (2008). *Functions of matrices: theory and computation*. Philadelphia: Society for Industrial and Applied Mathematics.

[8] Higham, Nicholas J. (2002). *Accuracy and stability of numerical algorithms*. Philadelphia: Society for Industrial and Applied Mathematics.

Optimal Stochastic Control of Life Insurance and Investment in a Financial Market

F. Martins¹, A. A. Pinto^{1,2} and D. Pinheiro³

¹ Department of Mathematics, Faculty of Sciences, University of Porto, Portugal.

² LIAAD- INESC TEC

³ Brooklyn College, City University of New York

In this work we analyse a consumption, investment and life insurance purchase problem, in a very general model of a financial market with stochastic coefficients that are not necessarily Markov processes, and that we assume to be complete.

We use duality tools from convex analysis to obtain optimal consumption, investment and life insurance purchase under very general utility functions. We analyse the case of deterministic coefficients, deducing a mutual fund result, and the Hamilton-Jacobi-Bellman equation on that case, and we obtain explicit solutions for utility functions with constant relative risk aversion (CRRA).

Effect of hypertonic sodium chloride on intracranial pressure and cerebral perfusion pressure

Maria João Silva¹, Celeste Dias², A. P. Rocha¹ and A. Rita Gaió¹

¹ Department of Mathematics, Faculty of Sciences, University of Porto, and CMUP, Portugal.

² Department of Intensive Care, Neurocritical Care Unit, Hospital São João, Porto, Portugal

This work examines the effect of hypertonic saline (HTS) administration on the treatment of intracranial hypertension (IHT) through linear mixed-effects models for longitudinal data. Although mannitol has been used as the standard treatment in patients with severe brain injury, recent studies revealed that HTS can be more effective and therefore a good substitute of mannitol [1].

We studied the effect of HTS bolus on the evolution of brain hemodynamics over time, namely on intracranial pressure (ICP), cerebral perfusion pressure (CPP) and cerebrovascular pressure reactivity index (PRx). Mathematically, the phenomena were modelled by Linear Mixed-Effects regression Models (LMEM) for longitudinal data [2]. The observations were grouped according to two levels of experimental units, namely the individual and the bolus within the individual. Time averages of the three variables of interest were calculated at baseline (60min) and at every 30 min of the 210 min after the beginning of the drug infusion, defining a total of 8 consecutive time points. Random effects of final models were considered for the intercept only, for the time slope only, or for both of them, at the individual and bolus level. We also evaluated models with different correlation matrixes and residual variances, and time structures for the mean predictor up to order 2. Comparison between models was based on the likelihood ratio test for nested models and on the Bayesian Information Criteria (BIC) otherwise. Statistical analysis was performed with R 2.15.3 software [3] and statistical significance was considered at $p < 0.05$.

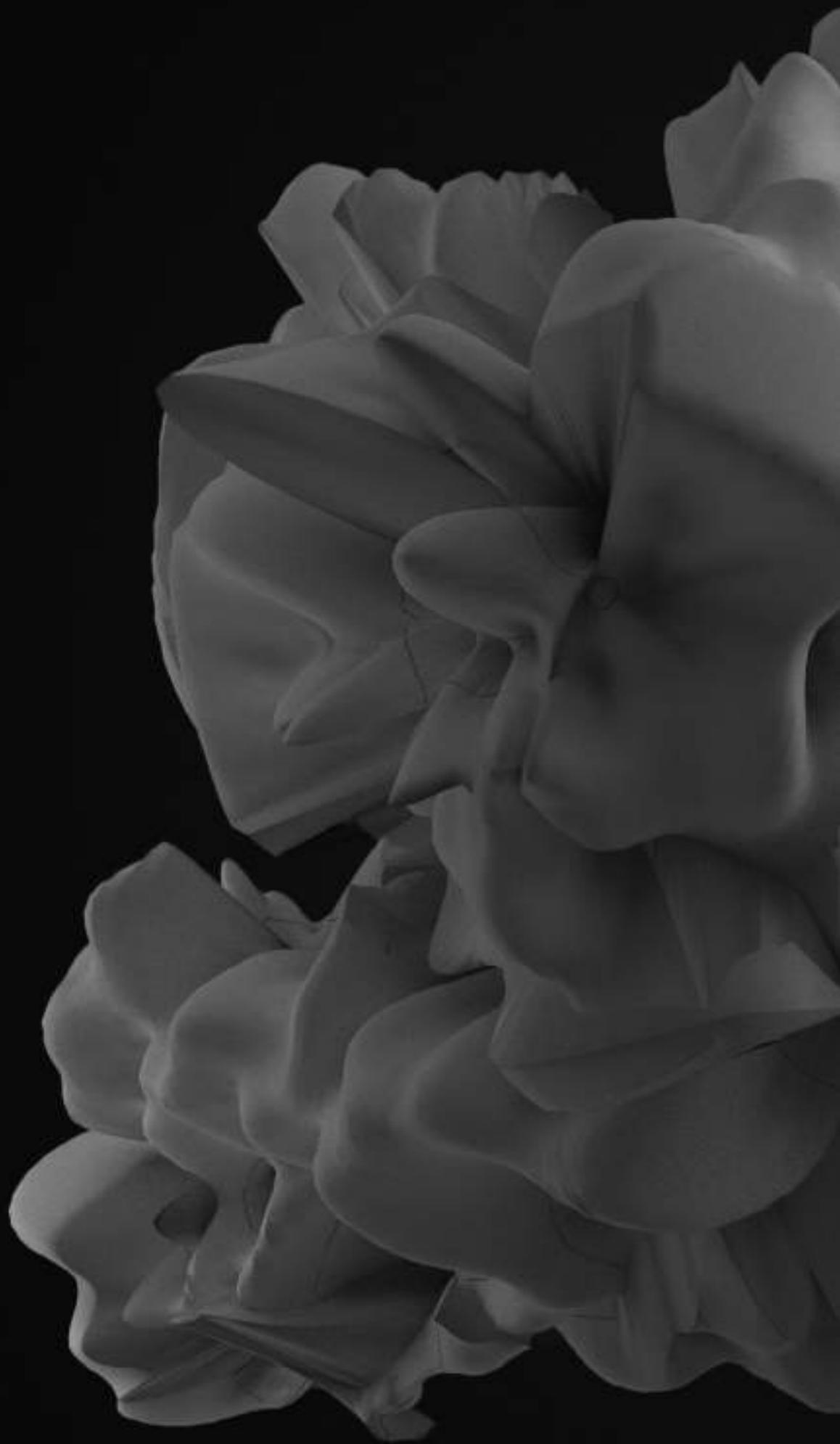
A total of 11 adult multiple trauma patients with severe head injury (9 males; mean age $40 \pm 11,0$ y. (range 21-64)) were analysed. A total of 99 HTS boluses were administered, with a median of 6, range 1 to 31 and with only 20% of the administrations above 11.

The results revealed that ICP and CPP improved significantly over time and were both explained by a quadratic model. At baseline ($t=1$) the value for ICP was estimated at 20.5 mmHg, then the mean curve decreased until 14.3 mmHg at 128 min and afterwards it started to increase again reaching a final value of 16.8 mmHg. Regarding CPP, the mean curve was convex with a baseline value of 85.1 mmHg, a maximum value of 88.2 mmHg attained at 119 min and a final value of 86.3 mmHg. Cerebrovascular reactivity index PRx was better described with decreasing linear models. Autoregulation index PRx impaired during IHT and significantly recovered after HTS bolus staying below 0.2.

Management of intracranial hypertension with 20% HTS bolus improves intracranial pressure and cerebral perfusion pressure with recovery of impaired autoregulation.

References:

- [1] Marko, N. F. (2012), *Hypertonic saline, not mannitol, should be considered gold-standard medical therapy for intracranial hypertension*, Critical Care (16:113).
- [2] Pinheiro, J. and Bates, D. (2000), *Mixed-Effects Models in S and S-PLUS*, Springer-Verlag, New York.
- [3] R Development Core Team (2012), *R: A language and environment for statistical computing*, ISBN 3-900051-07-0, retrieved from <http://www.R-project.org>.





**PARALLEL
ORAL
SESSIONS**



A1

BIOMEDICINE III

III
PARALLEL
ORAL
SESSIONS

Quality of life and psychological state after Heller myotomy in achalasia: What do patients think?

T. Fonseca¹, L. Ferreira-Pinto¹, M. Figueiredo-Braga PhD² and S. Carneiro PhD³

¹ Faculty of Medicine, University of Porto, Portugal.

² Dep Clinical Neurosciences and Mental Health, Faculty of Medicine, University of Porto

³ Faculty of Medicine, University of Porto and Department of General Surgery, Hospital São João, Porto

Background: Achalasia is an esophageal motility disorder characterized by dysphagia. Physical and psychological consequences of the disorder affect patient's daily routine, and compromise quality of life [1]. The gold standard procedure and the most effective surgical technique to treat achalasia is minimally invasive Heller myotomy with partial fundoplication. The aim of this study was to assess quality of life, mental health status and the emotional impact of achalasia in patients treated with Heller myotomy.

Methods: Between 2002 and 2012, 54 patients with achalasia were submitted to laparoscopic Heller myotomy at our surgical department. From this group we have selected 45 patients who were sent a set of questionnaires (9 patients were excluded due to underage or lack of clinical information). Postoperative evaluation was performed using a customized version of an achalasia disease-specific quality of life questionnaire (achalasia-DSQoL) [2]. Quality of life and the presence of depressive and anxiety symptoms were assessed using the Portuguese versions of the Medical Outcomes Study SF-36 and the Hospital Anxiety and Depression Scale (HADS). Statistical analysis was performed using SPSS 21.0[®].

Results: The study is still running and forty nine percent (n=22) of the total sample patients is already evaluated. Our preliminary data show that the mean age was 55 (standard deviation (SD) 19) years old and 36% were males. At the time of surgery, mean age was 49 years (SD 20). Dysphagia was the most prevalent symptom (94% of patients). No differences between physical and mental health domains have been found concerning quality of life through SF-36 Health Survey (p=0.877). A Kruskal-Wallis analysis revealed a significant difference between normal and both mildly and moderately anxious patients concerning mental health (p=0.04), without statistically significant relation with physical health domains. Depression level presented no significant association with either physical or mental health SF-36 scores.

Discussion and Conclusion: These preliminary results show that the patients submitted to Heller myotomy present a good quality of life both in the physical and mental domains, supporting that this procedure is a safe and reliable treatment for achalasia. Further analysis is needed to relate the quality of life, the severity of the disease and the presence of symptoms after the surgery, and to elicit the presence of psychological symptoms in this population.

References:

- [1] Madureira FA, Madureira FA, Loss AB, Madureira D (2009) *Quality of life after Heller-Dor's cardiomyotomy*. Revista do Colegio Brasileiro de Cirurgioes 36 (3):193-198
- [2] Urbach DR, Tomlinson GA, Harnish JL, Martino R, Diamant NE (2005) *A measure of disease-specific health-related quality of life for achalasia*. The American Journal of Gastroenterology 100 (8):1668-1676

Assessment of amylin's nociceptive effect in an animal model of neuropathic pain

L.S. de Almeida¹, F.L. Neto^{1,2} and C.S. Potes^{1,2}

¹ Department of Experimental Biology, Centre of Medical Research, Faculty of Medicine, University of Porto, Portugal.

² Morphophysiology of the Somatosensory System Group, Institute for Molecular and Cell Biology, Porto, Portugal.

Neuropathic pain results from injury in central or peripheral nerves of the nervous system and is characterized by an increased pain response to a noxious stimulation (hyperalgesia) and the onset of numbness, experience of pain from a non-noxious stimulus (allodynia), and spontaneous pain. Amylin is a peptide expressed in C-peptidergic neurons of the dorsal root ganglia. Several studies suggest a role for amylin in nociception and its expression is down-regulated following sciatic nerve transection, but data is still controversial. Thus, this study aimed at clarifying the effect of amylin's administration in neuropathic pain as well as at determining its site of action.

Neuropathic pain was induced by the spared nerve injury (SNI) model and its development was assessed by the von Frey, pin prick, acetone (days 7, 10, 14, 17 and 21) and cold plate (day 21) acute pain behavioral tests. The effects of acute (day 14) and chronic (days 14-21) subcutaneous (SC) amylin administration were evaluated by the same nociceptive tests. Furthermore, c-Fos expression and ERK1/2 activation in the ipsilateral dorsal horn of L4-5 spinal cord segments were assessed after 7 days of chronic SC amylin administration. In order to determine if amylin's action is mediated by the activation of amylin receptors in the spinal cord, the effect of intrathecal (IT) acute and chronic amylin administration as well as the effect of an acute IT injection of the antagonist of amylin's receptors (AC187) was also evaluated.

No significant results were obtained after an acute SC amylin administration. However, a tendency to the attenuation of mechanical allodynia and hyperalgesia induced by an acute IT injection of AC187 and amylin, respectively, was observed. These results may indicate an anti-nociceptive role of these peptides mediated by amylin's receptors in the spinal cord. In the acetone test, chronic SC amylin administration over 7 days caused a progressive and significant increase of the time response of neuropathic rats, compared to control neuropathic animals treated with saline, indicative of cold allodynia worsening. The same result was not obtained after IT administration of amylin, which suggests that the pro-nociceptive effect observed is not mediated via amylin's receptors located in the spinal cord, but probably at a supraspinal level. A strong tendency for an increased activation of ERK1/2, known to be involved in the maintenance of neuropathic pain, was also detected in neuropathic animals after chronic SC amylin treatment, which may be associated with the increase of cold allodynia in these animals. c-Fos expression was not significantly altered after chronic SC amylin administration.

Thus, the effect of amylin in chronic neuropathic pain seems to depend on the duration of the treatment and the route of administration, suggesting a distinct action at different levels of the nervous system.

Mitochondrial regulation of epigenetics and its role in human diseases

R. Coelho^{1,2}, J. Lima^{1,3}, P. Soares^{1,3}, V. Máximo^{1,3}

¹Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), 4200-465 Porto, Portugal.

²Institute of Biomedical Sciences of Abel Salazar, University of Porto, 4050-313 Porto, Portugal.

³Department of Pathology and Oncology, Medical Faculty of the University of Porto, 4200-319 Porto.

Mitochondria have a central role in energy uptake and energy production, and as consequence they play a key role in a wide variety of pathological conditions, such as cancer, neurodegenerative diseases, diabetes and aging. Indeed, mitochondrial dysfunction, and especially mitochondrial dysfunction caused by mutations in mtDNA have been implicated in a wide range of age related pathologies. The mitochondrial activity is largely dependent on the environmental availability of nutrients, namely carbohydrates, proteins and fats, and their conversion, mainly by glycolysis and oxidative phosphorylation (OXPHOS), in energy rich compounds, such as adenosine triphosphate (ATP), acetyl- Coenzyme A (acetyl-CoA), S-adenosyl-methionine (SAM), and nicotinamide adenine dinucleotide (NADH). ATP, acetyl-CoA, SAM, and NAD⁺/NADH, in turn, are the high-energy substrates for protein (including histones) phosphorylation, acetylation and deacetylation reactions, and DNA and protein methylation, thus contributing to the regulation of several cell signalling pathways and cell epigenetic landscape. Thus, the interrelationship between mtDNA, mitochondrial activity and cell metabolism have an essential role in the maintenance of the levels of essential co-factors for cell epigenetic mechanisms regulation, such as DNA methylation and protein acetylation.

The main goal of this study was to unveil how pathogenic mutations in mtDNA and/or mitochondrial variants may lead to epigenetic changes, namely alterations in the pattern of nuclear DNA methylation and/or acetylation of histones and other proteins.

Our data, showed that mitochondrial dysfunction, caused either by mtDNA mutations or absence of mtDNA, as well as the presence of specific mitochondrial variants (different mtDNA haplogroups) induce changes in cell metabolic profile, complex I activity, DNA methylation and protein acetylation. Furthermore, we observed that mitochondrial dysfunction caused by depletion of mtDNA, leads to the readjustment of several cellular processes through the acetylation of several proteins, allowing the cell to survive and maintain its homeostasis.

Anatomical region differences and age-related changes on calcium and magnesium levels in the human brain

**H. Correia¹, P. Ramos¹, A. Santos^{2,3}, N. Rosas Pinto³, R. Mendes³,
T. Magalhães^{2,3} and A. Almeida¹**

¹ REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

² CENCIFOR – Centro de Ciências Forenses, Portugal.

³ National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal.

Calcium (Ca) and magnesium (Mg) play a central role in neuronal physiology. Disturbances in the brain Ca homeostasis have been implicated in the pathogenesis of several acute and chronic neurodegenerative diseases (ND) and in brain aging; Mg brain levels seem to be decreased in elderly people and such depletion, particularly in the hippocampus, may represent an important pathogenic factor of some ND.

Most of the current information about the relationship between metals and human brain functioning is based on animal studies or relies on determinations in body fluids. Direct studies in normal and pathological human brain tissue are scarce, limited to a few brain areas and/or involve a small number of subjects. Additionally, and regarding the specific topic of the brain region differences, most of the published post-mortem and in vivo studies are focused solely on iron.

Based on this background, the main goal of our study¹ was to directly quantify Ca and Mg levels in 14 different areas of the human brain in order to evaluate a) the regional anatomic differences and b) age-related changes in Ca and Mg levels. Ca and Mg were measured in the brain tissue of adult individuals (n = 42; 71±12, range: 50–103 years old) without a known history of neurodegenerative, neurological or psychiatric disorder.

Considering the mean values for the 14 regions, Mg was present at ca. 2.3-fold higher levels than Ca (mean ± sd: 527±34 µg/g versus 226±53 µg/g). Calcium distribution within the brain showed to be quite heterogeneous: highest levels were found in the occipital (306±156 µg/g) and frontal cortex (287±78 µg/g), while lowest levels were found in the medulla (186±70 µg/g) and cerebellum (145±42 µg/g). Higher Ca levels were found in women than in men (248±59 µg/g vs. 213±46 µg/g; p < 0.05). A tendency for Ca levels to increase with age in all studied brain regions and in both genders was also observed. On the contrary, Mg presented a highly homogeneous distribution and seems to remain quite unchanged irrespective of aging².

References:

[1] Ramos, P. et al. (2014). *Iron levels in the human brain: a post-mortem study of anatomical region differences and age-related changes*. Journal of Trace Elements in Medicine and Biology, 28(1), 13-17.

Acknowledgments: The authors thank to Universidade do Porto and Santander Totta for financial support through the project “TRAIN : Trace elements in human brain: age-related changes and anatomic region specific differences” (PP_IJUP 2011 342).

¹ Conducted within the Course Unit “Projeto I”, Master in Pharmaceutical Sciences, FFUP 2013.

² Results already published: Correia, H., Ramos, P. et al. (2014). *A post-mortem study of the anatomical region differences and age-related changes on Ca and Mg levels in the human brain*. Microchemical Journal, 113, 69-76.

Protein structure and sequence complexity relationship: effect on protein interactome.

J. Oliveira¹, F. Martins¹, R. Gonçalves¹, I. Rebelo^{1,2}, E. Tejera^{1,2}

¹ Department of Biological Sciences/Biochemistry, Faculty of Pharmacy, University of Porto, Portugal.

²IBMC, University of Porto, Portugal.

In the present study, we explore the relationship between sequence and structural complexity and how this complexity is related to protein specificities in the interaction network. The influence of sequence complexity on the interactome had been explored through several approaches; however, there is limited information in terms of structure complexity, especially protein fractality.

Based on the human protein-protein interaction network (PPI) we obtained several protein structures from PDB database also considering several criteria (e.g. chain number, protein length). We also extract the protein sequence and the secondary structure information using the DSSP software. Computations were made using several R-packages implementation (bio3D, irefR), home-made codes and Cytoscape functionalities. Various centrality indexes were calculated in the PPI and compared with sequence and protein complexity indexes.

Our results indicate a significant correlation between protein and sequence complexity. Moreover, protein structure complexity is related to the number protein domains, the specific biological processes and also the degree of protein centrality of the PPI. In general, less complex proteins tend to more interactivity capabilities.

Trace element imbalances in hemodialysis patients – final results of a 16-month monitoring study in a Portuguese population

L. Araújo^{1,2}, H. Rodrigues², P. Ramos¹ and A. Almeida¹

¹ REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

² Clínica Laboratorial dos Arcos de Valdevez Lda, Portugal.

Most trace elements are important from both a biochemical and toxicological point of view. Some are particularly toxic, potentially noxious to the human body, while others are “essential”, i.e., well-defined signs and symptoms appear when a deficient intake exists and they attenuate or disappear when an adequate intake is re-established.

Patients with chronic renal failure submitted to hemodialysis therapy are at increased risk of imbalances in trace elements levels, which mainly results from the kidney failure itself, that predisposes to their accumulation (although losses can also occur in some situations, such as proteinuria), as well as from the dialysis process, which can also lead to significant losses of trace elements, contributing to a deficiency status with biological consequences, or be responsible for situations of overload, with potential toxicity [1,2].

Despite the extensive literature about the implications for the human body of imbalances on trace element status, there are still not very detailed studies regarding specifically hemodialysis patients (with the notable exception of aluminum).

Based on this background, we conduct a study aiming to evaluate, over a long period of time, the trace elements status in a population of individuals diagnosed with chronic renal failure performing regular hemodialysis therapy (mean of three times/week), in order to establish a link between the results, the underlying disease process and the therapy itself³. Blood samples were taken at several sampling time points (3 for Se; 8 for Pb and Mn; 6 for Cu and Zn), between January 2011 and April 2012, from ca. 50 hemodialysis patients. The work has focused on Cu, Zn, Se, Mn and Pb.

The results showed that patients with chronic renal failure undergoing hemodialysis therapy tend to suffer from imbalances in the blood levels of important trace elements. With the exception of Mn, for which normal values (and no significantly different from the control group) were obtained, significant imbalances were observed for the other elements studied: increased levels of Pb (patients: 14.8 ± 4.8 $\mu\text{g/dL}$, $n=304$; controls: 3.8 ± 2.5 $\mu\text{g/dL}$, $n=44$) and decreased levels of Cu (patients: 98.1 ± 23.2 $\mu\text{g/dL}$, $n=268$; controls: 126.6 ± 31.2 $\mu\text{g/dL}$, $n=62$), Se (patients: 82.5 ± 28.8 $\mu\text{g/L}$, $n=137$; controls: 129.5 ± 28.4 $\mu\text{g/L}$, $n=30$) and Zn (patients: 60.3 ± 12.4 $\mu\text{g/dL}$, $n=272$; controls: 91.0 ± 16.8 $\mu\text{g/dL}$, $n=62$).

References:

- [1] Covic, A., Gusbeth-Tatomir, P. (2009), *Trace elements in end-stage renal disease - unfamiliar territory to be revealed*, BMC Nephrology, 10, 12.
- [2] Tonelli, M., et al. (2009), *Trace elements in hemodialysis patients: a systematic review and meta-analysis*, BMC Medicine, 7, 25.

³ Master in Clinical Analysis, FFUP 2013.



A2

CHEMISTRY II

III
PARALLEL
ORAL
SESSIONS

Human salivary protein HPLC profile on different days: differences between genders

E. Brandão¹, N. Mateus¹ and V. de Freitas¹

¹CIQUP, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

In general, saliva is composed of proteins, electrolytes and small organic compounds. However, whole saliva represents a mixture of the secretions of the major (submandibular, sublingual and parotid) and minor salivary glands, together with the crevicular fluid, bacteria and cellular debris [1]. The secretions from the different glands have been shown to differ considerably and to be affected by different forms of stimulation, circadian rhythms, diet, age, gender, several disease states and pharmacological agents. Some authors discuss that saliva samples collected at the same time of day might be expected to be less variable than samples collected at different times of day due to the influence of circadian rhythms [2, 3]. Salivary proteins (SP) have been grouped into 6 structurally related major classes namely, histatins, basic proline-rich proteins (bPRPs), acidic proline-rich proteins (aPRPs), glycosylated proline-rich proteins (gPRPs), statherin and cystatins. This work focused on specific families of proteins (PRPs and statherin) due to their importance in the development of astringency sensation. So, in this study, it was investigated possible differences in the human salivary protein profile on different days and between genders.

Human saliva was collected on four different days at 2 p.m. from six volunteers, divided in two groups: three males and three females, designated group M and group F, respectively. All samples of saliva were treated with trifluoroacetic acid solution (10% aqueous TFA), centrifuged (10500 rpm, 5 min) and the supernatant (acidic saliva, AS) was analyzed by high-performance liquid chromatography (HPLC).

From the results obtained, it seems to be a trend of variation of the several families of SP on four different days within the two groups. However, the group M seems to show a higher variability in comparison with the group F which can result, probably, from a greater intra- and inter-individual variability. It was observed significant differences between the two groups in the amount and variation of the several families of SP. The group M presents a larger amount of all PRPs, especially bPRPs, while statherin seems to be the family of SP more constant on different days and between genders (no significant differences were observed).

[1] Ekström, J. K., N.; Castagnola, M.; Messana, I. (2012), *Dysphagia, Medical Radiology*, Springer-Verlag Berlin Heidelberg, New York, pp. 19-47.

[2] Dodds, M. W. J., Johnson, D. A., Yeh, C. K. (2005), *Health benefits of saliva: a review*, Journal of Dentistry, 33 (3), 223-233.

[3] Dawes, C., (1972), *Circadian-rhythms in human salivary flow-rate and composition*, Journal of Physiology, 220 (3), 529-545.

Location of caffeic acid and its alkyl esters in liposomes

C. Silva¹, L. Palagi^{1,3}, M. Ferreira², M. Costa¹, C. Bravo-Díaz⁴, S. Losada-Barreiro^{1,4}, P. Gameiro², F. Paiva-Martins¹

¹CIQUP, ²REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal

³Department of Chemistry, University of Genova, Italy

⁴Faculty of Chemistry, Department of Physical-Chemistry, University of Vigo, Spain

The knowledge of the interaction of the antioxidant with lipid membranes is crucial to understand the distribution of these molecules in the body, and to establish relationships between the physico - chemical properties and their biological effects. These interactions depend both on membrane properties and also on the compounds physicochemical properties. Thus, the interaction of an antioxidant with the membrane depends on its lipophilicity.

Caffeic acid, a strong radical scavenging molecule, has an hydrophilic nature and therefore have very low diffusion into biomembranes. Therefore, it was our aim to increase the hydrophobicity of caffeic acid by synthesizing caffeic acid esters with different alkyl chain sizes and to study the interaction of these esters with a model membrane.

It was synthesized the methyl (C1), ethyl (C2), octyl (C8) and hexadecyl (C16) caffeates [1]. In order to mimic the biological membranes, is was used DMPC liposomes (L- α - dimyristoylphosphatidylcholine), a synthetic phospholipid containing choline.

Fluorescence anisotropy of probes and fluorescence quenching studies were used to identify the location of acid caffeic and its caffeates in the liposomes [2]. DPH and TMA-DPH, locate in the hydrophobic regions and at the fourth carbon atom in the transient region between hydrophobic and hydrophilic parts of the bilayer respectively, were used as fluorescence probes. Such differentiated incorporation of the probes gives insight into the structural changes caused by incorporation of antioxidants.

In the studies by fluorescence anisotropy, it was found that caffeates interacted with the interior of the membrane in the same way except for C1, which changed to a lesser extent the anisotropy of the probes. In fluorescence quenching studies, it was found that at the concentration of 100 μ M, the order of interaction with the probe DPH, located inside the liposome, was C8 > C2 > C1 = C16. At a concentration of 200 μ M, there were no differences between the compounds C8, C2 and C16 but C1 showed a smaller activity on fluorescence quenching of the probe. Caffeic acid did not change both probes anisotropy and did not show any activity on the fluorescence quenching of the probe DPH at both concentrations tested proving its more external location.

References:

[1] Menezes, J.C.M.D.S, Kamat, S.P., Cavaleiro, J.A.S, Gaspar, A.garrido, J.,Borges ,F. (2011) Syntesis and antioxidant activity of long chain alkyl hydroxycinnamates, *J Med Chem*, 46, 773-777.

[2] Paiva-Martins, F; Gordon, M. H.; Gameiro, P. (2003) Activity and Location of Olive Oil Phenolic Antioxidants in Liposomes, *Chem. Phy. Lipids*, 124, 26-34.

Correlation between the efficiencies of caffeic acid alkyl esters and their distribution in emulsions.

M. Costa¹, C. Santos¹, S. Losada-Barreiro², C. Bravo-Díaz², L. S. Romsted³, F. Paiva-Martins¹

¹ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal

² Faculty of Chemistry, Department of Physical-Chemistry, University of Vigo, Spain

³ Department of Chemistry and Chemical Biology, State University of New Jersey, USA

Recently published results for series of homologous antioxidants (AO) of increasing alkyl chain length show a maximum in AO efficiency followed by a significant decrease for the more hydrophobic AOs, typically called the “cut-off” effect [1].

A recently developed kinetic method [2] was applied in an intact emulsion to determine the distribution of caffeic acid alkyl esters (from C1 to C16) in a model food emulsion composed of stripped olive oil, acidic water and Tween 20. Their AO efficiency was also determined by employing an accelerated oxidative test (Schaal Oven Method) applied to emulsions.

Results showed that a large percentage of the antioxidants are located at the interface of the emulsion and this percentage increases with the increase in the emulsifier volume (Φ_I). Both AO efficiencies and partition constants for distributions of caffeic acid alkyl esters between the oil and interfacial regions, P_O^I , show a maximum at the C8 ester (Fig. 1). Our results also show that the efficiency of the C3-C16 derivatives in emulsions with a low Φ_I is higher than that in emulsions with higher Φ_I showing that the overall antioxidant activity of compounds in emulsions will depend on the balance between the increase of their percentage at the interface and their actual concentration (mol/L) at the interface. Therefore, AO that are already present at higher percentage at the interface at low Φ_I may decrease their antioxidant efficiency with the increase of Φ_I .

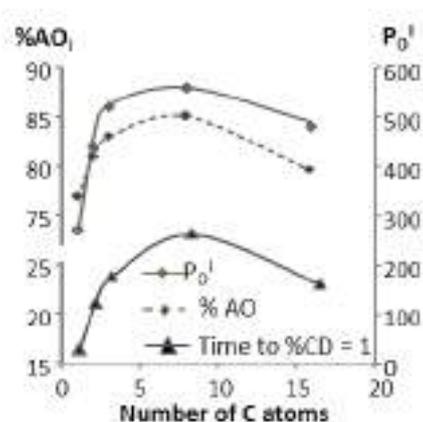


Figure 1 - Variation in the time to reach 1% conjugated dienes, values of P_O^I and %AO₁ with the number of C atoms.

In conclusion, the model provides a natural interpretation for both the maximum and the “cut-off” effect. Here we demonstrate that in olive oil emulsions both AO efficiencies and partition constants for distributions of caffeic acid alkyl esters between the oil and interfacial regions, P_O^I , show a maximum at the C8 ester (Fig. 1).

References:

- [1] Laguerre, M., López-Giraldo, L., Lecomte, J., Figueroa-Espinoza, M., Baréa, B., Weiss, J., et al. (2010). Relationship between Hydrophobicity and Antioxidant Ability of "Phenolipids" in Emulsion: A parabolic Effect of the Chain Length of Rosmarinate Esters. *J. Agric. Food Chem.*, 58, 2869-2876.
- [2] Losada-Barreiro, S., Sánchez Paz, V., Bravo Díaz, C., Paiva Martins, F., Romsted, L. S. (2012). Temperature and emulsifier concentration effects on gallic acid distribution in a model food emulsion. *J. Colloid. Interface Sci.*, 370, 73-79.

Optimization of Chlorogenic Acid Extraction from Lamb's Lettuce

S. Abrunhosa, L. F. Guido

REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Lamb's lettuce (*Valerianella locusta*) is a green leafy vegetable, which is well appreciated in France, Britain, Germany and Italy¹. Lamb's lettuce is achieving importance as a major source of phenolic compounds, especially chlorogenic acid (CGA). The multiple pharmacological effects of CGA, due to its antioxidant properties, are widely recognized. Classically, CGA are a family of esters formed between certain trans-cinnamic acids, like caffeic acid, *p*-coumaric acid or ferulic acid, and a quinic acid. The commonest individual CGA is 5-O-caffeoylquinic acid (5-CQA)².

The extraction of bioactive compounds, including CGA, by ultrasound assisted extraction (UAE) is an efficient, low cost, and easy to handle technique³.

An ultrasonic method for the extraction of chlorogenic acid from Lamb's lettuce's fresh leaves was investigated and optimized, within four extraction variables, namely: solvent / sample ratio (1:30 – 2:30), methanol / water ratio used as solvent (60-100% methanol), extraction time (20-40 minutes) and number of extractions (1-3). The characterization and quantification of CGA was carried out by HPLC/DAD (high-performance liquid chromatography/diode array detector) that enables continuous monitoring and recording of the ultraviolet and visible absorption spectrum⁴.

The experimental design (Fractional Factorial Design) combined 16 points in a factorial design to a level of center $\alpha = \pm 1.000$ and a central point with six replicates.

The analysis of effects highlights the variables with higher influence on the result (CGA content), which were found to be the solvent's methanol ratio and extraction time, followed by the number of extractions.

Since many factors can influence the outcome of the results, the response surface methodology (RSM) was applied to take advantage of the mathematical models that represent the relationship between the response and the input variables, studying their interactions. The found optimum extraction conditions were: 100% methanol; sample:solvent ratio (1:30) and 3 steps of 40 minutes (each) extraction time.

These results also suggest that lamb's lettuce is an excellent natural source of CGA which therefore strengthens its role in the food and pharmaceutical industry.

Acknowledgments

The authors greatly acknowledge the financial support through the Programme QREN - NORTE-07-0124-FEDER-000069 - Ciência do Alimento.

References

- [1] Pellegrino, C., Gilardi G., Gullino M. L. and Garibaldi A. (2010), *Phytoparasitica*, 38(2): 159-165.
- [2] Clifford, M. N. (2000), *J Sci Food Agric*, 80: 1033-1043.
- [3] Casazza, A. A., Aliakbarian, B., Mantegna, S., Cravotto, G. and Perego, P. (2010), *Journal of Food Engineering*, 100(1): 50-55.
- [4] Thompson, R. and LoBrutto, R. (2007) *HPLC for Pharmaceutical Scientists*, John Wiley & Sons, Inc, 14.

Phase Behaviour and Heat Capacities of 1-Benzyl-3-methylimidazolium Ionic Liquids Series

F.M.S. Ribeiro,¹ P.B.P. Serra,^{1,2} M.A.A. Rocha,¹ M. Fulem,²
K. Ruzicka,² L.M.N.B.F. Santos¹

¹CIQ, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, R. Campo Alegre 687, P-4169-007, Porto, Portugal

²Department of Physical Chemistry, Institute of Chemical Technology, Prague, CZ-166 28 Prague 6, Czech Republic.

In this work, the thermal behaviour and the heat capacities and their temperature dependence of four 1-benzyl-3-methylimidazolium derivatives (Fig.1), [Bnmim][PF₆], [Bnmim][BF₄], [Bnmim][NTf₂] and [Bnmim][C₂F₄HSO₃] are presented.

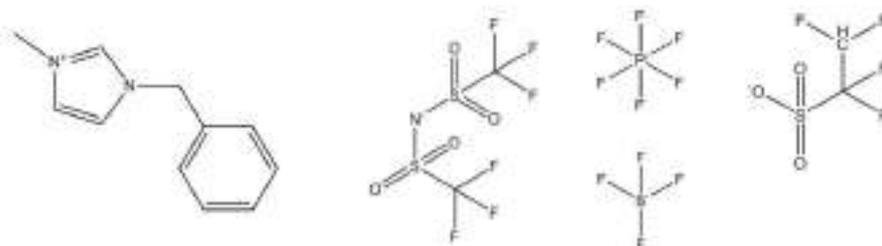


Figure 1 - Chemical structure of the four 1-benzyl-3-methylimidazolium derivatives.

The heat capacities of the studied ionic liquids were measured using two different calorimetric methodologies. The thermal phase behavior was performed in the temperature range of (183 – 423) K for the four ionic liquids using a TA Instruments Q1000 and heat capacities in the temperature range of (253 – 353) K were measured using a Setaram μ DSC IIIa microcalorimeter, in Prague. The heat capacities at $T = 298.15$ K were measured, using a high-precision heat capacity drop calorimeter, in Porto. [1,2,3] Based in the obtained results, it was possible to evaluate the anion and cation effect through a comparative analysis with the [C_{N-1}C₁im][NTf₂] [4] and [C_{N-1}C₁im][PF₆] [5] ionic liquid series. Two different polymorphic forms of the [Bnmim][C₂F₄HSO₃] were additionally found and characterized.

References:

- [1] Konicek J., Suurkuusk J., Wadsö I., *Chemica Scripta* (1971), 1, 217 – 220.
- [2] Suurkuusk J., Wadsö I., *J. Chem. Thermodynamics* (1974), 6, 667 – 679.
- [3] Santos L., Rocha M., Rodrigues A., Štejfa V., Fulem M., Bastos M., *J. Chem. Thermodyn.* (2011), 43, 1818-1823.
- [4] Rocha M., Bastos M., Coutinho J., Santos L., *J. Chem. Thermodyn.* (2012), 53, 140-143.
- [5] Serra P., Ribeiro F., Rocha M., Fulem M., Ruzicka K., Santos L., *Phase Behaviour and Heat Capacities of [C_{N-1}C₁im][PF₆] ionic liquid series*, (not published).

Insights on the Nanostructuration of Ionic Liquids by Infrared Spectroscopy

Inês C. M. Vaz, Marisa A. A. Rocha, Luís M. N. B. F. Santos

Department of Physical Chemistry, Faculty of Sciences University of Porto, Portugal.

Ionic Liquids (ILs) are salts with a melting point lower than 100°C. They are usually constituted by an organic cation and an organic or inorganic anion. Due to their unusual properties (low vapour pressures, high thermal stabilities) and wide range of applications they have been gaining popularity [1].

Aiming to understand the interplay between their structure and their properties, they have been extensively studied. It had been already evidenced both experimentally [2, 3] and by molecular simulations [4] that ILs are nanostructured.

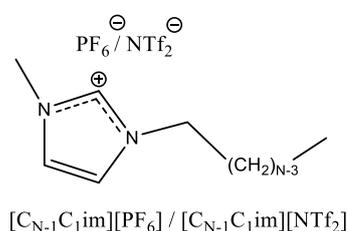


Figure 2. Chemical structure of the studied compounds.

In this work the nanostructuration of ILs was explored by FTIR spectroscopy in the [C_{N-1}C₁im][NTf₂] and [C_{N-1}C₁im][PF₆] ILs series (fig.1) giving some additional insights concerning the effect of the alkyl side chain length and the anion effect (fig.2) in their nanostructuration and the CASL (critical alkyl length size).

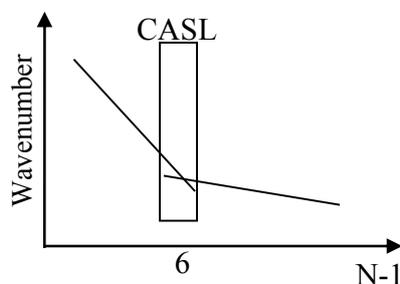


Figure 2. Schematic representation of the variation of wavenumber of C-H stretching vibrations with the variation of the alkyl side chain length.

References:

1. Plechkova, N.V. and K.R. Seddon, (2008), *Applications of ionic liquids in the chemical industry*, Chemical Society Reviews, 37 (1), 123-150.
2. Rocha, M.A.A., et al., (2012), *Heat capacities at 298.15K of the extended [C_nC₁im][Ntf₂] ionic liquid series*, The Journal of Chemical Thermodynamics, 53 (0), 140-143.
3. Rocha, M.A.A., et al., (2011), *High-Accuracy Vapor Pressure Data of the Extended [C_nC₁im][Ntf₂] Ionic Liquid Series: Trend Changes and Structural Shifts*. The Journal of Physical Chemistry B, 115 (37), 10919-10926.
4. Canongia Lopes, J.N.A. and A.A.H. Pádua, (2006), *Nanostructural Organization in Ionic Liquids*. The Journal of Physical Chemistry B, 110 (7), 3330-3335.



A3

ENVIRONMENT II

III
PARALLEL
ORAL
SESSIONS

***Solanum nigrum* L.'s Glutathione-S-transferase (GST) and Gamma-glutamyl cysteine synthetase (γ -ECS) evaluation in response to two steroidal hormones**

A. Pinto, F. Fidalgo and J. Teixeira

BioISI – Biosystems & Integrative Sciences Institute, Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal.

Progesterone is a natural female hormone that regulates mammal metabolism. The presence of this compound has also been naturally found in certain plants and it has been reported to accumulate in the environment due to anthropogenic activities. It is believed that progesterone can regulate plant growth processes and resistance to stress, however, its precise role in plants remains unknown [1]. Brassinosteroids (BRs) are a class of plant-steroidal hormones that play key roles in regulating broad aspects of plant growth, development and responses to various biotic and abiotic stresses [2]. To understand the effect of progesterone in *Solanum nigrum* L. and the effect of the a pre-treatment with BRs in these plant responses, 3 different treatments were performed: control (1); plants grown in the presence of 0,768 μ M Progesterone (2); and plants grown in the presence of 0,768 μ M Progesterone but pre-treated with foliar applications of 10 μ M BRs (3). All plants were exposed to these conditions for 30 days. Then, glutathione-S-transferase (GST) and Gamma-glutamyl cysteine synthetase (γ -ECS) activities were determined in shoots and roots, as well as the relative accumulation of their encoding mRNAs by semi-quantitative RT-PCR. In shoots, treatment (3) lead to a higher and significant activity for both enzymes (2.6x and 2.4x for GST and γ -ECS, respectively), but in roots both enzyme activities significantly decreased (2.0x and 1.2x for GST and γ -ECS, respectively). GST activities significantly decreased in treatment (2) in shoots and roots by 2.4x and 1.8x, respectively. There were no significant changes in γ -ECS activities in this treatment for both organs analyzed. RT-PCR analysis suggests that BRs regulate these enzymes at transcription, while progesterone does it post-translationally. These results support that BRs prepared the plants for a possible stressful situation by increasing the activity and amount of the analyzed antioxidant enzymes (γ -ECS and GST). In the case of the progesterone treatment, further studies must be performed in order to understand whether this compound has a positive effect on other antioxidant enzymes, thus making the action of γ -ECS and GST not so required by the plants.

References:

- [1] Erdal S., Dumlupinar R., 2011a. Mammalian sex hormones stimulate antioxidant system and enhance growth of chickpea plants. *Acta Physiologiae Plantarum* 33: 1011–1017
- [2] Yang, C.-J., Zhang, C., Lu, Y.-N., Jin, J.-Q., Wang, X.-L., 2011. The mechanisms of brassinosteroids' action: from signal transduction to plant development. *Mol. Plant* 4: 588–600.

Progesterone: a hormone with a role in plant defence against stress?

A. Cardoso, F. Fidalgo and J. Teixeira

BioISI - Biosystems & Integrative Sciences Institute, Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal.

Progesterone (PRG) is a mammalian steroid hormone that has been reported to be present in plants [see ref. in 1]. Furthermore, PRG and other sexual hormones have been recently considered emerging pollutants as more sites are reported to have increased levels of such compounds being accumulated [2]. Brassinosteroids (Br) are also steroid hormones that belong to the same class of PRG and that play a protective role in plants against stress. In this work, the responses to PRG exposure and the protective role of Br to such exposure were evaluated in *Solanum nigrum* L.. Plants were grown hydroponically for one month under three conditions: Control (C); 0.768 μ M PRG (P) supplied in the nutrient solution; and treated with a 10 μ M Br foliar application before the PRG treatment (P+Br). Plant stress biomarkers lipid peroxidation, proline levels and photosynthetic pigments were then assessed. Also, glutamine synthetase (GS) activity was determined in order to evaluate if these steroids influenced plant nitrogen metabolism. Lipid peroxidation significantly decreased in shoots and roots in both treatments, suggesting the absence of stress. Proline levels significantly decreased in roots in both treatments, especially in the presence of PRG (P), and suffered no changes in shoots, supporting the above-mentioned observation. Also, no changes in photosynthetic pigments were observed, strengthening this hypothesis. It was detected a significant increase of GS activity both in shoots and roots of PRG exposed plants (P) and in roots of plants grown in P+Br. Such results indicate that PRG promotes the activity of GS, possibly by inhibiting the formation of NO that may occur with the reduction of NO₃⁻ by nitrate reductase. In this way, the production of ammonia from NO₃⁻ reduction was enhanced, leading to a higher concentration of ammonia, the substrate for GS. Taken together, the results obtained suggest that PRG may have a role in *Solanum nigrum* L. defence against stress, which is consistent with results obtained for wheat and *Arabidopsis thaliana* [3,4]. As so, a combination of PRG and Br may be a possible cost-effective approach to maintain plants that highly tolerate stressful conditions.

References:

- [1] Janeczko, A. (2012), The presence and activity of progesterone in the plant kingdom, *Steroids* 77 169-173.
- [2] Jin W., Huang B., Wang B., Wang D., Zhao S. And Pan X. (2013), Simultaneous Determination of Androgens and Progesterone in Surface Water and Sediment by Gas Chromatography-Mass Spectrometry, *Chin J Anal Chem* 41 205-209.
- [3] Janeczko, A., Oklešťková, J., Siwek, A., Dziurka, M., Pocięcha E., Kocurek, M. and Novák, O. (2013), Endogenous progesterone and its cellular binding sites in wheat exposed to drought stress, *J Steroid Biochem Mol Biol* 138 384-394.
- [4] Janeczko, A., Tóbiás, I., Skoczowski, A., Dubert, F., Gullner, G. and Barna B. (2013), Progesterone moderates damage in *Arabidopsis thaliana* caused by infection with *Pseudomonas syringae* or *P. fluorescens*, *Biologia Plantarum* 57 169-173.

Copper phytoremediation by *P. australis* enhanced by autochthonous bioaugmentation

T. Oliveira^{1,2}, A. P. Mucha², I. Reis², P. Rodrigues², C. R. Gomes^{1,2}, C. M. R. Almeida²

¹ Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal.

² CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal.

Estuarine areas are target of several sources of pollution, including metals, which can accumulate in the environment, presenting a threat. Most of the estuaries present large salt marsh areas, which represent a very important ecosystem. So, it is of major importance to restore these areas. Several physical and chemical techniques are used, however they have many limitations. Phytoremediation (the use of plants in association with rhizosphere microorganisms) is presented as an alternative. In addition, bioaugmentation can be used to potentiate phytoremediation by plants.

The aims of this work were to evaluate the potential of the salt marsh plant *Phragmites australis* for phytoremediation of Cu contaminated sediment and to evaluate if this potential can be enhanced by bioaugmentation with autochthonous microorganisms resistant to Cu.

Plants were collected in an estuarine area and placed in vessels while an equal number of vessels were left unplanted. The sediment from both planted and unplanted vessels was contaminated with Cu and autochthonous microbial consortia resistant to Cu (prepared in the laboratory) were added to half of the vessels. Vessels were kept in greenhouses for two months, under tidal simulation. After this, metal concentrations on plant structures and sediments were determined, as well as metal speciation on sediments.

Regarding metal concentrations in plant structures, no statistically significant differences were observed between plants with and without bioaugmentation, except in the stems of the plants. In fact, the addition of the microbial consortia showed an increase of metal translocation.

Higher metal concentrations were observed in the sediments with plants independently of bioaugmentation. This demonstrates the influence of plants in the retention of metal in its rhizosphere, preventing metal lixiviation from sediments.

In relation to metal speciation in sediment, a tendency to increase the total bioavailable Cu was observed in the vessels with bioaugmentation, both planted and unplanted. This observation reinforces the benefits of using bioaugmentation to potentiate phytoremediation by plants.

In conclusion, this work demonstrated that *P. australis* is capable of phytoremediate Cu contaminated sediments and that autochthonous bioaugmentation can change metal bioavailability in the rhizosphere and can increase the phytoextraction potential of this plant.

ACKNOWLEDGMENTS: Research partially supported by the European Regional Development Fund (ERDF) through COMPETE - Operational Competitiveness Program and national funds through FCT, under PEst-C/MAR/LA0015/2011, REEQ/304/QUI/2005 and PHYTOBIO (PTDC/MAR/099140/2008).

Response of microorganisms from constructed wetlands to veterinary drugs

J. Fernandes^{1,2}, **A.C. Pereira**^{1,2}, **I. Lourinha**¹, **I. Reis**², **P. Carvalho**², **C.M.R. Almeida**²,
M.C.P. Basto^{1,2}, **A.P. Mucha**²

¹ Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal.

² CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal.

Pharmaceuticals, like veterinary drugs, are classified as emerging environmental pollutants that have been detected in various aqueous matrices such as wastewater, surface water and groundwater.

Veterinary drugs, or their active compounds, enter in the water system through effluent discharges derived from livestock industry. Among these, antibiotics are of main concern because when released in the environment they can cause not only serious toxic effects but can also promote antibiotic resistance.

Conventional methods of wastewater treatment are generally not capable to remove efficiently emerging pollutants present in wastewaters. Constructed wetlands are a potential alternative to remove these compounds. This technology is designed to mimic natural wetlands, being based on the interactions among soil/sediment, plant and microorganisms to remove the contaminants of the effluent.

The aim of this research was to study the response of the microbial community from constructed wetlands microcosm to two veterinary drugs (enrofloxacin (ENR) and tetracycline (TET)), present in livestock industry wastewater.

The microcosms were set up in plastic containers with layers of gravel, lava rock and sediment, and kept under greenhouse conditions. Each microcosm was wrapped in aluminium foil to avoid light penetration into the substrate and was left to acclimate for one week.

With the containers previously prepared were tested three treatments: one treatment only with wastewater (control) and two with wastewater doped with 100 µg L⁻¹ of ENR or TET. For each treatment were used planted (with *P. australis*) and unplanted microcosms.

The study was conducted over twelve weeks. Weekly, wastewater in each microcosm was drain out and new wastewater was added. At this time water samples were collected to quantify the levels of antibiotics (by HPLC) and sediment samples were characterized in terms of their microbial community structure (by ARISA).

The results demonstrated removal efficiencies of 98% for ENR and 94% for TET. Regarding the microbial community structure along the experiment no significant differences (ANOSIM, $p > 0.05$) were observed between control systems and those exposed to ENR or TET.

This study points to the importance of constructed wetlands for the removal of veterinary antibiotics from livestock wastewaters, showing promising results regarding its application for the remediation of the environmental impact of livestock industry.

Acknowledgments: Research partially supported by the European Regional Development Fund (ERDF) through COMPETE - Operational Competitiveness Program and national funds through FCT, under PEst-C/MAR/LA0015/2011 and SFRH/BD/44934/2008.

Toxicity testing of emerging contaminants under single and combined exposure using zebrafish and sea urchin embryo bioassays

T. Torres¹, R. Martins^{1,3}, I. Cunha¹ and M. M. Santos^{1,2}

¹ CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal.

² Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

³ Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, Porto, Portugal.

In the past decade, many emergent organic compounds, including Pharmaceuticals and Personal Care Products (PPCPs) have been detected in water at levels that can negatively impact aquatic ecosystems. The recent knowledge of their occurrence has raised concerns about human health effects and ecosystem risks. Although these compounds are frequently detected at concentrations that are not likely to induce adverse effects in humans and may be too low to cause acute effects in other organisms, there is still a serious lack of information about the effects in non-target species, particularly considering chronic exposure or effects resulting from interactions between them.

Pharmaceuticals and Personal Care Products enter the aquatic environment from different point and non-point sources and Wastewater treatments plants cannot ensure complete removal of many compounds, and therefore they may be present at significant concentrations in effluents. Hence, it is essential to understand the effects of these substances on aquatic organisms. Owing to the large number of new chemicals that must go through toxicity testing, short-term early-life-stages have been frequently used as an alternative to long-term exposures due to its high sensitivity and logistic advantages.

In addition to single exposures, if we aim at improving risk assessment of emerging contaminants it is important to understand the effects resulting from an actual environmental exposure and the mechanisms of toxic action involved, considering the diversity of contaminants present in mixture and their possible interactions.

Thus, the main aim of the present work was to assess the toxicity of five emergent chemicals, both under single and combined exposure: simvastatin, sertraline, triclocarban, propylparaben and 4-MBC during the embryonic development of zebrafish (*Danio rerio*) and the sea urchin (*Paracentrotus lividus*).

All selected compounds induced significant effects on the embryonic development of both test species after individual exposure. These effects were compound and concentration dependent. However our results show that sea urchin embryos were more sensitive than zebrafish embryos. Regarding the relative toxicity, simvastatin showed the highest toxicity in zebrafish bioassay, while triclocarban was the most toxic compound in sea urchin bioassay. Furthermore, significant effects were also reported in sea urchin and zebrafish embryos exposed to combinations of these compounds.

In conclusion, this study highlights the risk of these compounds to aquatic ecosystems. Hence, it is important to conduct more comprehensive studies on possible chemical interactions in the environment and the mechanisms involved in order to perform more reliable risk assessment and to implement guidelines for the protection of the aquatic environment.

Effects of macroalgae invasive species and climate-driven stressors on estuarine sediments nitrogen biogeochemistry.

Monteiro, MA, Rábano, O., García C., Salgado, P., Mucha, AP, Arenas, F., Magalhães, C.

Ecosystems Functioning and Biotechnology laboratory – ECOBIOTEC
Interdisciplinary Centre of Marine and Environmental Research (CIIMAR-UP), Rua dos Bragas, N°
289, 4050-123 Porto, Portugal

There is strong scientific consensus that coastal marine ecosystems are threatened by several factors such as pollution, habitat destruction, eutrophication, overfishing, and climate change (e.g. increasing sea temperatures, changing circulation patterns, increased storminess and ocean acidification) or by introduction of non-indigenous species. While it is generally accepted that the effects of climate change and invasive species may simultaneously impact coastal ecosystems, there are limited quantitative evidences about how it will modify the structure and function of coastal communities. In this study we studied the interactive effect of exotic macroalgae degradation and climate-driven stressors on estuarine sediments nitrogen biogeochemistry. A set of microcosm experiments with estuarine sediments were set up in order to manipulate temperature (15°C and 20°C) and the decomposition of exotic (*Gracilaria vermiculophylla* and *Sargassum muticum*) and native (*Ulva lactuca*, *Fucus vesiculosus*, *Zostera marina*, *Ascophyllum nodosum*) macroalgae. In these treatments, changes in inorganic nitrogen compounds were evaluated over time by measuring the net fluxes of NH_4^+ , NO_3^- and NO_2^- . While no differences were observed in the degradation of exotic and indigenous macroalgae in terms of the benthic nitrogen fluxes, the results obtained demonstrate that algae decomposition leads to an increase of NH_4^+ availability caused by a stimulation of the ammonification rates. In addition, our findings revealed a significant impact of the temperature on the processes involved on the nitrogen recycling, since high temperatures promoted a clear increase on the release of nitrogen compounds (NO_3^- and NO_2^-) to the water column. Our results suggested that high temperatures may cause a decrease on the efficiency of the nitrogen cycle processes that are responsible for the ablation of these compounds within natural impact systems, promoting a possible uncontrolled growth of algae and consequently a dysfunction of the estuarine ecosystems.



A4

**PSYCHOLOGY &
EDUCATION
SCIENCES II**

**III
PARALLEL
ORAL
SESSIONS**

School experiences and *queer* youth cultures: different ways of building (homo)sexual citizenships in schools

H. Santos¹, M. Ferreira¹ and S. M. da Silva¹

¹ Department of Educational Sciences, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

The homophobic violence, in their multidimensional shapes, poses some challenges at the level of how the different genders and/or sexualities, subjectivities and identifications of young students are conceptualized, resignified and effectively lived in public schools. It is supposed that the School can be a *democratic safe place* where senses for a plain *citizenship* are built. Although it is nowadays a contested concept, citizenship keeps a central core that refers to equality after a State, rights and duties, involving the sexuality field: a “sexual citizenship” that defies notions of what is public and what is private (Richardson, 2000) and it is implied in sex education law. In that sense, in my Master research, I was interested, through the description of their micro-experiences (*maneuvers*), in how non-heterosexual boys could build their *sexual citizenships* in a homophobic and heteronormative school *panorama*.

Methodologically our main method was *semi-structured interviews* (auscultation). We used strategically an *ethnographic approach* (observation) characterized by participant observation and writing of field notes (Silva, 2004) to obtain trust. The participants are seven young boys between 17-23 years old. Our theoretical perspective is located on Sociology of Youth and Education, Pos-structuralist Feminism and *queer* theory. There was some ethical cares as anonymity, confidentiality and informed consent.

Using the Max Weber’s idea of *ideal types* (Weber, 2005), we defined four types of sexual citizenships: a) *pretending citizenships* (Leandro and Manuel): working class young boys that reproduce gender norms against those gays/bisexuals that does not follow the *hegemonic masculinity* (“the crazy queers”). They not only escape from bullying but they also practice it; b) *citizenships of respectability* (Francisco and Rodrigo): young boys from middle classes who have strong connections to LGBT activism, have *politically correct* discourses about sex education, their engagement depends on their economic recourses and they reproduce *homonormative* violences; c) *subaltern (or precarious) citizenships* (Fábio): young boys who are the traditional victims of the homophobic physical aggressions and tell stories of educational disaffection and d) *reclaimed (subversive or queer) citizenships* (André): young boys passing their time in gay coffee shops and bars (*gay community*) who tell oppositional discourses against schools and its heteronormativity through their cultural expression.

References:

- [1] Richardson, D. (2000), *Constructing Sexual Citizenship. Theorizing Sexual Rights*, Critical Social Policy, 62, Vol. 20(1), 105-135.
- [2] Silva, S. M. da (2004), *Doubts and Intrigues in Ethnographic Research*, European Educational Research Journal, Vo. 3, Nº 3, 566 -582.
- [3] Weber, M. (2005), *The Protestant Ethic and the Spirit of Capitalism*, Routledge: New York.

Women as commercial sex clients: an exploratory study about female patrons of male striptease

C. Marques¹, A. Oliveira²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

² Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Striptease has been, in the last few decades, the target of several investigations which makes it and its actors one of the most studied of the sex industry.

However, according to the literature review carried out, most of these studies focus on female striptease; male striptease for women and the female costumers themselves being nearly ignored. Therefore the present investigation consists in an exploratory study about what motivates the female patrons of male striptease and the meanings of such experience.

In order to accomplish these objectives, a qualitative methodology was chosen, which resorted to semi structured interviews and field observation. Interviews were conducted with 27 female clients and 31 hours of participant observation in the context of male striptease were accomplished.

It was concluded that the clients of male striptease seem to not recognize nor value the sexual component related to the activity and perceive the experience as an opportunity to socialize with their female friends and enjoy a show they find entertaining, which elevates the homosocial component associated with this experience. It was also noted that in the context of male striptease there are some aspects that indicate the transgression of gender roles seen as women play a more active role. Furthermore, it was found that the participants regard male striptease as a professional occupation, but not a type of sex work, and they do not identify themselves as clients of commercial sex either. Overall the participants of this study demonstrated a tendency to distance the experience of the male striptease show from its sexual component which may possibly indicate an internalization of the main gender roles.

This study appears to be an important contribution to the research of this subject and a support to eradicate some of the stereotypes and prejudices associated with the female audience of male striptease.

Inhabit on the streets: a study about identity and survival of homeless people

I. Viegas¹, M. Pinto²

¹ Faculty of Psychology and Science Education, University of Porto, Portugal.

² Faculty of Psychology and Science Education, University of Porto, Portugal.

The phenomenon of homelessness is presented in contemporary societies as an unavoidable reality. However, in spite of its increase, there are still few studies in our country on this subject, in particular those that privilege a comprehensive and phenomenological approach.

This work aims to understand, from the perspective of its actors – homeless people -, (a) which survival strategies are used; (b) what is the relationship between them and the chronological, human and physical environment and (c) in which way does the homeless condition has an impact in identity and self-esteem.

Using a qualitative methodology approach, 70 hours of field observation were completed and 10 people living on the streets of Porto (ages between 26 and 61 years old) were interviewed.

The result refutes the vision of the homeless as a homogeneous group and questions some stereotypes, like the one that coats this condition solely with losses, self-destruction and suffering, and pointing to the possibility that this experience can contain a potential positive factor. Another of the main conclusions is the acknowledgement that dignity, personal realization, sense of self and positive self-esteem are a basic need such as those physical and material.

Body image in infertility

R. Antunes¹ and R. Barbosa²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal

² Center of Psychology, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Infertility is considered by WHO as a public health problem, characterized as the absence of a pregnancy after one year of regular intercourse without using contraception. Knowing that infertility and the treatments themselves may have important implications in physical terms, it is surprising that there are very few studies that explore how individuals experience their body, particularly at the level of body image.

This research paper seeks to establish itself as an interface between body image and infertility in women, supported by quantitative and qualitative analysis. Aims to understand the impact of infertility on women quality of life particularly in their body image.

70 women have participated in this study, with different types of diagnostic, aged between 26 and 44 years of age ($M = 33.83$, $SD = 3,76$). The instruments used were the *Body Image Scale* for the assessment of body dissatisfaction, the *FertiQol International* to assess the impact of infertility on quality of life and a sociodemographic questionnaire, clinical and psychosocial. Data were collected through an online survey.

The results suggest the existence of significant variables that have an impact on body dissatisfaction of these women, including the time of diagnosis, the perception of a good marital relationship, BMI, satisfaction with treatment and quality of life, all negatively associated. It is further than 32 % of the variance in body dissatisfaction is explained by the dimensions of quality of life and health care dimensions, and by BMI, although only the relational quality of life is a significant predictor. It was noticed also that the meanings attributed by these women with infertility are mostly negative and associated with the experience of loss and incompleteness. Likewise, in the living body of this experience, emphasizes the significance of motherhood, frustration and powerlessness in a body that doesn't work, emphasizing strong dissatisfaction by physical implications of treatments.

Finally, the way women experience their bodies and how they deal with the changes resulting from the treatment process will be an important dimension for attention by health professionals in monitoring women diagnosed with infertility.

Keywords: Body Image; Infertility; Quality of Life.

The influence of the incarceration experience in the development of social (re) integration perspectives in women serving sentences

I. Nascimento¹, J. Silva²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

² Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

The present study aims to explore, based on the point of view of women in a situation of incarceration, the extent and manner in which occurrences in their daily life, during the time they were deprived of freedom, become important in the way they project their life when returning to freedom. The participants in this study were ten women serving their sentence in the Prison of Santa Cruz do Bispo, with a convicted legal status. Through semi structured interviews with the inmates, the goal was to obtain relevant data concerning the periods of their lives (1) before (experiential background), (2) during (experiences in prison) and (3) after incarceration (prospects for the future when returning to freedom). The information collected, after being transcribed, was object of analysis and discussion by the inmates. The results of this study suggest that inmate women view their future return to freedom in a predominantly positive way, optimistically anticipating their life experiences post incarceration, with a special focus on their insertion in the social structure of employment and/or professional training, goal for which they rely on the support of family members (Hariston, 2002) [1]. Apparently, the experiences of these women during the period of incarceration, namely, the labor (Gomes, 2003) [2], academic and formative occupations (Provedoria da Justiça, 1996) [3] with which they had the opportunity to get involved, as well as visitations and flexibility measures (Santos et al., 2003) [4] of which they were object, significantly and favorably influence their expectations and future life projects for when they finish their sentence. Considering these results, it is, therefore, justified to create, in a prison environment, conditions for the experimentation and integration of the meaning and future implications of activities of (direct and indirect) exploration of self and the reality occurring there, which will allow the inmates to capitalize on their incarceration time, in the form of psychological gains susceptible of being invested (and reinforced) in a successful social reintegration process.

References:

- [1] Hariston, C. F. (2002). *Prisoners and families: Parenting issues during incarceration. A paper prepared for the from prison to home: the effect of incarceration and reentry on children, families and communities*. Washington DC: The Urban Institute.
- [2] Gomes, C. (2003). *Reinserção Social dos Reclusos – Um contributo para o debate sobre a reforma do sistema prisional*. Observatório Permanente da Justiça Portuguesa. Centro de Estudos Sociais. Faculdade de Economia. Universidade de Coimbra.
- [3] Provedoria da Justiça. (1996). *Relatório sobre o Sistema Prisional*. Lisboa.
- [4] Santos, B. S. et al. (2003). *A Reinserção social dos reclusos: um contributo para o debate sobre a reforma do sistema prisional*. Observatório permanente da justiça portuguesa. Centro de Estudos Judiciais. Faculdade de Economia. Universidade de Coimbra.

Between two worlds: Narratives about psychotherapists' Countertransference

F. Pereira¹, E. Costa²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

² Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

The psychotherapist as a person who has conflicts or unresolved issues throughout their developmental history that may influence their management of clinical practice, can be distinguished by their different developmental stages, characterized by different challenges and needs. Given the inevitability of countertransference and the scant research that exists on the narratives of psychotherapist before these processes, there is an urgent need to understand the clinical implications of its maintenance, affected by the perception of different degrees of mastery.

The main objective of this study was descriptive and comparative analysis of Apprentices and Senior psychotherapists on: maintenance of countertransference processes, based on the five factors present in the Countertransference Factors Inventory (Self-Insight, Self-Integration, Empathy, Anxiety Management and Conceptual Skills); the different manifestations of countertransference (Behavioral, Cognitive and Emotional) that may arise, and the effects perceived by psychotherapists on the maintenance of these processes in the course of the psychotherapeutic relationship. Starting from a semistructured interview guide, three psychotherapists in each group were interviewed, and through a qualitative methodological approach sought to analyze the narratives of the participants in relation to the maintenance of Countertransference.

The main results suggest the centrality of Self-Insight in reflective processes that in self-help, raising awareness of the different dynamics present in psychotherapy; Self-Integration was associated with confronting issues of identification with the client or with episodes of its developmental history; Empathy was analyzed as important in understanding the client's needs and timings, even though starting from intuitive processes; the Anxiety Management was associated with greater emotional detachment, and Conceptual Skills as being related to Empathy and Anxiety Management, encouraging a diagnosis' reframing. Behavioral manifestations have focused on overly affectionate or hostile behaviors; the cognitive ones were experienced in evoking invasive memories from clients who arouse the greatest concern; the emotional, in the case of Apprentices, were associated with fear, anguish and anxiety and in Senior's irritation.

Maintenance's effects of were perceived as beneficial to the psychotherapeutic relationship, strengthening the alliance between the two parties, but in management's absence of countertransference reactions the psychotherapeutic process was impaired.



A5

**BIOLOGICAL
SCIENCES III**

III
PARALLEL
ORAL
SESSIONS

Inhibitory effect of beer marinades on formation of heterocyclic amines (HAs) in chargrilled pork loin

P. Moreira¹, O. Viegas^{1,2}, and I.M.P.L.V.O. Ferreira²

¹ Faculdade de Ciências da Nutrição e Alimentação, Universidade do Porto, Portugal.

² REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências químicas, Faculdade de Farmácia, Universidade do Porto

Heterocyclic Amines (HAs) have been receiving heightened attention as a dietary risk factor for human cancer. The International Agency for Research on Cancer classified several HAs as possible or probable human carcinogens and recommended the reduction of exposure to these compounds [1].

HAs are particularly present in the crust of pan-fried, grilled or chargrilled meat and fish. They can be formed through pyrolysis reactions of amino-acids and sugars in the presence or absence of creatin(in)e, involving free radicals formation pathways.

The most promising mitigation strategies on HAs are the addition of antioxidant compounds. If applied *via* marinades has the advantage that the cooked food, are not over spiced and the cooked product do not acquire negative sensory properties [2,3].

In the present work, the effect of marinating meat with Pilsner beer, non-alcoholic Pilsner beer and Black beer (coded, respectively as PB, P0B and BB) on the formation of HAs in chargrilled pork was evaluated and compared with unmarinated meat. Antiradical activity of marinades (DPPH assay) was also assayed.

BB exhibited strongest scavenging activity (68.0 %), followed by the P0B (36.5 %) and the PB (29.5 %). A significant reduction on the radical-scavenging activity (compared to T0) after four hours of meat marinating was observed only for BB.

It was found in all marinated samples a significant reduction of the total amount of HAs. The marinade with BB proved to be the most effective in the inhibition of HAs formation, being the content found in these samples significantly lower than that found in the others.

A positive correlation was observed between antiradical activity and the reduction of total HAs formation.

Authors acknowledge financial support from Projects IJUP from University of Porto.

References:

[1] IARC (International Agency for Research on Cancer) (1993), *Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins*, in IARC Monogr. Eval. Carcinog. Risks Hum., IARC Publications, Lion, Vol. 56, pp. 163-242.

[2] Viegas, O., Amaro L.F., Ferreira, I.M.P.L.V. and Pinho, O. (2012), *Inhibitory effect of antioxidant-rich marinades on the formation of heterocyclic aromatic amines in pan-fried beef*, Journal of agricultural and food chemistry, 60 (24), 6235-40.

[3] Gibis, M., Weiss, J. (2010), *Inhibitory effect of marinades with hibiscus extract on formation of heterocyclic aromatic amines and sensory quality of fried beef patties*. Meat Science, 85, 735–42.

Quantification of sugars in Specialty malts by HPLC-IR

C. Almeida¹, O. Viegas^{1,2}, S. Meireles³, T. Brandão³ and I.M.P.L.V.O. Ferreira¹

¹REQUIMTE – Departamento de Ciências Químicas, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto

²Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto

³UNICER, Bebidas de Portugal SGPS, SA, Leça do Balio

Specialty malts, rarely used in levels above 5%, have an appreciable impact on the final beer characteristics as they contribute to the colour and flavour of several beer styles. These malts are produced by adapting the usual drying process of base malts. Specialty malts include kilned malts/high temperature, wheat malts, roasted malts, kilned and roasted malts [1,2].

The germination of grains during the malting leads to an increasing content of reducing sugars and amino acids when compared with barley composition. However during thermal treatment of green malt, Maillard reactions occur and influence the levels of carbohydrates and amino acids. This undoubtedly affects several aspects of the fermentation process [1,2].

The goal of the present work was the quantification and comparison of sugar profile of different specialty malts by high performance liquid chromatography using refractive index detector. Sugar extraction was performed with ethanol-water 50:50 (v/v) and addition of Carrez I and II for sample clean-up. Glucose, fructose, sucrose, maltose, maltotriose and maltotetraose were quantified in pilsen malt and specialty malts (caramel, chocolate and wheat) malts.

Sucrose was the most abundant sugar in pilsen malt, followed by glucose and maltose. Caramel malt presented the highest amounts of maltose and glucose, but also presented appreciable amounts of sucrose, maltotriose and maltotetraose. Chocolate malt was very poor in soluble sugars. Compared with Pilsen malt sugars, the lower content found in chocolate malt clearly reveals that sugars were used as precursors of thermally generated compounds. Wheat malt presented low amount of sugars, being sucrose the most abundant followed by maltose, maltotriose, and traces of other oligosaccharides that were not identified. The thermal conditions during the kilning and/or roasting clearly affect the sugar composition of final malt product.

This work was supported by Project PP-IJUP2012-UNICER- 13.

References:

[1] Yahya, H., Linforth, R.S. and Cook, D.J. (2014), *Flavour generation during commercial barley and malt roasting operations: a time course study*, Food Chemistry, 145, 378–387.

[2] Coghe, S., D'Hollander, H., Verachtert, H. and Delvaux, F.R. (2005), *Impact of Dark Specialty Malts on Extract Composition and Wort Fermentation*, 111(1), 51–60.

Characterization of Proteolysis in Specialty malts by chromatographic methods

C. Pereira¹, A. Glębowska¹, A. Melo¹, S. Cunha¹, S. Meireles², T. Brandão² and I.M.P.L.V.O. Ferreira¹

¹REQUIMTE - DEPARTMENT OF CHEMICAL SCIENCES, LABORATORY OF FOOD SCIENCE AND HYDROLOGY, Faculty of Pharmacy, University of Porto, Portugal.

²UNICER, Bebidas de Portugal SGPS, SA, Leça do Balio

Specialty malts are produced by adapting the usual drying process of pilsner malt and are important ingredients for the production of several beer styles. Specialty malts include kilned malts/high temperature, wheat malts, roasted malts, kilned and roasted malts. Several studies indicate that these malts provide colour and flavour to wort and beer, and also influence wort fermentation and foam stability [1], however, no studies were found concerning its proteolysis. Proteins are among the malt components that are essential for beer quality. Hordeins are the most abundant proteins, but albumins and glutelins (globulins) are also relevant proteins [2]. Extensive proteolysis occurs during malting. The aim of this work was the characterization of proteolysis of specialty malts by chromatographic methods, four types of malt were analysed: pilsner, caramel, chocolate and wheat malt.

Total protein content was evaluated by Kjeldahl method. Hordeins; albumins and other soluble proteins were characterized by size-exclusion high performance liquid chromatography (SE-HPLC) and the protein profiles of the different malts were compared. Free amino acids were quantified by GC-MS followed derivatization with MTBSTFA.

Total protein content of malts ranged between 9.34 – 13.42 %, it was in agreement with expected values. In SE-HPLC chromatograms peaks with TR 2-15min belongs to proteins with molecular weight 205-51 kDa, peaks from TR 15-26min corresponds to proteins and polypeptides >6.6 kDa and < 51 KDa. Peaks between TR 26 to 38 min are from polypeptides <6.6kDa. These limits were established by comparison with molecular weight standards separated by SE-HPLC. Similar qualitative protein profiles were observed in all samples for albumins, the more prevalent albumins stay between 51-6.6kDa (85-95% for Pilsener and caramel and 50-60% for chocolate and wheat). Chocolate and wheat malts contain higher percentages of high molecular weight proteins (205-51 kDa). Hordeins had different qualitative and quantitative profiles. In chocolate malt a high relative percentage of proteins (47.6%) presented molecular weight between 205-51kDa, followed by proteins and polypeptides with 51-6.6 kDa (4.3%). Caramel and Pilsner presented 99.9 to 69.5% of proteins in the interval of 51-6.6 kDa, whereas wheat malt presented more than 90% of hordeins between 205-51kDa.

Additionally, different profile of free amino acids was observed among speciality malts.

Acknowledgements: This work was supported by Project PP-IJUP2012-UNICER- 13.

References:

- [1] Yahya, H., Linforth, R.S. and Cook, D.J. (2014), *Flavour generation during commercial barley and malt roasting operations: a time course study*, Food Chemistry, 145, 378–387.
- [2] Silva, F., Nogueira, L.C. Gonçalves, C., Ferreira, A.A., Ferreira, I.M.P.L.V.O. and Teixeira N. (2008), *Electrophoretic and HPLC methods for comparative study of the protein fractions of malts, worts and beers produced from Scarlett and Prestige barley (Hordeum vulgare L.) varieties*, Analytical, Nutritional and Clinical Methods, 106 (2), 820–829.

The invasive behaviour of *Cylindrospermopsis raciborskii* in Portuguese Freshwater Systems

A. Matos^{1,2}, C. Moreira^{1,2}, V. Vasconcelos^{1,2} and A. Antunes^{1,2}

¹ CIMAR/CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal.

² Biology Department, Faculty of Sciences, University of Porto, Rua do Campo Alegre, Porto 4169-007, Portugal.

Cyanobacteria are photosynthetic organisms that inhabit a diverse range of ecological niches. These microorganisms have the capacity to produce toxins, also known as cyanotoxins, which are secondary metabolites. These metabolites represent a human health concern because they have been linked to cases of human sickness and death, as well as various incidents of animal mortality. *Cylindrospermopsis raciborskii* is a filamentous, heterocystous, freshwater cyanobacterium known to produce both cylindrospermopsin and the paralytic shellfish toxin. *C. raciborskii* blooms were associated with the death of cattle in regions of northern Australia and human poisoning incident in Palm Island, Australia, in 1979. Since these incidents the study of this cyanobacterium species has become relevant on a worldwide scale. Known to be a tropical species, *C. raciborskii* is currently an invader in temperate environments, including Portugal. Previous surveillance studies have revealed its presence in the South and Center part of the country lacking its presence in the North part.

In this study seven Portuguese freshwater systems located in the North and Center regions of Portugal were screened for the presence of this cyanobacterium species from May to September 2012 and from May to October 2013. The molecular approach was the followed procedure in study, which consisted initially in a DNA extraction followed by the Polymerase Chain Reaction (PCR) to detect the presence of *C. raciborskii* in the sampled regions. The positive results were sequenced in order to confirm the DNA amplification results.

The preliminary data showed that *C. raciborskii* is present in some of the sampled regions, particularly in the North region of Portugal becoming this the first study that reports the presence of this species in this region of our country. This work emphasizes the invasive behaviour of *C. raciborskii* in Portuguese freshwater systems and the need to continue the screening of this cyanobacterium in these regions.

Growth performance, antioxidant and immune responses in European seabass fed dietary probiotic supplementation under different rearing temperatures.

L. F. Pereira^{1,2}, J. Gonçalves^{1,2}, M. J. Peixoto², A. Domingues², S. B. Fonseca², R. Ozório^{1,2}.

¹ ICBAS - Abel Salazar Biomedical Sciences Institute, University of Porto, Portugal.

² CIIMAR - Interdisciplinary Centre of Marine and Environmental Research.

Aquaculture production has increased significantly over the past three decades, playing an important role as a major protein source, reaching 60 million tonnes in 2010, and traducing to nearly US\$119 billion revenue [1].

The increase in production was attained mostly by maximizing stock density. Such rearing strategy are prone to poor water quality, reduced growth rates and disease outbreaks. Antibiotics and chemical treatments are the common choice as disease control strategy. However, these choices are banned by restrictions in European countries [2], since they have proven to be hazardous to food fish and to the environment.

In pursuit for alternatives to the chemical approach, probiotics have been tested as a promising biological solution. Probiotics are defined as "live organisms which when administered in adequate doses confer benefits on the health of the host" (FAO, 2001), such as improvements in host nutritional retention [3], antagonistic properties to bacterial proliferation [4] and modulation of immune system responses [5]. Temperature plays a major role in the prophylactic use of probiotics, since fish are poikilothermic animals.

The current study was carried out in the Aquatic Engineering Laboratory, at ICBAS-UP (see Figure).

This trial was designed to evaluate the use of dietary probiotic supplementation in European seabass (*Dicentrarchus labrax*), one of the most consumed marine fish in Southern Europe. Fish were fed on a multi-species commercial probiotic (AquaStar® Growout), reared under 3 different temperatures (17 ° C, 20 ° C and 23 ° C) for 70 days. Several growth parameters and biomarkers of the immune and oxidative stress were determined.

Results of growth performance showed significantly better weight gain, food conversion rate (FCR) and daily growth index



between temperatures, but not between dietary treatments. Fish fed probiotic diets, showed improved results in immune system (lysozyme activity) and oxidative stress (catalase), when compared to unsupplemented (control) group.

References:

[1] FAO (2012), The state of world fisheries and aquaculture 2012.

[2] Regulation (EC) 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal feed. Official Journal of the European Union No 125 of 18/10/2003.

[3] Taoka, Yousuke, et al. "Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system." *Fisheries Science* 72.2 (2006): 310-321.

[4] Gaggia, Francesca, Paola Mattarelli, and Bruno Biavati. "Probiotics and prebiotics in animal feeding for safe food production." *International Journal of Food Microbiology* 141 (2010): S15-S28.

[5] Irianto, Agus, and Brian Austin. "Probiotics in aquaculture." *Journal of Fish Diseases* 25.11 (2002): 633-642.

The evolution of the opsin gene family in cave fish (*Astyanax mexicanus*)

M. Roque¹, R. Borges^{1,2}, and A. Antunes^{1,2}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² Interdisciplinary Centre for Environmental and Marine Investigation, Portugal

The cave fish (*Astyanax mexicanus*) has, as its name implies, clear adaptations to its cave lifestyle. During its development, there was an adaptation of the visual organs in the sense of a structural simplification. Its most striking features are the absence of eyes and pigmentation. However, the characterization at the genetic level of these adaptations lacks depth studies. Here we studied the molecular-based mechanism of photic systems adaptation in fishes, always bearing in mind, the peculiarities of the cave fish. In vertebrates, the integration of photic stimulus is mediated by a specific group of photoreceptor genes - the opsins. Opsins are the universal photoreceptor molecules of all visual systems in the animal kingdom [1]. The strategy used was the determination of the syntenic configuration of the opsin genes and the study of the presence/absence of the opsin genes in the cave fish genome. We also intend to determine which evolutionary and adaptive phenomena were responsible for these patterns. As gene locations are usually conserved among species, we can track the opsin's evolutionary history such as gene losses, gene duplications and function-altering amino acid substitutions, which can contribute to understanding the diversification of biological functionalities [1]. So far we studied the presence and absence of the opsin genes in fishes using the NCBI and Ensembl public databases. We conclude that fishes present opsin genes representative of all the opsin gene family. The opsin gene family (Fig. 1) can be roughly subdivided into 4 major groups: ciliary opsins (visuals, non-visuals and encephalopsins), rhabdomeric opsins (melanopsins), photoisomerases and neuropsins, which will be studied in greater detail to establish their syntenic configurations. In some families, fishes present several duplicated copies. In the future, we intend to implement synteny analyses to determine the opsin genes that are under or over represented in fishes when compared to other vertebrates groups. We aim to understand the biological consequences of gene number variations and, ultimately, their adaptive significance.

References:

[1] Y. Shichida and T. Matsuyama (2009), Review, Evolution of opsins and phototransduction, The Royal Society B. 369, pp. 2881-2895.



A6

GEOGRAPHY & HISTORY

III
PARALLEL
ORAL
SESSIONS

Life and death in the Benedictine Community of the Monastery of the Salvador of Paço de Sousa (1625-1826)

M. Vieira¹

¹Faculty of Letters, University of Porto, Portugal.

The congregation of the Black Monks of Saint Benedict of the Reign of Portugal, created in 1569, it had 22 monasteries. This study concentrated on the Monastery of the Salvador of Paço de Sousa, the monastery that housed the congregation of the 13th May, 1579. The study of the Benedictine community from the beginning of the 17th C until the beginning of the 19th C is an attempt to define the sociological profile of the members of this community and their itineraries. This information is projected in the interpretation of the patrimony with means and technological resources placed at the service of the Cultural Tourism initiatives.

The information in the *Livro de Óbitos do Mosteiro de Paço de Sousa*, in the *Fundo Monástico Conventual – os Estados do Mosteiro de Paço de Sousa* and in the *Memórias do Mosteiro de Paço de Sousa* established a universe and biographical profile of the monks that died in the Monastery of the Salvador, between 1625-1834.

The study of this community was divided into the following segments: 1) biographical data of the monks (nationality, filiation, date of death); 2) mobility in the monastic spaces of the congregation, from the time they took the habit until their death; 3) intellectual profile (levels of academic education); 4) social profile (measured by their family relations); 5) positions held and the relation of social provenience and academic education. On a methodological level, a scientific-historical investigation was conducted and a bibliographical revision on the Benedictine Congregation of Portugal from the reformation of the 16th C until its extinction in the 19th C, overall on the Congregation of the Black Monks of the Reign of Portugal in the Monastery of the Salvador of Paço de Sousa, especially in their monastic community. A data base of prosopographic orientation was developed through the elaboration of biographic records as well as a technological interface through the application of multimedia research software to access established biographies.

The historical investigation intends on demarcated, extensive bibliography and primary sources to develop an interpretative project to fulfill the objectives of patrimonial mediation and cultural tourism, based on the systematization of data to be introduced in normalized areas of a historical data base, a risky process with which the historical scholar know-how is compatible with its disclosure. Thus, the possibility of linking the historical investigation to the new technologies with multimedia means is believed to be proven, and leveraging mediation projects strongly grounded in research routes. It is necessary a multidisciplinary training in the completion of this type of project: there is the necessity of searching for technical support and orientation in the technological area from statistics and cartography. It is essential to form a team that includes technological technicians, historians, tourism technicians and involve public and private entities linked to the patrimony and to tourism so as to guarantee economic viability and the sustainability of the project.

Municipalities and Military Orders in the Middle Ages. Relations of dependence and confrontation in XII-XIV centuries.

Emanuel Cardoso Pereira¹

¹ Master in Medieval History. Department of History and Political and International Studies (DHEPI), Faculty of Arts, University of Porto, Portugal.

This communication aims to exhibit the results of the dissertation in medieval history presented at FLUP in November 2013. The work consisted on the intersection of military, municipal and on the Military Orders historiography in the twelfth to fourteenth centuries, and revisit the municipal charters (called “cartas de foral”), as well as royal sentences to understand the conflicts between the municipalities and the Military Orders.

We conclude that the Military Orders started a fruitful strategy of settlement, through the adoption of the royal legal codes, in their municipal charters, and trough the establishment of a vast commandaries networks to sustain their castles and fortifications, operating, thus, as an original model of settlement sustained through these tree mechanisms, which allowed them prepare more successful attacks on Muslim lands and a better consolidation in their territories.

On these territories, the Military Orders could capture settlers to their lands, allowing them to its social mobility and, perhaps, even integrate to the institutional structures of the Military Orders. In these territories they have also stimulated the economic exploitation and transaction of goods, collecting them important taxes, through the agricultural production, maritime activities and livestock, and, also collect taxes from the tolls of goods which circulate in the kingdom. Thus, these incomes contributed to sustain the structures of these institutions, as well as for the management of their fortifications and garrisons to fight against Muslims.

As guarantee of the Military Orders feudal rights in these areas, especially in the municipal areas, the Military Orders were represented by their officials, including, bailiffs and commanders who participate in the collect of their taxes and court issues, safeguarding the interests of the Orders. However, the Military Orders recognize the autonomy of the municipal council, thus, they could choose their magistrates, especially, judges, mayors and other officials (according to each council), safeguarding the rights of the residents of municipalities.

Finally, after the Christian Reconquest in Portugal, the strategic deployment of the Military Orders in the territory based on the three pillars of settlement that we observed, led to an environment of tension between the Military Orders and their municipalities, as well as between the Military Orders and royal municipalities, those who coexisted with the commandaries of the Military Orders. The nature of these processes was very diverse. The nature of the issues in dispute could be economic, administrative or jurisdictional nature.

The works of João Antunes in Northern of Portugal

Rúben Ribeiro¹

¹ Master in Art History Portuguese, Faculty of Letters, University of Porto, Portugal.

The text that presents in this space is the result of research carried during the Masters in Art History Portuguese [1]. The chronological period is between the second half of the seventeenth century and the first decade of the century XVIII, and the physical space allowed to better understand the religious architecture in northern Portugal.

The method of project development, this fell in a first reading on the relationship between political and economic system with the cultural environment during the time period, to then enter a search architecture itself. The following steps result from natural analysis, visualization, and a photographic record of their spaces, ending the observation manuscripts (previously published and unpublished) and the writing of the dissertation.

Retreating over a dramatic period in Portugal, the war with Spain between 1640 and 1668, we know that all resources were directed to build a defensive architecture and with little influence on the development of new architectural styles both in religious and civil architecture. After this cycle, it would be during the reign of king Pedro II, who would know a new phase of economic growth, largely due to the arrival of gold and diamonds from Brazil. This king, maintained an organized royal house, and an alignment of the more privileged classes, such as the nobility and clergy. The seventeenth century presents an architecture organized on ways and also includes a rich interior in various materials. The compositional lines are visually simple, but inside, this leads us to devotion towards the carving, painting and sculptures.

We know that the church of Santa Engrácia (1682), draft of João Antunes, will present a new aesthetic, since it integrates a circular planimetry, and the use of noble materials such as marble collaborating with the lighting system. However, the aim of our works is the production of João Antunes in the north, and thus the works studied were the church Senhor Bom Jesus da Cruz (Barcelos), which keeps a close proximity to the plant as to the church of Santa Engrácia, the sacristy of the Cathedral of Braga, with a rectangular plant, and integrating a pure and clear granite, the baptistery of the Cathedral of Porto, through the floor of black, red and white stones, as well as the initials projects for the church of Nossa Senhora da Vitória (Porto). Another issue that is being investigated on my part refers to the inspection of the architect to dock in Porto, since it presented difficulties to the navigability of the Douro river merchants.

The results of this dissertation support previously data published by Joaquim Oliveira Caetano and Nuno Vassallo e Silva, in «*Breves notas para o estudo do arquitecto João Antunes*», 1993. However, new documents were analyzed and published in the dissertation, especially involving the project for the presbytery and tribune of the church Nossa Senhora da Vitória, in Porto.

References:

[1] Ribeiro, Rúben R. G. (2013), *A atividade do arquiteto João Antunes no norte de Portugal*, Porto.

Auto dealers in Presidente Prudente, Brazil

F. R. dos Santos¹, M.E.B. Sposito (supervisory)²

¹ Department of Geography, Faculty of Sciences and Technology, Universidade Estadual Paulista (UNESP), Brazil.

² Department of Geography, Faculty of Sciences and Technology, Universidade Estadual Paulista (UNESP), Brazil.

Introduction

The research in progress aims to identify the factors that contribute to the process of dealers concentration in two major commercial and services axis in the city of Presidente Prudente (Brazil): the extending of Manuel Goulart Avenue and Joaquim Constantino Avenue. In this research considers that dynamic is related of the role of population growth and territorial in the south of the city, associated with deploying multiple closed allotments, revealing the multiple dimensions of the recent trend of socio-spatial segregation of urban space, according Villaça (2001)

Methodological procedures

The research is being conducted from reading of texts related to the automotive industry and the production of urban space, with emphasis on topics like concentration and functional specialization and/or socioeconomic, emphasizing the emergence and strengthening of new commercial and services areas, reconstituting the urban center at multiple scales.

In a second moment was held interviews with the managers of dealers and commercial establishments linked to the automotive sector, aiming support of refute the hypotheses regarding the concentration of dealers in the urban space.

Analyses and Discussion of the results

All the dealers in the city of Presidente Prudente are located in just two avenues: Joaquim Constantino and Manoel Goulart extending. The possible reasons for this concentration in urban space is given by the distance from the main center, relative proximity to residential areas of high and medium occupational standard, the large flow of vehicles and people and the proximity of the exits of the city.

Conclusion

The study of spatial logics of automotive company's offers elements for understanding the process of urban restructuring. At the city scale is occurring big changes, because before there was a trend of dealers concentration in the commercial and services axes which unfolded from the main center, has most recently deploying them in more distant axes. The principal axe is marginal to the highway, meeting consumer demands of more affluent groups, who settled in the southern residential area of the city of Presidente Prudente.

References

Villaça, Flavio. (2001), The intra-urban space in Brazil. Sao Paulo: Studio Nobel.

Research and knowledge networks in the *European Network for Housing Research*

Joana Pinheiro¹

¹ MRCOT, Department of Geography, Faculty of Arts, University of Porto

This research aims at analyzing the broad lines of inquiry that dominate in Europe in the field of housing.

The information source was selected one of the major research networks Iropaia in housing - European Network for Housing Research.

The aim is then to answer the following questions for research in housing:

What position assume the different continents in the system of international research? In which institutions are centralizing research? Institutions are centralized are or not European? What are the themes that dominate in European research? Authors who dominate the European network analyzed? The authors are dominant Europeans?

To answer all these questions were compiled in a database, all communications relating to the ENHR years above referred. This database contains all communications organized around the following attributes year, title, subject, author (s), institution (s) of author (s) and country (ies) of the author (s).

In terms of relational attributes, the fact that each communication in each annual conference to be classified into different thematic groups, allows an analysis focused on thematic affiliations.

In this sense, in terms of knowledge networks for research in the field of housing in Conferences of the European Network for Housing Research analyzed:

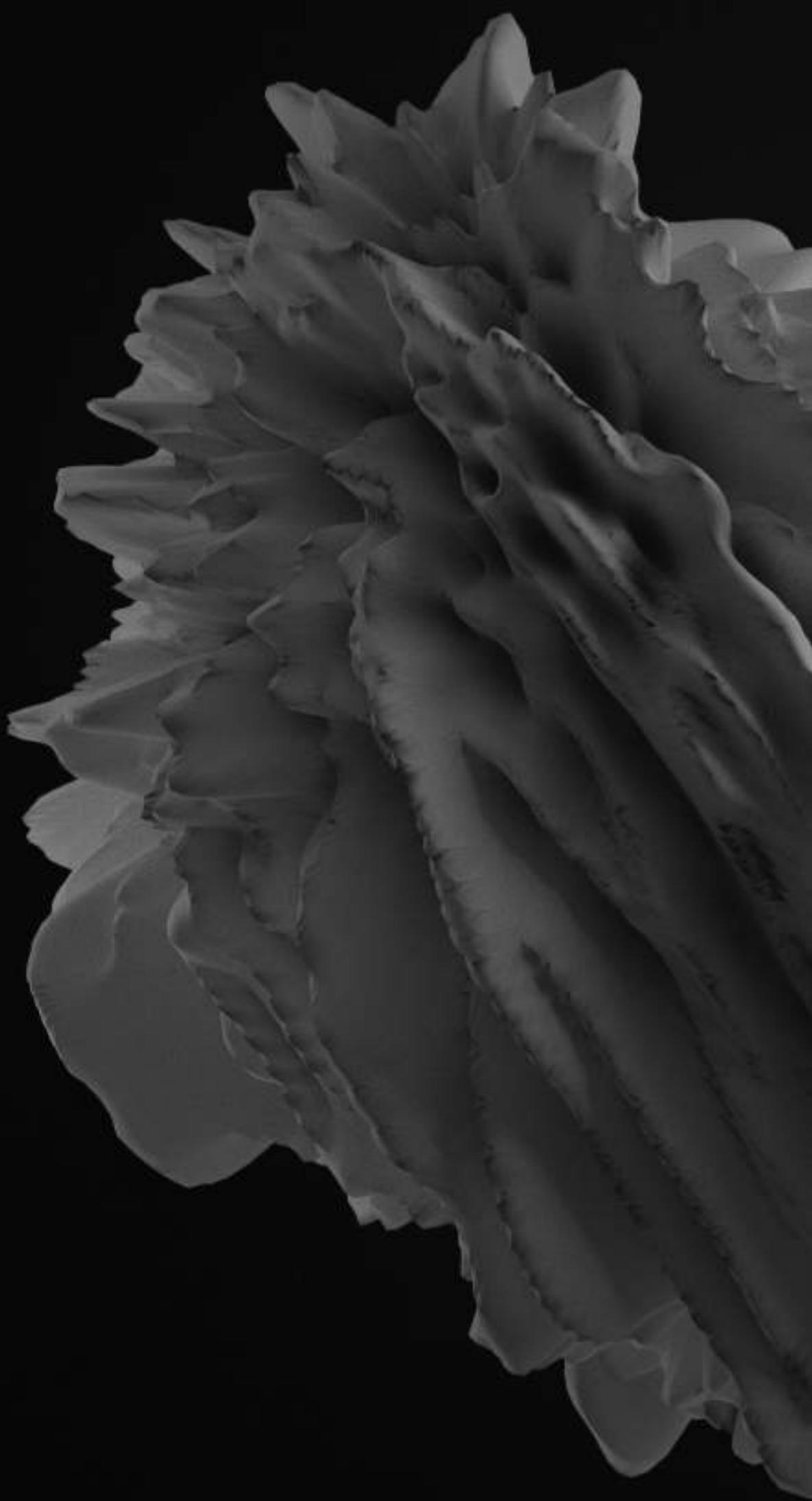
The centers and the geographic proximity by identifying the countries and continents of the central and peripheral research;

The centers and the nearby organization by identifying the central and peripheral institutions;

The centrality and cognitive nearby by identifying the authors integrated into subthemes of ENHR Conference, evaluating the authors strongly specialized and authors that connect different research topics.

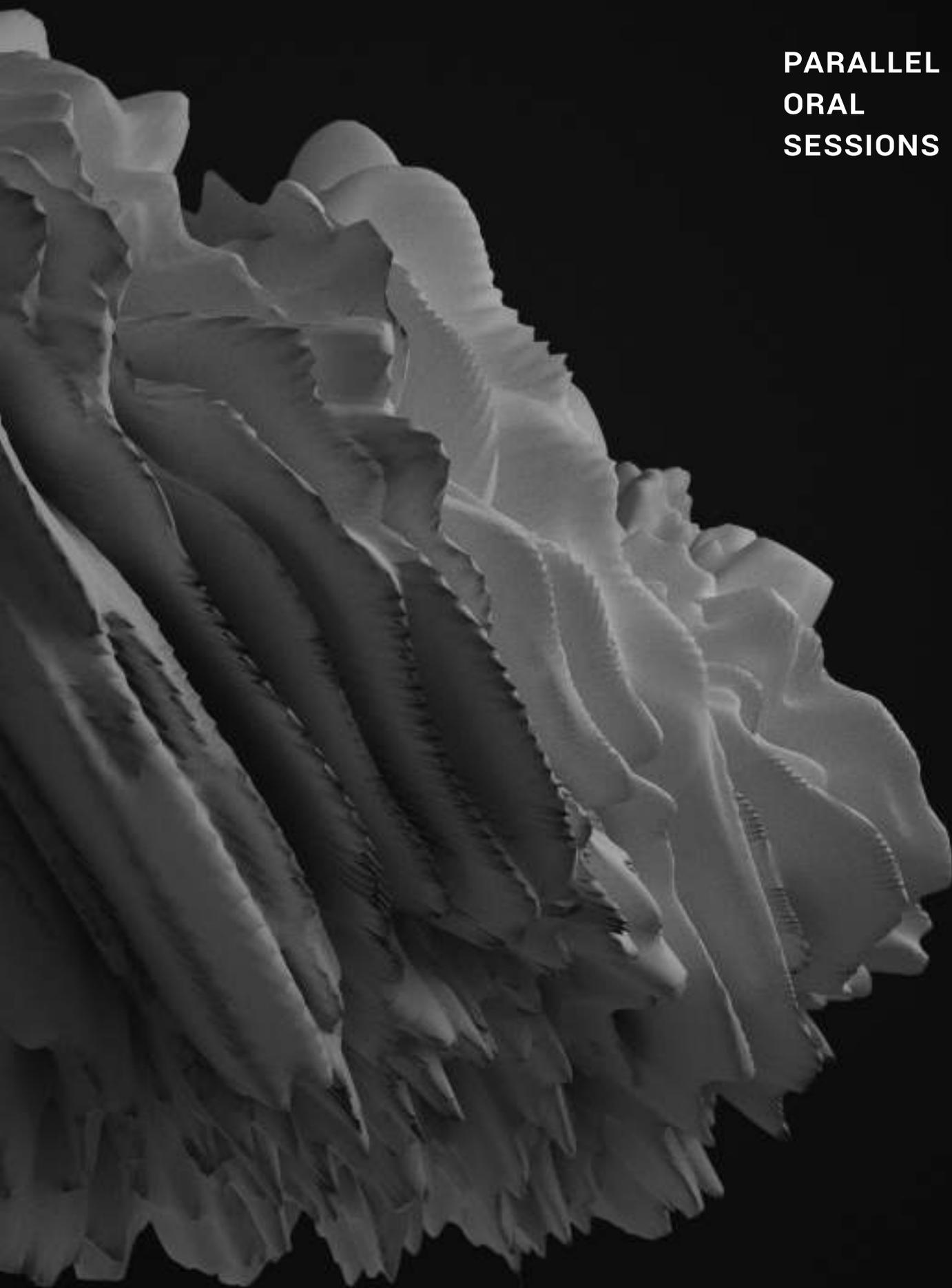
Network analysis was supported in NodeXL program, a support tool to the study of social networks.

Keywords: Housing, knowledge networks, Europe



IV

PARALLEL
ORAL
SESSIONS



A1

BIOMEDICINE IV

IV
PARALLEL
ORAL
SESSIONS



Sustained release dosage forms: a thiamine hydrochloride tablet formulation based ethylcellulose

A.S. Brandão, M. Constante, M. Costa, M. Brilhante, M. Estanqueiro, J.M. Sousa Lobo

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

A sustained release dosage form is a pharmaceutical form designed to modulate the release of an active ingredient [1]. Matrix systems still appear as one of the most employed for this purpose [2]. In this work a pharmaceutical formulation was rationalized and developed, in order to achieve a controlled and prolonged release system using thiamine hydrochloride, known as vitamin B₁, as a model drug. This active ingredient is highly unstable, being easily degraded by alkaline pH, heat, oxygen, light and radiation. It is required, therefore, new techniques for enhancing stability and bioavailability of this unstable drug.

An ethylcellulose matrix was chosen and the group started working on a functional and compatible formulation that would present the requirements desired. Ethyl cellulose is an inert organic polymer which, as the name suggests, is an ethyl derivative of cellulose, meaning the hydroxyl groups repeating on the several glucose units are converted into ethyl ether groups. The final formulation developed by the group was composed of corn starch (50%, w/w), stearic acid (1.5%, w/w), talc (3.5%, w/w), microcrystalline cellulose (23%, w/w), Ethocel™ standard 10 premium ethylcellulose polymer (20%, w/w) and thiamine hydrochloride (2%, w/w) [3].

The tablets have been prepared by direct compression. With the aim to evaluate the obtained tablets we proceeded to the accomplishment of some quality control assays including weight variation, hardness, friability, and dissolution tests. The tests were done in agreement with Portuguese Pharmacopoeia 9 [4].

The obtained results were in accordance with the Pharmacopoeia specifications, namely, with regard to uniformity of weight and friability. The tablets presented an average hardness acceptable for the proposed used. Regarding the dissolution assay, a maximum cumulative drug released were about 50%, after 90 minutes.

To sum up, the formulation had very positive results. Its effectiveness was clearly shown in all aspects tested to attain tablets with prolonged and controlled release, assuring itself as one possibility to orally deliver substances such as thiamine hydrochloride.

References:

- [1] Pezzini, B.R., Silva, M.A.S., Ferraz, H.G. (2007). Formas farmacêuticas sólidas orais de libertação prolongada: sistemas monolíticos e multiparticulados. *Revista Brasileira de Ciências Farmacêuticas*, 43 (4), 491-502.
- [2] Sánchez-Lafuente, C., Faucci, M.T., Fernández-Arévalo, M., Álvarez-Fuentes, J., Rabasco, A.M. and Mura, P. (2002), Development of sustained release matrix tablets of didanosine containing methacrylic and ethylcellulose polymers. *International Journal of Pharmaceutics*, 234, 213-221.
- [3] Rowe,R.C., Sheskey, P.J. and Owen, S.C. (2009). *Handbook of Pharmaceutical Excipients*, 6th Ed Pharmaceutical Press and American Pharmacists Association, Washington.
- [4] Farmacopeia Portuguesa 9: Ministério da Saúde, INFARMED, I.P., 2009.

New 7 α -allylandrostanes as Aromatase Inhibitors: Biological Effects in Hormone-dependent and Hormone-resistant Breast Cancer Cells

J. Maurício^{1,2,3}, C. Amaral^{1,4}, C. Varela⁵, E. Tavares da Silva⁵, F. Roleira⁵, S. Costa⁵, G. Correia-da-Silva^{1,4} and N. Teixeira^{1,4}

¹Laboratory of Biochemistry, Department of Biological Sciences, Faculty of Pharmacy and

²Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

³IPO Porto – Portuguese Oncology Institute of Porto FG, EPE, Portugal

⁴Institute for Molecular and Cell Biology (IBMC), University of Porto, Portugal.

⁵CEF, Center for Pharmaceutical Studies, Pharmaceutical Chemistry Group, Faculty of Pharmacy, University of Coimbra, Portugal.

The most common subtype of breast cancer is the hormone-dependent cancer (ER⁺). Targeted therapies include selective estrogen receptor modulators, down-regulators and aromatase inhibitors (AIs). AIs inhibit the aromatase enzyme, preventing the conversion of androgens to estrogens. However, there are drawbacks concerning their use: the development of acquired resistance and the occurrence of bone loss [1,2].

The focus of our group is to identify new potent steroidal AIs that can overcome endocrine resistance and bone loss. Recently, we described the steroidal compounds (7 α -allylandrostanes) as potent AIs in placental microsomes: 7 α -allylandrost-4-ene-3,17-dione (**6**), 7 α -allylandrost-4-en-17-one (**9**), 7 α -allyl-3-oxoandrosta-1,4-dien-17 β -ol (**10**) and 7 α -allylandrosta-1,4-diene-3,17-dione (**12**) [3]. In this work, the anti-aromatase activity and the biological effects were studied in an ER⁺ aromatase-overexpressing human breast cancer cell line (MCF-7aro). The anti-proliferative effects were also explored in an AI-resistant cancer cell line (LTEDaro). Moreover, in order to understand the involvement of autophagy in AI-acquired resistance, the effects of an autophagic inhibitor (3-methyladenine, 3-MA) was also studied. Our results demonstrate that AIs **9**, **10** and **12** are able to inhibit aromatase and induce a decrease in MCF-7aro cell viability in a dose- and time-dependent manner, which was accompanied by the presence of chromatin condensation and membrane blebbing. The results also showed that the new AIs induce a decrease in LTEDaro cells viability suggesting that they can revert AIs-acquired resistance. In addition, in both cell lines a reduction in cell viability was also observed when cells were treated with the new AIs plus 3-MA. The study of these new AIs is important to provide new insights for the finding of new AIs more potent and with fewer side effects than the AIs currently used in clinic.

Acknowledgements: FEDER Funds through COMPETE and FCT project FCOMP-01-0124-FEDER-020970 (PTDC/QUI-BIQ/120319/2010) and C. Amaral and C. Varela PhD grants (SFRH/BD/48190/2008; SFRH/BD/44872/2008); Dr. Shiuan Chen (Beckman Research Institute, USA) for cell lines.

References:

- [1] Eroles, P., et al. (2012). *Molecular biology in breast cancer: intrinsic subtypes and signaling pathways*. Cancer Treat Rev 38(6): 698-707.
- [2] Lonning, P. E. and H. P. Eikesdal (2013). *Aromatase inhibition 2013: clinical state of the art and questions that remain to be solved*. Endocr Relat Cancer 20(4): R183-201.
- [3] Varela, C. L., et al. (2013). *Design, synthesis and biochemical studies of new 7 α -allylandrostanes as aromatase inhibitors*. Steroids 78(7): 662-669.

Hypoxia-dependent regulation of galectin-3 in mammary tumours

C. Ribeiro^{1,2}, **JT. de Oliveira**^{1,2,3}, **C.Gomes**¹, **C. Paulo**^{1,2} and **F.Gartner**^{1,2}

¹ Institute of Molecular Pathology and Immunology at the University of Porto, Portugal

² Department of Pathology and Molecular Immunology, ICBAS, Portugal.

³ Faculty of Veterinary Medicine of Lusófona University, Lisbon, Portugal

Galectin-3 is a well-studied member of beta-galactoside-binding animal lectins family implicated in multiple cellular functions [1]. The expression of galectin-3 has been frequently associated with several cancers [2, 3]. This lectin has multiple different roles in tumorigenesis such as cell-cell and cell-ECM adhesion, angiogenesis promotion, cell proliferation and apoptosis resistance [1, 4-6]. However, the mechanisms that regulate galectin-3 in the tumour microenvironment are not clear. In the tumour microenvironment different cellular stressful conditions are found, such as hypoxia, oxidative stress and pH alterations [7]. Hypoxia is known to lead to genetic adaptive changes through the transcriptional induction of a series of genes via the Hypoxia Inducible Factor (HIF) [8]. In order to evaluate the putative hypoxia regulation of galectin-3, we evaluated the galectin-3 and GLUT-1 (hypoxic marker) expression in a series of spontaneous mammary tumours and observed a co-expression of both in hypoxic areas. These results are in agreement with in vitro studies that show an increase in cytoplasmic galectin-3 under hypoxic conditions. Upon 24 hours of hypoxia exposure, galectin-3 is secreted to the extracellular space. Both hypoxia and oxidative stress induce an increase in galectin-3 mRNA levels. Simultaneous, treatment with hypoxia and reactive oxygen species (ROS) in the CMT-U27 cell line seems to result in a synergic effect on galectin-3 mRNA expression. Moreover, when catalase is added to the culture medium under hypoxic conditions galectin-3 mRNA levels decreased.

In conclusion, we showed that galectin-3 mRNA is very sensitive to small changes in the microenvironment, namely in hypoxic environment where galectin-3 mRNA has significantly increased, probably in a ROS-dependent manner. After long period of exposure to low oxygen levels, galectin-3 is secreted into the extracellular space and possibly plays important roles in tumor aggressiveness both in invasion and angiogenesis. Therefore hypoxic cells which express galectin-3 may be associated to tumor aggressiveness.

References:

- [1] Zou, J., et al. (2005), *Peptides specific to the galectin-3 carbohydrate recognition domain inhibit metastasis-associated cancer cell adhesion.*, *Carcinogenesis*, **26**(2), p. 309-18.
- [2] Ahmed, H., et al. (2009), *Evidence of heavy methylation in the galectin 3 promoter in early stages of prostate adenocarcinoma: development and validation of a methylated marker for early diagnosis of prostate cancer.* *Transl Oncol*, **2**(3), p. 146-56.
- [3] Ahmed, S., et al. (2007), *Adenoma of anogenital mammary-like glands.* *J Am Acad Dermatol*, **57**(5), p. 896-8.
- [4] Nakahara, S., et al. (2006), *Characterization of the nuclear import pathways of galectin-3.* *Cancer Res*, **66**(20), p. 9995-10006.
- [5] Yoshii, C., et al. (2002), *Welder's pneumoconiosis: diagnostic usefulness of high-resolution computed tomography and ferritin determinations in bronchoalveolar lavage fluid.* *Intern Med*, **41**(12), p. 1111-7.
- [6] Shekhar, M.P., et al. (2004), *Alterations in galectin-3 expression and distribution correlate with breast cancer progression: functional analysis of galectin-3 in breast epithelial-endothelial interactions.* *Am J Pathol*, **165**(6), p. 1931-41.
- [7] Cairns, R.A., I.S. Harris, and T.W. Mak, (2011) *Regulation of cancer cell metabolism.* *Nat Rev Cancer*, **11**(2), p. 85-95.
- [8] Abbondati, E., et al. (2013), *An Immunohistochemical Study of the Expression of the Hypoxia Markers Glut-1 and Ca-IX in Canine Sarcomas.* *Vet Pathol*.

Aged female diet anti-oxidant supplementation: effect on placental bed and reproductive outcome

AI Soares^{1,2}, JP Castro^{1,2}, L Matos^{1,2}, H Almeida^{1,2} and E Silva^{1,2}

¹ Department of Experimental Biology, Faculty of Medicine, University of Porto, Portugal. ²Ageing and Stress Group, IBMC - Instituto de Biologia Celular e Molecular, Portugal.

In placental bed, where placenta and uterus interact, ROS can react with biomolecules and alter cell function. Loss of redox homeostasis may lead to abnormal placental development and reproductive capacity loss. As mammal ageing is a risk factor the establishment of a cellular redox imbalance, it was hypothesized supplementing antioxidants to the diet of aged female mice before and during pregnancy might improve the reproductive ability.

Uterine samples of mice aged 11–15 weeks or 43–45 weeks were obtained. Protein carbonylation was determined in uterine epithelium by fluorescent immunohistochemistry techniques. Reproductive outcome was evaluated by counting the number of viable foetus and re-absorption sites. SOD activity and expression were determined in placental bed by spectrophotometry and western blotting, respectively. Females aged 43–45 weeks were treated, prior to and during pregnancy, with a SOD mimetic (TEMPOL, 1 mM) or a NOX inhibitor (apocynin, 5 mM) and reproductive outcome was re-evaluated. Results are presented as mean \pm standard error mean.

The non-pregnant uterus of the aged female mice was hypertrophied and contained cysts, when compared to young mice. Moreover, protein carbonylation was increased. Pregnant aged females showed an age-related decreased in the number of viable foetuses [young females 6.0 ± 1.2 (n=9) and aged females 2.0 ± 0.4 (n=6), $P=0.03$ (Student's t test)]. This decrease was accompanied by early and late foetal loss and re-absorption. Total SOD activity was significantly increased in placental bed of reproductively aged female mice [1.6 ± 0.2 fold increase $P=0.04$ (Student's t test)]. This increase in SOD activity was accompanied by an increase in the expression of SODI and SODII. In the same tissue, no age-related changes were observed in total protein carbonylation. Anti-oxidant treatment increased the number of foetuses from aged female mice [control 2.0 ± 0.4 (n=9), TEMPOL 3.5 ± 0.9 (n=4) and apocynin 4.2 ± 0.6 (n=5), $P=0.05$ (ANOVA)]. Moreover, treatment with a SOD mimetic increased significantly foetuses weight [control 1.30 ± 0.06 (n=9) and TEMPOL 1.52 ± 0.01 (n=4), $P=0.02$ (Student's t test)].

The age-related alterations observed in uterine tissue indicate a local imbalance in redox homeostasis that is accompanied by impaired reproduction. Anti-oxidant treatment ameliorated aged female reproduction outcome and point to an important role of SOD in foetuses weight.

A new selective activator of PKC δ discovered using a yeast-based assay

C. Bessa¹, N. Harilal¹, C. Pereira², C. Maciel¹, J. Machado¹, J. Gonçalves³, P. Rijo^{4,5}, M. F. Simões⁴ and L. Saraiva¹

^{1,3}REQUIMTE, ¹Laboratory of Microbiology, Department of Biological Sciences, ³Laboratory of Pharmacology, Department of Medicine Biology, Faculty of Pharmacy, University of Porto, Portugal

²Institute of Molecular Biology (IBMC), University of Porto, Portugal

⁴iMed. UL, Faculty of Pharmacy, University of Lisbon, Portugal

⁵CBIOS, University Lusófona, Lisbon, Portugal.

The protein kinase C (PKC) is a family of serine/threonine kinases with at least ten isoforms divided into three major subclasses: classical (cPKC α , β I, β II and γ), novel (nPKC δ , ϵ , η and θ) and atypical (aPKC ζ and λ ν). This family regulates a wide range of cellular processes, such as cell proliferation and death. Particularly, PKC δ has emerged as a crucial therapeutic target in several apoptotic-diseases, including cancer [1]. Therefore, the discovery of selective modulators of individual PKC isoforms has been the focus of an intense research. However, this task has been hampered by the difficulty to carry out an independent analysis of individual PKC isoforms in mammalian cells. To circumvent this limitation, *Saccharomyces cerevisiae* cells expressing individual mammalian PKC isoforms have been used to search for selective modulators of individual PKCs. In this yeast-based assay, PKC activators cause an inhibition of the yeast cell growth, without affecting the growth of control yeast (transformed with the empty vector).

In this work, the yeast-based assay was used to study the modulatory activity of four semisynthetic diterpenic compounds (C1-C4), from the Simões' group (iMed. UL), on mammalian PKC α , β I, δ , ϵ and ζ , as described [2]. The results showed that compound C1 had no effect on PKCs, whereas compounds C2 and C3 induced a significant growth inhibition in all PKCs tested, without affecting the growth of control yeast. Notably, compound C4 significantly inhibited the growth of yeast expressing PKC δ , without interfering with the growth of yeast expressing PKC α , β I, ϵ or ζ , and control yeast. In addition, the C4-induced growth inhibition of yeast expressing PKC δ was associated with an increase of DNA fragmentation (apoptosis), without loss of plasma membrane integrity (necrosis). The selective activation of PKC δ by C4 was further confirmed using an *in vitro* PKC assay. In conclusion, herein two putative non-selective activators of PKC isoforms (C2 and C3) and a PKC δ -selective activator (C4) were identified. As a whole, C4 represents the first non-peptide selective activator of PKC δ and a promising compound to be explored as an anticancer agent.

Acknowledges: This work was supported by FCT through REQUIMTE (PEst-C/EQB/LA0006/2011). C. Bessa is recipient of a FCT fellowship (SFRH/BD/87109/2012).

References:

[1] Silva R.D. *et al.* (2012), *Yeast as a powerful model system for the study of apoptosis regulation by protein kinase C isoforms*, Current Pharmaceutical Design, 18 (17), 2492-2500.

[2] Coutinho, I. *et al.* (2009), *Selective activation of protein kinase C-delta and -epsilon by 6,11,12,14-tetrahydroxy-abieta-5,8,11,13-tetraene-7-one (oleon U)*, Biochemical Pharmacology, 78 (5), 449-459.

Determination of HIF-1 α relevance in the immunopathology associated with *Mycobacterium avium* infection

M. Cardoso¹, R. Appelberg^{1,2} and M. Borges^{1,3}

¹ Laboratory of Microbiology and Immunology of Infection, Institute for Molecular and Cell Biology, University of Porto, Portugal

²ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, University of Porto, Portugal.

³Biological Sciences Department, Faculty of Pharmacy, University of Porto, Portugal

Mycobacterial infections are characterized by the formation of granulomas. Granulomas are well-organized aggregates of immune cells, namely infected macrophages. The granuloma's main function is to constrain and prevent dissemination of the mycobacteria while concentrating the immune response to a limited area. In some cases these granulomas undergo central necrosis leading to their caseation. The mechanisms leading to granuloma caseation are still poorly understood [1,2]. It has been reported that reduced vascularization of granuloma may be one essential mechanism for caseation. Some studies have been demonstrated signs of severe hypoxic regions at the center of the granuloma. The hypoxia-inducible factor -1 α (HIF-1 α) has been shown to be important in some diseases, such as cancer and infections. HIF-1 α is able under hypoxic conditions to transcriptionally regulate gene expression, allowing macrophage adaptation, by enhancing their phagocytic activity and antigen presentation capacity [3]. The Appelberg laboratory has developed a study model that mimics the human pathology of *Mycobacterium tuberculosis*, using the mouse model and *Mycobacterium avium* infection. This model consists of C57BL/6 (WT) mice infected intravenously with a low dose of a highly virulent strain of *Mycobacterium avium* (ATCC 25291), which develops granulomas that, at 4 months of infection, exhibit central necrosis. To determine HIF-1 relevance during *M. avium* infection we used a mouse strain deleted of HIF-1 α under Cre-lox system in the myeloid cell lineage (HIF1 α KO). Infected mice were euthanized at different times during the infection and the lungs, liver and spleen were aseptically collected. Bacterial loads were determined in the organs of infected animals. Morphometric analysis of granulomas was performed in haematoxylin-eosin stained liver sections. The localization pattern of macrophages in the livers from infected mice was studied by evaluating the expression of F4/80 cell marker, by immunohistochemistry. The analysis of liver and spleen cell populations were determined by flow cytometry analysis. IFN-gamma and HIF-1 α production has been evaluated *ex-vivo* by ELISA. The results obtained indicate that HIF1 α KO mice are more predisposed to the infection and the onset of necrotic granulomas is faster.

References:

[1] O'Garra, A., Redford, P.S., McNab, F.W., Bloom, C.I., Wilkinson, R.J. and Berry, M. (2013), *The immune response in tuberculosis*, Annu. Rev. Immunol. 31:475-527.

[2] Flório, M., Cooper, A.M., and Appelberg, R. (2002), *Immunological basis of the development of necrotic lesions following Mycobacterium avium infection*, Immunology 106: 590-601.

[3] Harper, J., Skerry, C., Davis, S.L., Tasneen, R., Weir, M., Kramnik, I., Bishai, W.R., Pomper, M.G., Nuermberger, E.L. and Jain, S.K. (2012), *Mouse model of necrotic tuberculosis granulomas develops hypoxic lesions*, J Infect Dis 205(4):595-602.

A2

ECONOMICS & MANAGEMENT

IV
PARALLEL
ORAL
SESSIONS

Impact Assessment of the Possible Free Trade Agreement between EU and Mercosur

A. Bandeira¹, S. Monteiro¹, V. Morais¹, and A. Silva¹

¹ Faculty of Economics, University of Porto, Portugal.

This paper is focused on the trade relations between EU and Mercosur in the last two decades and the possible outcome of an FTA agreement between those. That FTA is currently in a renewed power negotiations period, after the past setbacks.

To explore the main questions, the approach will focus on the following intermediate goals: explain the historical context of the last two decades of trade between the two trade unions; define and quantify the impact of the nowadays trade barriers between EU and Mercosur; identify the main sectors of trade that these days are the most relevant between the two and the ones that will be more affected by the FTA; evaluate the models used on the referenced bibliography, specially through the statistical methods, the impact and quality of the pre-assumptions made on those. All of these intermediate goals are the basis theory support to achieve the main goal that focus on having an impact assessment on the trade changes that will outcome of the FTA.

The preparatory work and the data from previous years matched with the expected conclusions, although with a smaller impact. The EU's imports from Mercosur are concentrated only in two sub-sectors - food and live animals, and in crude materials – accounting more than 65% of total goods imports. At the same time, EU exports to Mercosur are concentrated mainly in machinery and transport equipment and chemical goods, reaching a share of more than 70% of total EU exports to Mercosur.[1]

The collected data from the forecast proved that agricultural commodities is the sector that will raise the most with the scenarios of trade opening studied, looking from a EU imports perspective. This represents a raise of 0.37% of Mercosur GDP, although the share of this impact comes 70% from a Doha Round Agreement, and only 30% from the FTA with EU concessions. Manufactures exports of EU to Mercosur are the most affected sector. With the increase of Manufactures and Services exports from EU to Mercosur, the estimated GDP impact is 0.16/0.17 % positive in EU, but more than 90% of the gain comes out of Doha Agreement Impact.[2]

Ultimately, a Doha Round Agreement it's the biggest platform of benefit that the two unions can get. The impact of the FTA, however it goes, it's almost irrelevant compared to the benefits possible from a positive outcome of the Doha Round negotiations as it's observed by the low share that the FTA has in the bilateral trade increase.

References:

[1] Sunesen, E., and Thelle, M. (2011), *Assessment of barriers to trade and investment between the EU and MERCOSUR*, Copenhagen Economics, Copenhagen, pp. 12-14.

[2] Burrell, A., et all (2011), *Potential EU-Mercosur Free Trade Agreement: Impact Assessment*, Institute for Prospective Technological Studies, European Commission, Seville pp. 65-71.

Revaluation of Real Estate Assets Motivations and Consequences

Tomás Veloso¹

¹ Faculty of Economics, University of Porto, Portugal.

The theme of our work emerged after analyzing a news in “Jornal de Negócios” on October 6, 2013 titled "Banks threatened to cut up to 60 % in value of the property if not re-evaluated".

Our work focus on three key points: analysis of the reasons for the concern of the Bank of Portugal to see the real estate assets revaluated, the rules that sustained this review and the consequences for financial institutions.

As reasons for the concern of the Bank of Portugal, we studied the financial crisis triggered in 2008 from the U.S., in turn influenced by the subprime crisis. Easy access to credit and misconception of the constant valuation of real estate assets led, with the saturation of the real estate bubble, that several properties entered in lack of payment and do not express its true market value on the balance sheets of banks. In our country, the Bank of Portugal revealed the need for a revaluation of properties that are in this situation in order to update their value entered in the balance sheet of the banks.

We analyze, through the circulars issued by the Bank of Portugal in the years 2009 and 2013, the rules that this review is based and still the forms to reevaluate such assets and their components, according to the working paper in management from the University of Aveiro.

We refer to the usefulness of the financial ratios used by banks and we make a description and interpretation of them. Through the analysis of the presentation of results from four national banks, we compare them based on these same ratios.

Finally, we explain briefly how the banks may undertake, in accounting terms, the revaluation of its real estate assets and also present some possible ways of increasing its equity as a mean to maintain the minimum required by the Bank of Portugal to certain ratios.

Key-words: Real estate, Revaluation, Bank of Portugal, Ratios, Banks

JEL classification: G01, G21, G28

Collective action as model for social-ecological systems management: the Douro region in a climate change scenario

M. Barros¹, C. Chaves² and M. Cunha³

¹ Faculty of Economics, University of Porto, Portugal.

² Faculty of Economics, University of Porto, Portugal.

³ Department of Geosciences, Environment and Territory, Faculty of Sciences, University of Porto, Portugal.

Introduction

Climate change is expected to have a strong impact on wine production. Estimated projections for the Douro wine region indicate an increase in the range of average temperatures and the reduction of average precipitation by 2080. Adaptation to those scenarios is more efficient if strategies are implemented according to collective action models. Common Pool Resources (CPR) management is an issue that concerns policy makers and academics for a long time. Collective action theory, enunciated by [1], can be used in a variety of contexts and typologies. In the present case, we present a model based in a collective action strategy for managing a Social-Ecological System (SES) such as the Douro wine region, in a climate change scenario. Developed forecast model was used to estimate the impact climate change on Douro regional wine production. We propose the adoption of viticulture in integrated production rules with decentralized management, led by a viticulturists association.

Methodology

A forecast model for estimating the impact of biophysical variables on the annual variation in regional wine production has been developed over the period 1980-2010. The model considers the impact of diseases and a number of climatic variables determined for the key periods of grapevine growth cycle. This model was used to predict the impact on Douro wine production of the climate scenarios proposed by [2] for the years 2020, 2050 and 2080. Three alternatives were placed in this dissertation: the climate variables maintain the average values between 1980 and 2010, or suffer an increase of 10 a 20% regarding the referred average.

Results and Discussion

Appropriate statistical tests indicated that the wine forecast model explains about 62% ($R^2=0.62$; $n= 31$; $p<0.000$) of the inter-annual variability in Douro wine region. From the results, it's possible to infer that, whatever the scenario placed, wine production increases. However, we do not know the effects that extreme temperature increase could place in the vineyard physiology and the very limits of plants survival capacity.

Climate change will force actors to implement adaptation measures. We propose an adaptation model based in viticulturist practices based on integrated production system, regarding a collective action strategy. This is a bottom-up model, were small scale viticulturists gather around a viticulture technical association, suitable to overcome the effects of extreme scenarios.

References:

[1]Ostrom, E. (1990), *Governing the Commons: the evolution of institutions for collective action*, Indiana University.

[2]Jones, G.V. and Alves, F. (2012), *Impacts of climate change on wine production: a global overview and regional assessment in the Douro Valley of Portugal*, Int. J. Global Warming, Vol. 4, Nos. 3/4, pp.383–406.

Country Embeddedness: An exploratory study about the strengths that connect portugueses young adults to Portugal

R. Geraldes¹ & L. Pinto²

^{1 2} Department of Economics , Faculty of Economics, University of Porto, Portugal.

This study aimed to explore the concept of *country embeddedness*. Using a sample of Portuguese young adults (N=26) currently living in Portugal and abroad (*self-initiated expatriates*), this work explores the factors that favour or hinder the relationship with home-country and the differences between both groups.

Applying a qualitative methodology, with semi-structured interviews and focus groups, we were able to assess three dimensions in the current Portuguesees relationship with Portugal. The theory of *country embeddedness* [1] has shown to be adequate to this purpose.

The construct of *country embeddedness*, defined as the forces that embedded an individual to a country, in this case his/her home-country, has demonstrated to be pertinent and distinct. Contextual factors, combined with cultural and personal aspects determine the strength of the tie each individual keep with Portugal as the home country. Thus, the construct of *country embeddedness* is circumstantial and composite.

While Portuguese young adults perceive themselves as country embedded, they reported several other factors that further influenced their intentions to leave, stay abroad and/or return to Portugal. This finding corroborates the pertinence and distinctiveness of *country embeddedness*. Comparing both groups (e.g. highly skilled Portuguese self-initiated expatriates and residents), they revealed a strong connection with Portugal, being both country embedded, though Portuguese self-initiated expatriates showed also a high host-country embeddedness, which seemed to influence their plans to return.

So far, this is the first empirical study exploring the concept of *country embeddedness*, comparing Portuguese residents with Portuguese self-initiated expatriates. The findings are helpful to assist in the development of a scale of *country embeddeness*, providing also insights into the specific relationship young Portuguese adults build with the country whether residents or living abroad.

References:

[1] Lo, K. I. H., Wong, I. A., Yam, C. M. R., e Whitfield, R. (2012), "Examining the impacts of community and organization embeddedness on self-initiated expatriates: the moderating role of expatriate-dominated private sector", *The International Journal of Human Resource Management*, Vol. 3, n° 20, pp.4211-4230.

Portuguese Self-Initiated Expatriates and their Social Networks: composition, diversity and roles

R. Araújo¹ and L. Pinto¹

¹ Department of Management, Faculty of Economics, University of Porto, Portugal.

Portuguese self-initiated expatriates (SIEs) are reinforcing the international mobility of qualified professionals. An exploratory study was conducted to describe the social networks that Portuguese SIEs establish abroad: composition, diversity and roles.

SIEs decide to move abroad (Froese and Peltokorpi, 2012) and are hired under local contracts (Crowley-Henry, 2007). Yet, their relocation is temporary and at their own expenses. Thus, SIEs are known for their self-directedness (Doherty, 2012), namely regarding networking, instrumental to obtain social support (Johnson *et al.*, 2003; Osman-Gani and Rockstuhl, 2008; Stroppa and Spieß, 2011).

Accordingly, the development of Portuguese SIEs networks and their perceived roles have been addressed through semi-structured interviews to thirteen Portuguese qualified SIEs. Interview content analysis was performed after a semi-inductive analysis process.

SIEs build and maintain home and host social networks; in each country, the networks are both interpersonal and organisational. In Portugal, SIEs connect with family/partner, friends, recruitment agencies and diplomatic institutions. Abroad, the network is composed by friends, co-workers, acquaintances, partner, employers, diplomatic institutions and unions. Regarding diversity, host social networks tend to be diverse in terms of nationality, quantity and roles. Besides, the Portuguese diaspora is not a source of information or support, even if Portuguese SIEs do have one main element in the social networks and its presence is constant from the pre-departure stage onwards.

Overall, results show (1) the absence of a diaspora-based network, (2) the strength of the host country networks and (3) the existence of a main knot in the social network.

References:

- [1] Crowley-Henry, M. (2007), "The Protean Career: Exemplified by First World Foreign Residents in Western Europe?", *International Studies of Management and Organization*, Vol. 37, no. 3, 44-64.
- [2] Doherty, N. (2012), "Understanding the Self-initiated Expatriate: A Review and Directions for Future Research", *International Journal of Management Reviews*, DOI: 10.1111/ijmr.12005.
- [3] Froese, F. J. and Peltokorpi, V. (2012), "Organizational expatriates and self-initiated expatriates: differences in cross-cultural adjustment and job satisfaction", *The International Journal of Human Resource Management*, 1-15.
- [4] Johnson, E. C., Kristof-Brown, A.L., Van Vianen, A. E.M., De Pater, I. E. and Klein, M. R. (2003), "Expatriate Social Ties: Personality Antecedents and Consequences for Adjustment", *International Journal of Selection and Assessment*, Volume 11, Number 4, 277-288.
- [5] Osman-Gani, A. M. and Rockstuhl, T. (2008), "Antecedents and Consequences of Social Network Characteristics for Expatriate Adjustment and Performance in Overseas Assignments: Implications for HRD", *Human Resource Development Review*, Vol. 7, No. 1, 32-57.
- [6] Stroppa, C. and Spieß, E. (2011), "International assignments: The role of social support and personal initiative", *International Journal of Intercultural Relations*, 35, 234-245.

The 2014 FIFA World Cup as a Job Creator

S. Rêgo¹, R. Schiestl², J. Sarmento³, M. José Carvalho⁴

¹ Erasmus Student, Faculty of Sports, University of Porto, Portugal.

² Master Student, Faculty of Sports, University of Porto, Portugal.

³ PhD, Faculty of Sports, University of Porto, Portugal.

⁴ PhD, Faculty of Sports, University of Porto, Portugal.

The FIFA World Cup 2014 is an excellent opportunity to consolidate Brazil as an emerging country. To do this, the appropriate framework conditions to host an event of this magnitude must be created, investing in civil infrastructure (stadiums, airports, roads and ports) and services infrastructure (healthcare, lodgings, telecommunications and security). These sectors must be supplied with: inputs, labor force, infrastructure construction, increased inventory, equipment, services and technologies. The objective of this study is to analyze the amount of jobs generated by the FIFA World Cup 2014. It is important to know the impact that such a mega event will have in the Brazilian job market. The methodology adopted for this study was a review of nine scientific articles in journals which are indexed in EBSCO and SCIELO, with the keyword “megaevento”, two master degree dissertations and a document analysis of sources from the Ministry of Sports of Brazil. The results show that 332 thousand permanent jobs (2009-2014) and 381 thousand temporary jobs (2014) have been and will be generated, totaling 713 thousand jobs. Comparing the last World Cup in relation to the population index, Brazil has had an increase in job creation, employing 0.35%, while the 2010 World Cup in South Africa generated 150 thousand jobs, equivalent to 0.30% of its population. The 2006 World Cup in Germany generated 50 thousand jobs, equivalent to 0.06% of its population. Therefore, we can conclude that the World Cup in Brazil will create more jobs when compared with the two latest World Cup editions, when analyzed through the population index.

A3

**PSYCHOLOGY &
EDUCATION
SCIENCES III**

**IV
PARALLEL
ORAL
SESSIONS**



DOES THE STATUS OF AN INGROUP DEVIANT AND AN EFFECTIVE GROUP CONTROL INFLUENCE SOCIAL COHESION ON ATHLETES?

C. Morais, I. R. Pinto, J. M. Marques¹

¹ Laboratory of Social Psychology, Faculty of Psychology and Educational Sciences, University of Porto

Individuals are intrinsically motivated to maintain or achieve a positive social identity [1] and to reduce the uncertainty about the world by sharing and validating norms and values with the ingroup [2]. The Subjective Group Dynamics Theory [SGD, e.g. 2] argues that, in order to confirm the positive value of the ingroup, individuals resist to evidence that deteriorates validity of group norms and accept those that confirm it. In this sense, deviance within group is perceived as a threat to the norms and consequently to individuals' positive social identity. According to SGD, groups evolve in intense and negative reaction toward such members in order to restore the legitimacy of the violated norms and to enhance ingroup value [3].

However, recent research has demonstrated that not all ingroup members trigger such negative reactions. For instance, ingroup leaders can be forgiven when incur in serious transgression (transgression credit, 4). According to these authors, transgression credit is a manifestation of members' loyalty to the group (commitment to the group). On the other hand, prior investigations have demonstrated that deviant leaders are more negatively evaluated than deviant non-leaders [5]. In this line of research, members show stronger commitment to the group in derogating deviants with high intragroup status. Apparently, thus, there is no consensus about the psychosocial process underlying reaction to deviant leaders and the impact of these reactions in intragroup cohesion.

We conducted an experiment aiming to test the impact of deviant status and of reaction to deviance in members' commitment to the group (social cohesion). A 2 (Deviant Status: High vs Regular Member) x 2 (Group Reaction: Punishment vs No punishment) experimental design was used. Fifty-seven athletes were asked to give us their opinion about another (deviant) athlete of their group. Half of participants learned that the deviant was the captain (High Status) of a sport's teams within participants' sports club, while the other half did not have any information about the deviant status (assumed regular member status). Moreover, within each condition, participants were also informed that the sports club institutionally punished (vs not punished) the deviant leader/member.

Results showed that participants tend to agree less with punishment and to judge less negatively the deviant when the deviant is a regular member and had already been punished by the group. On the contrary, punishment of deviant leaders leads individuals to agree with such reaction. Results also show that the more participants perceive that the deviant caused a threat to their social identity, the more they agree to punish the deviant, the more they legitimate the violated norm and the more committed they are to the group. Altogether, these results suggest that reaction to deviance has impact on intragroup cohesion especially when the deviant member assumes a high intragroup status and the group is able to react effectively towards this deviant. Indeed, in such case, it seems that we can observe a more intensive concern for group protection.

References:

- [1] Tajfel, H., and Turner, J. C. (1979). *An integrative theory of intergroup conflict*, Austin, W.G. and Worchel, S. "The social psychology of intergroup relations", Brooks-Cole, Monterey, pp. 33-47.
- [2] Abrams, D., Randsley de Moura, G., Hutchison, P. and Tendayi Viki, G. (2005), *When bad becomes good (and vice-versa): Why social exclusion is not based on difference*, in Abrams, D., Hogg, M.A. and Marques, J.M. "The Social Psychology of Inclusion and Exclusion", Psychology Press, New York, pp. 161-189.
- [3] Marques, J. M., Abrams, D., Páez, D. and Hogg, M. A. (2001), *Social Categorization, Social Identification, and Rejection of Deviant Group Members*, in Hogg, M.A. and Tindale, S. "Blackwell Handbook of Social Psychology: Group Processes", Blackwell Publishers, Oxford, pp. 400-424.
- [4] Abrams, D., Randsley de Moura, G. and Travaglino, G. (2013), *A Double Standard When Group Members Behave Badly: Transgression Credit to Ingroup Leaders*, *Journal of Personality and Social Psychology*, 105(5), 799-815.
- [5] Karelaiia, N. and Keck, S. (2013). *When deviant leaders are punished more than non-leaders: The role of deviance severity*, *Journal of Experimental Social Psychology*, 49, 783-796.

Police officers' perceptions about prostitution and its actors: an exploratory study

S. Matias¹, and A. Oliveira¹

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Police and prostitution may be seen as opposite sides of a spectrum. The first has the duty to protect and serve the citizens against crime, while the second is an activity largely discriminated and ostracized, which often borders the criminal frontier. Furthermore, we may consider the high levels of violence against prostitutes, the misinterpretation of the law and the need for impartiality by the officers. Since the actors of prostitution, namely female and male prostitutes and their clients, are members of society who also need protection, it seems interesting to explore how policemen and women perceive and interact with them. Thus, we conducted an exploratory study in Porto whose aim was to understand the perceptions and attitudes of male and female police officers towards prostitution and its actors.

To achieve this objective we used an inquiry mainly composed of open ended questions. The data analysis was done through qualitative and quantitative methods. We are still conducting the study and the present data is preliminary.

The sample is composed of 34 active police officers (28 male and 6 female), who were reached through a snowball strategy. The ages of participants are between 30 and 56 years (Mean = 43) and they work as police officers for 20.9 years in average. The majority of the participants (65.65%) completed the high school.

The findings show that 79.42% of the participants know the portuguese legislation on prostitution and defend that it should be legalized, however they propose certain conditions such as regulation, inspection and control. Approximately half of the sample (47.06%) confirms to contact frequently with prostitutes while working. The representation they have of people who engage in prostitution is mostly negative, but whilst women are given motives associated with structural factors (61.77%), men are ascribed with intrinsic reasons (38.23%). When questioned about the attitudes they think police officers in general have toward prostitutes, 35.30% admit they are not the most correct, while 26.47% describe total indifference.

We can conclude that police officers know the phenomenon, contact with it and acknowledge that attitudes are not always the correct one's. which might be related with the broad negative representation about prostitutes.

Schooling as experience: narratives of Portuguese 9th grade students

T. Freires¹, F. Pereira², and C. Santos³

¹ Department of Education, Faculty of Psychology and Education Science, University of Porto, Portugal.

² Department of Education, Faculty of Psychology and Education Science, University of Porto, Portugal.

³ Department of Education, Faculty of Psychology and Education Science, University of Porto, Portugal.

The current research debates the results of a project entitled "The central role of the schooling experience as a structuring factor on young students' lives: biographical narratives of students from the 9th grade (Elementary School)", which is part of the Young Research at Porto University Program (IJUP) and conducted in partnership between the Faculty of Psychology and Educational Sciences (FPCEUP) and the Faculty of Letters of the University of Porto. In general terms, this research was aimed at understanding through the students' voice how school contributes to the development of diverse dimensions in each student life, in a search to identify the senses and meanings that these students address to school and to the experiences they live within it.

In order to meet our goals, 34 interviews of biographical type were carried out in a first phase with students of the 9th grade from public schools of North Portugal. From this group, 4 participants were invited to a second meeting to deepen the data. Among the criteria for being selected for the second phase, we took into account gender, age and interest (or lack of) in school activities. The collected material was analyzed according to the narrative approach [1] and through the use of content analysis techniques [2].

Results showed that School is established as a space for a life project, although it can be oblivious to the wishes and interests of students. It is by the arrangement of relationships between the institution, the community, family and friends that young students make sense of their educational processes [3]. If by one side, school may present itself as an obstacle to social activities, by the other one, all students recognize its importance. Data shows that students who perceive school as a concrete project adhere to its logic with a greater degree. Moreover, there's an explicit desire for humanization of relationships inside school [4], which may symbolize a way to lessen the oppression that school culture can imprint in the students' lives [5].

References:

[1] Goodson, I. (2013), *Developing narrative theory. Life Histories and personal representation.*, Routledge, London.

[2] Pereira, F. (2010). *Infância, educação escolar e profissionalidade docente. Um mapeamento social dos discursos em formação inicial de professores.*, Calouste Gulbenkian, Lisboa.

[3] Charlot, B. (2009). *A relação com o saber nos meios populares. Uma investigação nos liceus profissionais de subúrbio.*, CIEE /Livpsic, Porto.

[4] Freire, P. (2005). *Pedagogia do oprimido.*, Paz e terra, Rio de Janeiro.

[5] Abrantes, P. (2003). *Os sentidos da escola. Identidades juvenis e dinâmicas de escolaridade.*, Celta Editora, Oeiras.

Is it true that education and training courses can contribute to the transformation of the trainees' self-efficacy beliefs and career development? A case study in the area of table waiting

A. Graça¹, I. Nascimento²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

² Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

This research departs from the view that education and training courses have responsibility in the way the trainees (re)construct the image of themselves and of the world by providing experiences that will eventually help them to question and transform the present commitment through reconstructive exploration of career commitment [1]. It assumes, therefore, that this kind of training context is a privileged one in what refers to the review or validation of self-efficacy beliefs, self-concept and attributions of academic achievement and, consequently, in the career development. This includes the development processes of vocational interests and choice behavior in which the variables of self-efficacy, outcome expectations and goals are involved [2, 3].

The trainees and the trainers of an education and training course in the area of table waiting from a school located in Maia participated in this study. The trainees' self-efficacy beliefs, their self-concept and causal attributions were evaluated at the beginning and, two years later, at the end of the course. It was also evaluated the methodologies used by the trainers, previewed by the curriculum of their disciplines, and the progression of the trainees during the course.

The main goals of this study were to explore (1) to what extent the course is a context that offers experiences of exploration of the investment and thereby influences the (trans)formation of self-efficacy beliefs, the development of vocational interests and their future vocational choices; (2) if attending the course is associated with possible reconfigurations of the academic self-concept of the trainees according to the academic results achieved and the causal attributions of their academic success.

Results show that the methodologies used by the trainers provided experiences that potentiate and facilitate career development by stimulating the reconstructive exploration of investments and it seems to be a source of influence in the formation of vocational self-efficacy beliefs. The traineeship and the technological component disciplines, including the simulated practice, were important on that point because they provided direct experiences of mastery and success in professional activities as well as vicarious learning. On the other hand, self-efficacy beliefs and the outcome expectations apparently influenced the development of interests and goal setting in which the trainees lay their future vocational choices. Attending the course also gave them opportunities to change self-concept and causal attributions and so at the end of the course the self-concept was more independent from the academic results and the trainees had a more external attributive style for the academic results.

Referências:

[1] Coimbra, J. L., Campos, B. P., & Imaginário, J. L. (1994). *Career intervention from a psychological perspective: definition of the main ingredients of an ecological developmental methodology*. Paper presented on 23rd International Congress of Applied Psychology, Madrid.

[2] Lent, R. W., Brown, S. D., & Hackett, G. (1994). Towards a unifying social cognitive theory of career and academic interest, choice, and performance. *Journal of Vocational Behavior*, 45, 79-122.

[3] Lent, R. W., Brown, S. D., & Hackett, G. (2002). Social Cognitive Career Theory. In D. Brown & Associates (Eds.), *Career choice and development* (4th ed., pp. 255-311), San Francisco: Jossey-Bass.

Body image in persons with spinal cord injury: relationship with depression, anxiety, satisfaction with perceived social support and self-esteem

F. Costa¹ and R. Barbosa²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal

² Center of Psychology , Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

The onset of the spinal cord injury leads to significant changes in individuals life, leading to a series of biopsychosocial consequences. Being a lesion with direct implications on body image has become relevant to better understand how the body satisfaction of these individuals is affected and how these changes have an impact on the psychosocial adjustment of the injured individual. The current investigation, embedded in a essentially quantitative methodology, thus aims to explore the relationship between body satisfaction, psychosocial adaptation (anxiety and depression), as well with social support and global self-esteem satisfaction.

The sample of this research was collected in a non-probabilistic convenience sampling (snowball), with a total of 53 spinal cord injured (38 men and 15 women) with an average age of 39.87 years (SD = 14.09). We used the Portuguese version of the Body Image Scale, subscales of Anxiety and Depression of the Inventory Psychopathological Symptoms, the Scale of Satisfaction with Social Support Questionnaire and the Global Self-Esteem. Was also used a sociodemographic and clinical survey, with the inclusion of a question about the main perceived changes in their lives after injury.

Regarding results, the descriptive analyzes revealed some dissatisfaction with body image, low prevalence of symptoms of anxiety and depression, good global self-esteem levels along with positive values such as satisfaction with social support in this participants sample. It was observed strong and positive correlations between anxiety, depression and body dissatisfaction and negative correlations including self-esteem, overall satisfaction with perceived social support and body dissatisfaction. It was also found that the injured in whom the diagnosis of lesions occurred after 16 years-old and tetraplegic patients presented higher values of body dissatisfaction. The predictive model indicated that the psychopathological symptoms (anxiety), the age of occurrence of the injury, such as its extension, are the variables that best predict dissatisfaction with body image. Finally, the readaptation to the situation and the losses and difficulties of social integration were the most salient aspects of the participants speeches relating to meanings attributed to that life event and its implications.

From the clinical point of view, the emphasis is put on the importance of promoting positive feelings, reframing constructively the experience of the new body, as in social interventions.

Keywords: Spinal Cord Injury, Body Image, Anxiety, Depression, Global Self-esteem, Satisfaction with Perceived Social Support.

Automated software for prosody assessment in European Portuguese

F. Lima¹, B. Coelho¹, C. Guedes¹, P. Rodrigues², T. Rodrigues², J. N. Moutinho², M. Filipe¹, D. Freitas², S. Vicente¹

¹ Faculty of Psychology and Educational Science, University of Porto, Portugal.

² Faculty of Engineering, University of Porto, Portugal

Prosody - the expression of feelings and emotions through intonation and tone of voice - involves suprasegmental features of spoken language and is looked as an important component of face-to-face interaction and language processing [1]. Since there is a lack of prosodic assessment materials for clinical and research purposes, it is crucial to develop specific assessment tools to evaluate these abilities. Furthermore, the characterization and analysis of the prosodic acoustic patterns are user-dependent and technically demanding. To overcome these limitations, our aim is to develop a comprehensive prosodic battery with automated acoustic analysis, in order to assess both receptive and expressive prosodic traits in normative and clinical populations native speakers of European Portuguese.

A computerized version of the Profiling Elements of Prosodic Systems - Communication (PEPS-C; [2]) was developed for Portuguese speakers. The available PEPS-C is a test addressing both receptive and expressive language abilities. The tasks, in their computerized version, were built on two levels: formal and functional. The formal level includes four tasks for the evaluation of auditory discrimination and ability to listen to auditory stimuli and produce it verbally. The functional level contains eight tasks intended to assess the ability to express and understand expressive prosody among four communicative functions: (1) Turn-end - the ability to detect if an utterance is a question or statement, (2) Affect - the ability to express like or dislike about food and drink elements, (3) Chunking - the process of verbal phrasing, (4) Focus - the capacity to emphasize a particular word in an utterance. This software automatically extracts and analyses the acoustic parameters from the expressive tasks and compare acoustic data from a normative sample.

This new software will allow to optimize the process of collecting and analysing prosodic data. In addition, it provides important insight to the prosodic research, which in turn might enhance the clinical diagnosis and intervention.

References:

[1] Peppé S., Cleland, J., Gibbon, F., O'Hare, A., Martínéz Castilla, P. (2011). Expressive prosody in children with autism spectrum conditions. *Journal of Neurolinguistics*, 24, 41-53. doi: 10.1016/j.jneuroling.2010.07.005.

[2] Peppé, S., & McCann, J. (2003) Assessing intonation and prosody in children with atypical language development: the PEPS-C test and the revised version. *Clinical Linguistics & Phonetics*, 17(4/5), 345-354. doi: 10.1080/0269920031000079994

A5

BIOLOGICAL SCIENCES IV

IV
PARALLEL
ORAL
SESSIONS



Impact of abiotic factors in the invasion process: the case of *Artemia parthenogenetica* from Aveiro and Rio Maior saltworks

F.J. Sousa^{1,2}, F.M. Ferreira^{1,2}, and M.N. Vieira^{1,2}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² CIIMAR – Interdisciplinary Centre of Marine and Environmental Research

Unlike it was thought in the past, it is proven that aquatic ecosystems are more vulnerable and favorable to the occurrence of biological invasions. In fact, some of these ecosystems, like the hypersaline ecosystems, have been changed, in terms of salinity and hydrological dynamics, and are subjected to great pressure from invasive species. This is the case of the genus *Artemia*, a crustacean that inhabits salterns and salty lakes, whose dispersion mediated by man is 50,000 times greater than its natural rate of dispersion. The presence of this invasive species may remain unnoticed several decades before being found, being necessary molecular methods to identify them.

In Portugal there are only two salterns where these invasions haven't already happened: the salterns from Troncalhada (Aveiro) and Rio Maior. The aim of this study was to understand the better tolerance to the variation of some environmental factors of these *Artemia parthenogenetica* populations, in order to assess their susceptibility to invasion.

Various concentrations of salinity (70, 110, 150ppt), temperature (24, 29 and 34°C) and food (300000, 150000, 37500 cells ml⁻¹) and photoperiod (0, 12 and 24 hours of light) were tested and various reproductive parameters were monitored. *Artemia parthenogenetica* from Aveiro showed to be susceptible to invasion, unlike the population from Rio Maior, which is well adapted to conditions of 24°C, 150 ppt and 37500 cells ml⁻¹.

Acknowledgments: This work was financially supported by the project “Chemical wars: the role of chemically mediated interactions in the invasiveness potential of non-native *Artemia*” – Ref. PTDC/MAR/108369/2008 funded by the Portuguese Foundation for the Science and Technology.

Evolution of eye development genes in mammals

J. Fonseca¹, R. Borges^{1,2}, and A. Antunes^{1,2}

¹ Department of Biology, Faculty of Science, University of Porto, Portugal.

² CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Portugal

This study aims to determine which of the mammalian eye developmental genes were involved in the diurnal adaptations, following the nocturnal ancestral period that mammals have experienced. By studying the genes and proteins involved in this process we expected to disentangle the molecular processes determining the evolution of the eye-related genes and how they have contributed to the diurnal adaptations in mammals (such as, the increased iris size and orbit convergence).

We implement an *in silico* experimental design. We used Gene Ontology to determine the genes involved in the mammalian eye development. We found 317 genes that are related with photoreceptors and/or photoreception, signal transduction, eye morphogenesis and pigmentation and optic nerves. Sequences from the above genes were collected using Orthoman in distinct species of mammals. Additionally we have also calculated the alpha parameter and relative rate of evolution for each gene. The relative evolutionary rate describes the utility of a phylogenetic marker. The higher the rate value the faster the gene evolves. The substitution rate heterogeneity among-sites of the gene alignment is described by alpha. The lower the alpha value, the higher the heterogeneity among-sites. So far, we have determined important evolutionary signatures for the studied genes considering both the rate/alpha parameters. Our analyses show that the majority of the alignments had strong among-site variability while the markers were conservatives or evolved on a slower pace. But there were some exceptions such as the genes GRM6 and FZR1 that are fast-evolving, CCDC66 and RP1 which had a more evenly distributed variability and NES that is a fast-evolving gene with a high alpha value, i.e. with rather homogeneous substitution pattern among-sites. These exceptions can be of major importance, representing an outlier evolutionary behavior, potentially related to photic adaptation in mammals. In the future we intend to analyze these specific genes using a phylogenetic and adaptive approach.

Evaluation of ionic composition of water as chemical barrier against the invasive *Artemia franciscana*

F.M. Ferreira^{1,2}, **F.J. Sousa**^{1,2}, and **M.N.Vieira**^{1,2}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² CIIMAR – Interdisciplinary Centre of Marine and Environmental Research

The *Artemia* is a small crustacean that belongs to the *Artemiidae* family, Anostraca order, and subclass Branchiopoda. At the moment, there are 7 *Artemia* species described, 6 being bisexual and one parthenogenetic. Its presence all over the world is representative of its high adaptability and tolerance. Its global distribution, is also due to the fact that it can inhabit hyper saline environments, where the occurrence of predators is reduced, or even absent. Currently, in Iberian Peninsula, it is possible to find both parthenogenetic species and the bisexual species, *Artemia franciscana*.

In Portugal, *Artemia parthenogenetica* is the autochthonous species, however, it is endangered due to the introduction of the invasive species *Artemia franciscana*, which inhabits almost all biotopes, especially in the South.

It is necessary to preserve biodiversity in places where it is still possible to find the autochthonous species, and so, it's imperative to know the medium composition where *Artemia* live.

In order to do that, it is necessary to know the local conditions, especially the conditions of the medium.

This work is focused on the study of a possible chemical barrier caused by the ionic composition of the saline water from Rio Maior saltern, as the major cause preventing the invasion.

The main objective of this work was to determine the effects caused by the ionic composition of the water both in the autochthonous and invasive species of *Artemia*.

Various concentrations of calcium (0, 50, 150, 450 mg/L) and magnesium (0, 50, 100, 450, 1300 mg/L) were tested and survival was monitored.

The results show that the ionic composition of the water from Rio Maior saltern may work as chemical barrier against the invasive species, opening new perspectives for research, in order to better understand the process preventing the invasion.

This work was financially supported by the project “Chemical wars: the role of chemically mediated interactions in the invasiveness potential of non-native *Artemia*” – Ref. PTDC/MAR/108369/2008 funded by the Portuguese Foundation for the Science and Technology.

Protective effect of intranasal immunization against *Neospora caninum* infection established through the gastrointestinal tract

C. Santos¹, P. Ferreira^{1,2}, A. Correia^{1,2}, M. Vilanova^{1,2}

¹ICBAS – Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto. Largo Prof. Abel Salazar 2, 4099-003, Porto, Portugal. ² Instituto de Biologia Molecular e Celular – IBMC, Rua Campo Alegre, 4099-003 Porto, Portugal.

Neospora caninum is an intracellular apicomplexan parasite closely related to *Toxoplasma gondii*, which was first described as the etiologic agent of neuromuscular disease in dogs. The dog and other canids such as the wolf have been shown to be definitive hosts for *N. caninum* while bovines are the most economically relevant intermediate hosts. Infected cattle may suffer abortions or congenitally transmit the parasite to descendants. Moreover, horizontal transmission through the ingestion of oocysts is the privileged form of introducing *N. caninum* infection within a herd. Mainly due to cattle abortions, neosporosis is responsible for severe economic losses worldwide in dairy and beef industry, estimated at 1.2 billion dollars annually. However, no commercial vaccine is currently available to prevent neosporosis. Therefore, in this study, the efficiency of intranasal immunization with *N. caninum* membrane antigens plus CpG adjuvant in preventing *N. caninum* infection was evaluated in a murine model of neosporosis established by intragastric administration of *N. caninum* tachyzoites. Mice immunized with antigens plus adjuvant presented lower parasitic burden in the brain than sham-immunized animals. The immunization procedure led to an increase of intestinal parasite-agglutinating IgA antibodies. Moreover, opsonization of *N. caninum* tachyzoites with IgG antibodies purified from immunized mice sera reduced parasite survival in macrophage cells cultures. An IgG1/IgG2a ratio < 1 was detected in immunized animals either before or after infection, indicating a predominant Th-1 type immune response. Nevertheless, no increased production of IFN- γ by splenocytes or mesenteric lymph nodes T cells of the immunized mice. Altogether, these results show that mucosal immunization with *N. caninum* membrane antigens plus CpG adjuvant protected mice against *N. caninum* infection. In addition they also indicate that parasite-specific IgA and IgG antibodies may play a significant role in the achieved protection against this intracellular pathogen.

Supported by FCT/MCTES (PIDDAC) and co-funded by FEDER through COMPETE, PTDC/CVT/115126/2009 and FCOMP-01-0124-FEDER-014679. PF and AC were supported by FCT grants SFRH/BD/76900/2011 and SFRH/BPD/91623/2012, respectively.

The evolution of Star and Star-related proteins in vertebrates

Rui Miguel Cerqueira Coelho^{1,2}, L. Filipe C. Castro^{1,2} & Luísa Azevedo^{1,3}

¹FCUP - Department of Biology, Faculty of Sciences, University of Porto, Portugal

²CIMAR/CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal

³IPATIMUP-Institute of Molecular Pathology and Immunology of the University of Porto, 4200-465 Porto, Portugal

Steroidogenic acute regulatory protein (Star) and Star-related proteins such as MLN64, are key for the cholesterol transport from the outer mitochondrial membrane to the P450scc (cytochrome P450 cholesterol side chain cleavage). This step is required for the de novo synthesis of all steroid hormones.

Here we analyze the evolutionary history of this gene family. We examined all main vertebrate lineages through genome database mining to describe gene diversity and patterns of duplication and loss. Our results indicate that in addition to the described Star protein (StarA), a second Star-like sequence (StarB) is present in the genomes of teleosts, coelacanth, amphibians and birds. Comparative genomics shows that a strong degree of synteny conservation has been maintained throughout the evolution.

The findings now reported provide clear evidence that a) genome duplications in the ancestor of vertebrates, the so-called 2R were instrumental in the generation of Star protein diversity; b) the novel gene isoform we now report is retained in some lineages whilst lost in others such as mammals. The physiological and functional implications of the reported findings should be addressed in the future.

Polyphenol oxidase activity of *Valerianella locusta* plants along time after package opening

C. Soares, J. Teixeira

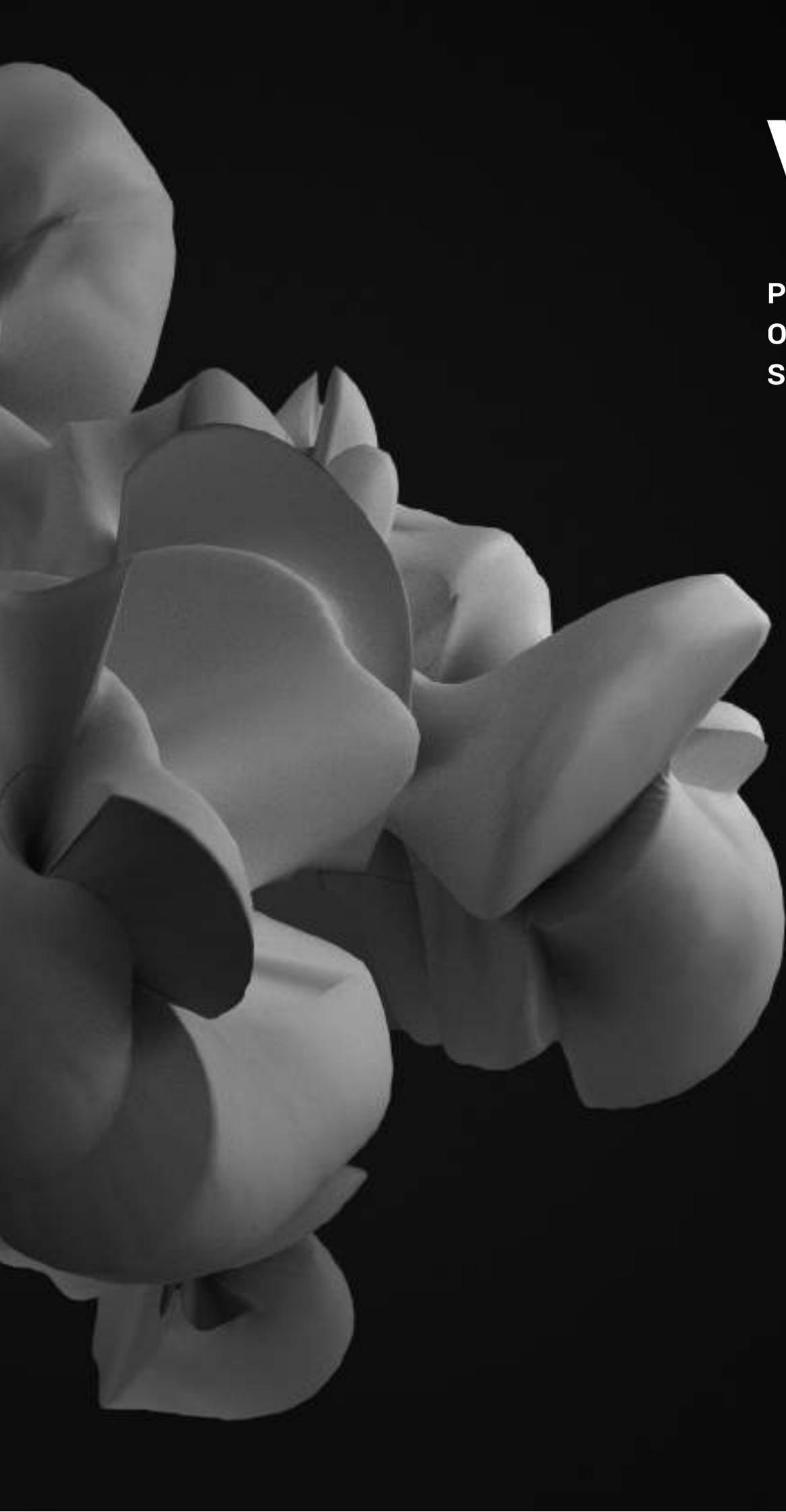
BioISI - Biosystems & Integrative Sciences Institute, Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal.

Polyphenol oxidase (PPO) is an enzyme that causes browning in fruits and vegetables. Although this causes prejudices in agriculture, it is an important defence mechanism against plant pathogens and herbivore insects [1]. PPO is a copper-containing enzyme that catalyzes o-hydroxylation of monophenols and/or the oxidation of o-diphenols to quinones in the presence of oxygen [2], causing a brown pigmentation in plants. When the tissue is damaged, the rupture of plastids (where PPO is located) leads to the enzyme coming into contact with the phenolic compounds released by vacuole rupture, the main storage organelle of these compounds [1]. Plant PPOs have broad substrate specificities [1], an example being catechol. Stress can occur in plants upon physical injury. This leads to an increase in reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), that are prejudicial to plants. The main objective of this study was to evaluate *V. locusta* plants' PPO activity and to estimate ROS production along time after package opening. In the present study, commercially obtained *V. locusta* plants were frozen with liquid nitrogen on the day of package opening, and two and four days later. PPO was then extracted and its activity was determined spectrophotometrically by following the formation of the reaction product derived from the oxidation of catechol. The results showed a significant decrease in PPO activity and an increase in H₂O₂ along with time. The increase in H₂O₂ suggests that plants may have been subjected to stress, producing ROS in consequence to the exposure to oxygen derived from the opening of the package. Regarding the decrease in PPO activity, it can be explained by the fact that other enzymes, like peroxidases, are using the same phenolic substrates, leading to less substrate available for PPO, consequently decreasing its activity. Another possible explanation is that PPO is being directly inactivated by the produced ROS. Therefore, future work will consist on determining guaiacol peroxidase activity and accessing PPO protein levels by western-blotting to test the mentioned hypothesis.

References:

- [1] Queiroz et al (2008) *Polyphenol Oxidase: Characteristics and Mechanisms of Browning Control*, Food Reviews International, Vol. 24:4, Pages 361-375
- [2] Kurt F. Schulbach, Jodie V. Johnson, Amarat H. Simonne, Jeong-Mok Kim, Yoonhwa Jeong, Yavuz Yagiz, Maurice R. Marshall(2013), *Polyphenol oxidase inhibitor from Blue Mussel (Mytilus edulis) Extract*, in Warren, E.G., Journal of Food Science, Vol. 78, Nr. 3, Pages C425-431





V

**PARALLEL
ORAL
SESSIONS**



A1

BIOMEDICINE V

V
PARALLEL
ORAL
SESSIONS

Role of adrenaline in the development of β_2 -adrenoceptors

M. Ferreira¹, A. L. Graça², J. Afonso², P. Serrão², M. Morato^{3,4}, F. Ferreirinha⁵, P. Correia-de-Sá⁵, S. N. Ebert⁶, D. Moura^{2,4} and M. Moreira-Rodrigues¹.

¹Laboratory of General Physiology, UMIB/ICBAS-UP; ²Department of Pharmacology and Therapeutics FMUP; ³Laboratory of Pharmacology, Department of Drug Sciences FFUP and REQUIMTE; ⁴Neuropharmacology, IBMC-UP; ⁵Laboratory of Pharmacology and Neurobiology, UMIB/ICBAS-UP; ⁶Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, USA.

It has been observed that there is a parallel time-course between the postnatal increase in adrenaline content of the adrenal medulla, and the development of β_2 -adrenoceptor-mediated effects, as previously described in the canine saphenous vein [1]. This view is also supported by the diminished response of β -adrenoceptors in blood cells from human newborns compared to adults [2]. The aim of this study was to characterize the β -adrenoceptor-mediated function and β_2 -adrenoceptor protein density in the aorta of adrenaline deficient mice, the phenylethanolamine-*N*-methyltransferase-knockout (Pnmt-KO) mice. In wild type (WT) and Pnmt-KO mice (129x1/SvJ, male, 8-12 weeks old) catecholamines were quantified by reverse-phase HPLC-ED. Aorta rings were mounted in a myograph to determine concentration-response curves to non-selective β , selective β_1 and β_2 -adrenoceptor-agonists in the absence or presence of selective β_1 and β_2 -adrenoceptor-antagonists. Aortic rings were also preincubated with ³H-noradrenaline to measure tritium overflow elicited by electrical stimulation in the presence of increasing concentrations of non-selective β and selective β_2 -adrenoceptor-agonists. β_2 -adrenoceptor protein density was evaluated by western-blotting and immunofluorescence confocal microscopy. Statistical analysis was performed by the Mann-Whitney test and Hill slope values were compared by the F-test. $p < 0.05$ was assumed to denote a significant difference. Adrenaline is absent in Pnmt-KO mice. The selective β_2 -adrenoceptor antagonist ICI-118551 antagonized the relaxation caused by isoprenaline in WT but not in Pnmt-KO mice. The potency and the maximal effect of β_2 -adrenoceptor agonist terbutaline were decreased in Pnmt-KO than in WT mice. ICI-118551 antagonized the effect of terbutaline in both groups, but the pA_2 was lower in Pnmt-KO than in WT mice. Isoprenaline and terbutaline induced concentration-dependent increases in tritium overflow in WT mice only. The protein density of β_2 -adrenoceptors was decreased in membrane aorta homogenates from Pnmt-KO as compared with WT mice. We confirmed that β_2 -adrenoceptor immunoreactivity was less evident in aortas of Pnmt-KO than in WT mice, by immunofluorescence confocal microscopy. In conclusion, adrenaline is crucial for the development of β_2 -adrenoceptors and associated β_2 -adrenoceptor-mediated vasodilation and facilitation of noradrenaline release. In the absence of adrenaline β_2 -adrenoceptors are not efficiently incorporated into cell membranes (internal or cytoplasmic membranes), thus hindering its functional activity.

Acknowledgements: *Fundação Professor Ernesto Morais* (2012, Porto, Portugal), Porto University (IJUP2011-219) and *Fundação para a Ciência e a Tecnologia* (FCT, grants REEQ/1264/SAU/2005 and PEst-OE/SAU/UI0215/2011).

References: [1] Paiva *et al.* (1994), *Naunyn Schmiedebergs Arch Pharmacol*, 350, pp. 28-33; [2] Thies *et al.* (1986), *Monatsschr Kinderheilkd* 134, pp. 453-458.

Alpha 1a adrenoceptor antagonist suppresses bladder pain and urothelial disruption in a model of bladder pain syndrome/interstitial cystitis

RD. Matos¹, F. Cruz^{2,3,4} and A. Charrua^{1,2,3}

¹ Department of Experimental Biology, Faculty of Medicine, University of Porto, Portugal.

² IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal

³ Department of Urology, Faculty of Medicine, University of Porto, Portugal

⁴ Department of Urology, Hospital São João, Portugal

BPS/IC patients present an increased sympathetic activity, characterised by an increase in tyrosine hydroxylase expression in the bladder, increased noradrenaline levels in the urine and elevated mean blood pressure and heart rate during bladder hydrodistention. Sympathetic overactivity can be observed in several human painful diseases and in rat pain models.

Previous studies showed that chronic adrenergic stimulation in rats induced pain behaviour, increased voiding frequency and urothelial disruption that mimic BPS/IC. In the present work we determined the alpha 1 adrenoceptor (AR) subtype implicated in the process.

A group of female Wistar rats were injected with 2.5mg/kg/day phenylephrine (PHE; s.c., for 14 days). Visceral pain behaviour and voiding pattern were analysed before and 14 days after PHE treatment. At day 15, animals were anaesthetised with urethane (1.2 g/kg) and cystometries were performed for 2h. Animals were then perfused with paraformaldehyde and the bladder and L6 spinal segment were harvested. These experiments were repeated in rats co-treated with PHE+ 0.2 mg/kg/day silodosin (predominant α 1A-AR antagonist) or with PHE+ 0.9 mg/kg/day naftopidil (predominant α 1D-AR antagonist). Bladder sections were stained with haematoxylin-eosin to analyse urothelium morphology, with toluidine Blue to study mast cells, and immunoreacted (IR) against Caspase 3 (active form) to investigate urothelial apoptosis. L6 spinal cord sections were IR against Fos.

PHE induced visceral pain behaviour which was completely counteracted by silodosin and partially by naftopidil. PHE induced an increase of bladder reflex activity from 0.4 \pm 0.1 to 2.5 \pm 0.8 bladder contractions/min, which was reversed by both silodosin (0.6 \pm 0.2 bladder contractions/min) and naftopidil (0.5 \pm 0.1 bladder contractions/min). PHE induced urothelial disruption in 53 \pm 15% of urothelium length. Silodosin decreased this length to 12 \pm 13%. In naftopidil-treated rats disruption occupied 82 \pm 14% of urothelial length. The areas of urothelial disruption showed an intense caspase 3-IR. PHE induced mast cell infiltration (17 \pm 6 cell/mm²) in the suburothelial layers. Silodosin and naftopidil did not change this infiltration (14 \pm 4 and to 20 \pm 5 cell/mm², respectively). At L6 spinal cord level, both silodosin and naftopidil reversed the PHE-induced increase in the number of spinal Fos expressing neurons.

Alpha 1a- is more effective than alpha1d-blockade to revert bladder pain and urothelial dysfunction induced by chronic adrenergic stimulation. These results may be relevant for the clinical use of specific alpha 1a adrenoceptors antagonists in BPS/IC.

Ecto-enzymes involved in the metabolism of adenine nucleotides in the rat ileum: distribution, enzymatic activity and neuromodulatory role

S. Gonçalves-Monteiro¹, M. Duarte-Araújo¹, C. Tavares¹, M.T. Magalhães-Cardoso¹, F. Ferreirinha¹, J. Sévigny² & P. Correia-de-Sá¹

¹Laboratório de Farmacologia e Neurobiologia, UMIB, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, Portugal, and ²Centre de Recherche en Rhumatologie et Immunologie, Centre Hospitalier Universitaire de Québec, Québec, QC, Canada

The myenteric plexus (MP) is the main responsible for gastrointestinal motility. We and others demonstrated that purines (ATP, ADP and ADO) play important neuromodulatory roles at the myenteric neuromuscular synapse. ATP transiently increases spontaneous ACh release, an effect that is cut-short by its extracellular catabolism by membrane-bound ectonucleotidases. ADP generated from the catabolism of extracellular ATP may restrain stimulation-evoked acetylcholine (ACh) release via the activation of inhibitory P2Y₁ [1]. On its own, ADO acts predominantly to facilitate evoked transmitters release (both ACh and substance P) via A_{2A} receptors located on myenteric nerve terminals (see *e.g.* [2,3]). Considering that among the several players of the “purinome” the distribution, enzymatic activity and neuromodulatory role of ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases) and ecto-5'-nucleotidase (E-5'-NTase) lack considerable attention in the myenteric plexus, we used the rat ileum to investigate this constrain.

Confocal microscopy studies demonstrate that the rat ileum lacks immunoreactivity against E-NTPDase1. E-NTPDase2 and 3 are abundantly expressed in PGP 9.5-positive myenteric ganglia and in vimentin-positive intramuscular interstitial cells. E-NTPDase2 immunoreactivity is also evident in GFAP-positive glial cells, whereas E-NTPDase3 immunoreactivity is co-localized predominantly with synaptophysin in myenteric nerve terminals. HPLC analysis of the extracellular metabolism of ATP (30 μM, $t_{1/2}=7\pm 1$ min, $n=6$) showed that it was delayed in the presence of POM-1 (100 μM, $t_{1/2}=12\pm 3$ min, $n=4$), a non-selective E-NTPDases inhibitor and ARL67156 (100 μM, $t_{1/2}=14\pm 1$ min, $n=4$), a preferential E-NTPDase1 and 3 inhibitor. In the presence of these two inhibitors the formation of ADP, AMP and ADO, as well as tissue deposition of phosphates (Wachstein-Meisel histochemical reaction) [2], was significantly ($P<0.05$) decreased when ATP was used as a substrate. Regarding E-5'-NTase, immunolabeling was predominant in smooth muscle fibers. Inhibition of E-5'-NTase with α,β -methyleneADP (AOPCP, 200 μM) prevented the deposition of phosphates in enteric muscular layers following incubation with AMP (100 μM). Inhibition of E-NTPDase1 and 3 with ARL67156 and E-5'-NTase with AOPCP reduced [³H]ACh release from stimulated (5Hz, 1350 pulses) myenteric neurons by $42\pm 4\%$, ($n=4$) and $36\pm 5\%$ ($n=3$), respectively. These results indicate that inhibition of the extracellular catabolism of released nucleotides and, thus, ADO formation downregulates transmitter release, implying that the nucleoside exerts a predominant facilitatory tonus as previously predicted [2,3]. In the presence of ARL67156, ADP formation might not be sufficient to activate inhibitory P2Y₁ receptors, because ARL67156-induced inhibition of evoked [³H]ACh release was not affected by the P2Y₁ receptor antagonist, MRS2179 (300 nM). Localization of E-NTPDase 2 and 3 at the myenteric neuromuscular synapse yields AMP formation from both ATP and ADP, which may be subsequently dephosphorylated into ADO by E-5'-NTase bound to smooth muscle fibers. This might explain why formation of ADO from the extracellular catabolism of released adenine nucleotides activates preferentially facilitatory (A_{2A}) receptors on myenteric nerve terminals, but not inhibitory A₁ receptors located on ganglion cell bodies [3]. Thus, the localization of substrate-specific E-NTPDases and E-5'-NTase at the myenteric neuromuscular synapse of the rat ileum affords some degree of selectivity to manipulate enteric motility.

[1] Duarte-Araújo *et al.*, 2009. *Br J Pharmacol.*, 156: 519-533; [2] Duarte-Araújo *et al.*, 2004. *Br J Pharmacol.*, 141: 925-934; [3] Vieira *et al.*, 2011, *Neurochem Int.* 59: 1043-55 ; [4] Lavoie *et al.*, 2011. *Am J Physiol (Gastroint Liver Physiol)*, 300: 608-620.

Work supported by FCT (FEDER funding, PEst-OE/SAU/UI0215/2011).

Macrophage-targeted nanoparticles: a novel approach towards rheumatoid arthritis theranostics

C. Moura^{1,2}, J. das Neves³, S. Reis² and B. Sarmiento^{3,4}

¹ FEUP – Faculdade de Engenharia da Universidade do Porto, Porto, Portugal

² REQUIMTE, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal

³ CESPUI/IIINFACTS – Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Instituto Superior de Ciências da Saúde-Norte, Gandra, Portugal

⁴ INEB – Instituto de Engenharia Biomédica, Porto, Portugal

Rheumatoid arthritis (RA) is one of the most common and severe autoimmune diseases related to joints. Regrettably, RA inflammatory process remains puzzling, and finding effective therapies for the disease as well as new means for its early diagnosis have been daunting tasks. Macrophages play a pivotal role in the features and progress of RA, and effective diagnosis and therapy may encompass the ability to target these cells. In this work, a new approach for RA theranostics is proposed, taking advantage of the vast potential of nanomedicine. This study aimed at the development of a nanoparticulate system for RA imaging and therapy by intravenous administration, based on poly(lactic-co-glycolic) acid (PLGA) nanoparticles (NPs), actively targeting macrophages.

PLGA NPs were prepared by a single emulsion-evaporation method and comprised the co-association of superparamagnetic iron oxide nanoparticles (SPIONs) and methotrexate (MTX), for RA imaging and therapy purposes, respectively. The NPs were further functionalised with a monoclonal antibody (mAb) against the macrophage specific cell surface receptor, CD64, which is overly expressed in RA. Different PLGA-based NPs were prepared in order to compare the effects of each component (MTX, SPIONs and anti-CD64 mAb) on NP properties. Physicochemical properties of the NPs were characterised regarding mean particle size, polydispersion, zeta potential and morphology, as well as by determining SPIONs and MTX association and anti-CD64 mAb conjugation efficiencies. Lastly, the cytotoxicity potential of developed NPs to murine RAW 264.7 macrophages was assessed using standard MTT and LDH assays.

NPs presented mean diameter values below 200 nm and charges lower than -16 mV in all cases. The association with either MTX or SPIONs did not affect the NPs properties significantly. However, the conjugation of the anti-CD64 mAb caused an expected slight increase on both size (130-160 nm to 160-200 nm) and surface charge (-30 mV to -20 mV). Transmission electron microscopy (TEM) confirmed the association of SPIONs within the PLGA matrix. Successful antibody conjugation was also suggested by TEM, as noted by the appearance of a characteristic 'corona' surrounding NPs. Both antibody conjugation and MTX association were further confirmed by Fourier transform infrared spectroscopy (FT-IR) and quantified as 35% (Bradford protein assay) and 75% (HPLC-UV), respectively. *In vitro* toxicity studies highlighted the high toxicity of MTX, since MTX-loaded NPs caused a significant reduction on the levels of RAW 264.7 cell metabolism and proliferation. Conversely, MTX-free NPs were only toxic when administrated at the highest concentration tested thus suggesting that PLGA NPs possess low toxicity and biocompatibility.

In conclusion, multifunctional anti-CD64 mAb-modified PLGA NPs for the combined delivery of MTX and SPIONs were successfully prepared and characterised. Proposed NPs have the potential to provide a new theranostic approach for RA management.

Delivery of indomethacin loaded liposomes to Arthritic Joints

M. Machado^{1,2}, C. Nunes ², S. Lima ² and S. Reis ²

¹ Faculty of Engineering, University of Porto, Portugal

² REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal

Rheumatoid Arthritis (RA) is a chronic, systemic, inflammatory autoimmune disease that targets preferentially the synovial tissue. It affects 1% of the population of the world and it is more common in woman than man, in a ratio of 3:1. RA is treated recurring to several drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) which have several negative drawbacks such as low bioavailability, high clearance rates, high and frequent dosing which increase the risk of side effects.

To reduce the side-effects of NSAIDs, four different pH responsive liposomal formulations were prepared by the thin-film method using 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), cholesteryl hemisuccinate (CHEMS), Stearylamine (SA) and indomethacin. Using DPPE and CHEMS in a ratio 7:3 multilamellar vesicles (MLV) and large unilamellar vesicles (LUV) were prepared, also formulations containing DPPE, CHEMS and SA in a proportion 7:2.5:0.5 in the MLV and LUV structure were produced. All the formulations contained 1 mg/mL of indomethacin, also placebo formulations were prepared. The formulations were physicochemical characterized in terms of size, zeta potential, entrapment efficiency and loading capacity; also morphology was assessed using Transmission Electron Microscopy. Liposomes stability was evaluated throughout a month in order to study changes in size and zeta potential. Drug release was evaluated for 48 hours at pHs 7.4 and 5. Also, in vitro studies using cell lines of macrophage and fibroblasts, Raw 264.7 and L929, respectively, were performed to evaluate the cytotoxic character of liposomal formulations. Finally, in vitro permeation studies were done using Franz diffusion cells to assess the permeability through the skin for a period of 8 hours.

LUVs possessed a size around 120 nm and MLVs 220 nm, zeta potential below -30 mV and EE of 60% for all formulations tested. In which concerns morphology, at pH 7.4 liposomes in both structures possessed a round-shape morphology, whereas at pH 5, the liposomes only formed small aggregates. In which concerns cytotoxicity, neither indomethacin nor liposomes alone had a cytotoxic effect, however all the formulations containing indomethacin present a cytotoxic effect. Last but not least, regarding the diffusion of liposomes, the indomethacin passage through the skin was favored by the encapsulation into the liposomes.

In conclusion, it is possible to say that pH responsive liposomes were successfully prepared and shown to be promising particles for the treatment of rheumatoid arthritis.

Acknowledgments:

We thank the financial support through the project PP-IJUP2011-279.

Oxidative stress parameters in the brain and peripheral organs following exposure of adolescent rats to an MDMA neurotoxic regimen

R. Feio-Azevedo¹, V.M. Costa¹, D. Barbosa¹, A. Gomes¹, F.C. Pereira², M. Duarte-Araújo³, J.A. Duarte⁴, E. Fernandes⁵, M.L. Bastos¹, F. Carvalho¹ and J.P. Capela^{1,6}

¹REQUIMTE (Rede de Química e Tecnologia), Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal ²Farmacologia e Terapêutica Experimental, Instituto Biomédico de Investigação da Luz e Imagem (IBILI), Faculdade de Medicina, Universidade de Coimbra, Portugal ³Biotério do Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, Portugal ⁴CIAFEL, Faculdade de Desporto, Universidade do Porto, Portugal ⁵REQUIMTE, Laboratório de Química Aplicada, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Portugal ⁶Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Portugal

3,4-Methylenedioxymethamphetamine (MDMA) is a widespread drug of abuse commonly used by adolescents for its known psychoactive properties. Both in humans and in laboratory animals, MDMA neurotoxicity has been disclosed [1]. As MDMA safety during adolescence lacks experimental data, in the present study we aimed to assess the toxicity and oxidative stress related changes in the cortex, striatum, cerebellum, and hippocampus and in three peripheral organs, (liver, heart and kidneys), in an adolescent rat model while mimicking the human binge administration.

Adolescent male Wistar rats (7 weeks-old) were housed in individual cages and kept in a controlled ambient (temp 22°C; 12h light/dark cycles). Animals were divided in two groups: control (n=5) and MDMA group (n=5) and received, respectively, an intraperitoneal injection of a saline (NaCl 0.9%) or 5mg/Kg MDMA every 2 hours, to a total of 3 doses [2]. On the day of the administrations, the intradermic temperature of each animal was monitored for 7 h, through a neck implantable transponder. Until sacrifice, animal weight and food/water consumption were registered daily. Seven days after exposure, brain areas were dissected and the peripheral organs were collected. Total and oxidized glutathione (GSH/GSSG), ATP and quinoprotein (protein-bound quinone) levels were determined in the brain areas. GSH and GSSG, ATP and malonaldehyde (MDA) levels were quantified in the peripheral organs.

After the second MDMA dose, the increase in temperature was significantly higher in the MDMA-treated group ($p < 0.0001$) whose temperature was kept significantly higher for 3 hours after the third dose. There was a significantly decrease ($p < 0.05$) on the body weight of the MDMA group on the first day post-MDMA administration, despite no observed differences on food or water intake/day/weight. In the brain areas, no differences were found in ATP content or GSH/GSSG ratio. However, quinoprotein levels were significantly higher in the cortex of the MDMA group ($p < 0.02$). In heart, liver, and kidneys no differences were found in the ATP levels or in the GSH/GSSG ratio. Moreover, only the liver had detectable MDA values, but without differences among the two groups.

In conclusion, MDMA binge administration induced a hyperthermic response in adolescent rats and a decrease of body weight in the first post-MDMA day. After 7 days following MDMA administration, no significant oxidative stress related changes were found in any organ, except for the cortex, where higher quinoprotein levels were found.

Acknowledgments: Supported by the project (PTDC/SAU-FCF/102958/2008), under the frame of “Programa Operacional Temático Fatores de Competitividade (COMPTE)” and “Fundo Comunitário Europeu (FEDER)”.

References:

- [1] Capela, J.P., Carmo, H., Remião, F., Bastos, M.L., Meisel, A. and Carvalho, F. (2009), *Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: an overview*, *Molecular Neurobiology* 39, 210-271.
- [2] Goni-Allo, B., Mathúna, B., Segura, M., Puerta, E., Lasheras, B., DeLaTorre, R., Aguirre, N. (2008), *The relationship between core body temperature and 3,4-methylenedioxymethamphetamine metabolism in rats: implications for neurotoxicity*, *Psychopharmacology* 197, 263-278.



A2

**PUBLIC
HEALTH &
EPIDEMIOLOGY I**

**V
PARALLEL
ORAL
SESSIONS**

Food Portion Sizes

A. Goios¹, T. Amaral¹, C. Afonso¹, A. Oliveira¹, A. Costa¹, J. Nogueira¹, L. Rodrigues¹, M. Liz¹

¹ Faculty of Food and Nutrition Sciences, University of Porto, Portugal.

Introduction: An essential step in measuring food intake is the quantification of the portion size of each food item consumed. In recent years, Portuguese dietary habits have changed substantially. Additionally, food supply has increased, which leads to an increased diversity of available foods. There are not among us updated descriptions of weight and food portions, so its development is of utmost importance.

Objective: To update the food quantification data published on “Food Portion Sizes” research developed in 1993 [1].

Methods: Food average portion sizes were obtained by a group of eight researchers. National and imported foods available in North of Portugal markets were used and weighed portions were calculated considering the different presentation forms and cooking methods, presenting the values of main forms of consumption of foods.

Results: Average portion sizes of 1102 foods were obtained. In global, 15284 weighing were conducted.

Conclusion: Present study provides an updated, quick and valid alternative to weights of foods and their portion sizes which are available in the North of Portugal. These data may be useful not only in food intake assessment but also in counseling and food education.

References:

[1] Amaral, T., Nogueira, C., Paiva, I., Lopes, C., Cabral, S., Fernandes, P., Barros, V., Silva, T., Calhau C., Cardoso, R. and Almeida, M. (1993), *Pesos e Porções de Alimentos*, Revista Portuguesa de Nutrição, 5 (2), pp.13-23.

Screening of virulence factors in swine *Enterococcus faecium* strains with zoonotic potential

Elsa Martins¹, Ana R. Freitas¹, Carla Novais¹, Teresa M. Coque², Luísa Peixe¹

¹ Department of Microbiology, Faculty of Pharmacy, University of Porto, Portugal.

² Servicio de Microbiología, Hospital Ramón y Cajal, Madrid, Spain.

Specific virulence factors (VFs) have been strongly associated with clinical *Enterococcus faecium* (Efm), but the prevalence of recently described cell wall-anchored surface proteins has been scarcely explored among animals. We aimed to determine the extended virulence profile of isolates from pig-related sources in different countries.

Representative Efm isolates (n=44) previously identified among a collection of multi-resistant strains from swine's faeces in Portugal, Denmark, Switzerland and the USA (n=18), and the animal setting in Portugal (different pig farms, n=26) were selected for this study. This collection includes isolates recovered during wide surveillance studies performed in these countries during 1995-2008, some of which are widespread among pigs of EU countries since the mid-1990s. Clonal relatedness was assessed by PFGE and MLST. Screening for 18 VFs included *esp*-Efm (enterococcal surface protein), *hyl*-Efm (hyaluronidase-like gene), IS16 (conferring genomic plasticity), and 15 predicted cell-wall anchored *E. faecium* surface proteins (Fms) with typical characteristics of MSCRACMM- (microbial surface components recognizing adhesive matrix molecules) and/or pilus-encoding genes (e.g. the collagen adhesins *acm* and *scm*, or the pilus cluster *ebpABC*-fm) was performed by PCR/colony hybridization. Efm strains Aus0004 and C68 were used as positive controls.

Isolates clustered into CC17 (18%), CC5 (50%) or were identified as different singletons. The distribution of the 18 VFs was highly variable (0-95%), but a high number (≥ 7 VFs) was identified among all isolates. The *esp* gene (2%, only identified in a ST132-clone also found in Portuguese hospitals) and IS16 (9%) were confined to CC17 isolates, while *acm* was randomly distributed in different lineages except CC5 (45%). *hyl* and *fms18* were not identified. The occurrence of the remaining 13 MSCRACMM- genes ranged from 14% to 93%. The complete *ebpA*(23%)-*ebpB*(95%)-*ebpC*(93%) operon was found in 23% of isolates including all CC17. The *fms11*(14%)-*fms19*(30%)-*fms16*(30%) cluster was present in 14% of isolates, most of which belonged to CC17 (none of these genes was found in CC5). A few isolates (11%) from different lineages but CC5 carried the entire *fms14*(14%)-*fms17*(80%)-*fms13*(80%) cluster. *fms21*(91%) and *fms20*(43%) coexisted in 43% of isolates from all lineages. *scm* was predominant (93%) and *fms15* was found in 73% of isolates including all from CC5. Nine (*acm/ebpB/fms17/fms13/fms16/fms19/fms21*) and 4 (*ebpB/scm/fms15/fms21*) of the 18 genes were present in 100% of the CC17 and CC5 isolates, respectively. Complete gene clusters were not identified in CC5. The same virulence profile was established for similar PFGE types.

Our results demonstrate the considerable variability of VFs among Efm from swine with the lowest prevalence/diversity being observed in CC5. Complete gene clusters important in adherence/biofilm formation were predominant, but not exclusive of CC17. The exclusive association of specific VFs (*esp*/IS16) with swine CC17 reinforces the possibility of strain transmission between human and animal hosts.

Nutritional intake adequacy in fourth grade children of Porto municipality –socioeconomic and anthropometric influences

S. Bastos¹, M. Liz Martins¹

¹ Faculty of Nutrition and Food Sciences of University of Porto, Portugal.

Introduction: An adequate nutritional intake in children and adolescents has been associated with a lower risk of developing obesity in child and adulthood, as well as health problems at long term. At the same time, lower socioeconomic level has been associated with nutritional intake inadequacy, as well as high prevalence of overweight.

Objective: Evaluate nutritional intake adequacy in fourth grade children of Porto municipality and the relation with socioeconomics and anthropometrics influences.

Methods: A stratified clustered sample was selected and all fourth grade children attending to 21 public primary schools in the municipality of Porto were involved. A 24-hour recall questionnaire was applied to children to evaluate nutritional intake. Sociodemographic (age, sex, level of social support and parents education) and anthropometric (weight, height, z-score for the body mass index) information were also collected. Nutritional composition was analyzed by *The Food Processor SQL*® program.

Results: 48,6% of the children were male and 42,3% were overweight or obese. Energy, proteins and sugar intakes were above recommendations for the aged group, only 12,7% of children met the recommendations for proteins and 3,8% for sugar. The breakfast and the lunch were the only meals that met the energy recommendations. It was observed higher energy and sugar intake through the day for children with financial support for school lunch and whose parents had a lower level of education. On the other hand, children with overweight and obesity revealed a lower energy intake.

Conclusion: Our findings suggested a nutritional inadequacy of children's diet during the day. It was found an association between high energy and sugar intake and a lower socioeconomic level. Nevertheless, there was not found any significative association with anthropometric factors.

Protozoa colonization in Portuguese population on peritoneal dialysis

I. Correia¹, J. Barbosa^{1,2}, L. Simões-Silva³, M. João Sousa^{3,4}, M. Pestana^{3,4,5}, C. Santos-Araujo^{3,4}, I. Soares-Silva³, B. Sampaio-Maia^{1,3}

¹Faculty of Dental Medicine, University of Porto.

² Faculty of Medicine, University of Porto.

³ Nephrology and Infectious Diseases Research and Development Group, INEB-(I3S), University of Porto.

⁴Department of Nephrology, São João Hospital Center, EPE.

⁵Department of Renal, Urological and Infectious Diseases, Faculty of Medicine, University of Porto.

Introduction: The prevalence of chronic kidney disease (CKD) is increasing worldwide. Peritoneal dialysis (PD) is a home-based and widely used therapy of renal replacement. Despite the improvements in this renal replacement therapy, peritonitis is still one of the most important causes of technique failure in peritoneal dialysis. However, up to 20% of peritonitis are of undetermined cause. In these patients with persistent culture-negative peritonitis, consideration should be given to unusual microorganisms, such as protozoa. So, the aim of the present study was to evaluate the presence of protozoa in PD effluent from Portuguese CKD patients undergoing PD.

Methods: Clinical and demographic information was collected from 41 PD patients. Samples of peritoneal dialysis effluent were also collected in an aseptic environment. For protozoa identification it was performed direct wet smear with Lugol's solution and smears staining with Giemsa and modified Ziehl-Neelsen's. PD patients presenting protozoa in PD effluent were further characterized regarding age, gender, educational level, profession, CKD etiology, diabetes and environment/social condition such as children cohabiting, domestic poultry, consumption water source, raw vegetable (especially lettuce) consumption and rivers or lakes swimming frequency.

Results: We found protozoa on dialysis effluent samples collected from 5 (12%) different PD patients. In 2 PD patients we found *Blastocystis hominis* and, in the other 3 PD patients we found *Entamoeba sp*, *Giardia sp* and *Endolimax nana*. All the protozoa-positive PD patients presented low education level, different CDK etiologies and were not diabetics. Interestingly, 40% were female, have contact with domestic poultry, consume water from their private well and normally eat raw fresh vegetables. Although similar information was obtained between genders, no correlations between specific protozoa microorganism and environmental/ social conditions were found.

Conclusion: In the present study, 12% of PD patients presented asymptomatic colonization of dialysis effluent with *Blastocystis hominis*, *Entamoeba sp*, *Giardia sp* or *Endolimax nana*, highlighting the need for a more systematic screening of protozoa in Portuguese PD population. The clinical impact of these sub-clinical infections should be further investigated. It is also noteworthy that there are no previous studies in this field in Portugal.

Syphilis hospitalizations in Portugal over the last decade

B. Sousa-Pinto¹, A. Freitas¹ and C. Lisboa²

¹Department of Health Information and Decision Sciences, Faculty of Medicine, University of Porto, Portugal

²Department of Microbiology, Faculty of Medicine, University of Porto, Portugal

Background: Several studies have reported an increase of syphilis incidence over the last decade, notably in Europe and in the United States of America [1-3]. However, information concerning syphilis epidemiology is missing in Portugal and, therefore, we aim to characterize syphilis-associated hospitalizations occurred in Portugal between 2000 and 2010.

Methods: A database containing all hospitalizations occurred in Mainland Portugal public hospitals was analysed. Congenital and non-congenital syphilis-associated hospitalizations were studied in what concerns to the patients' gender, age, district and comorbidities. Hospital mortality rates were also analysed. Rates per 100.000 inhabitants were calculated based on 2011 Portuguese census data.

Results: Between 2000 and 2010, there were a total of 7726 syphilis-related hospitalizations in Mainland Portugal. The rate of congenital syphilis hospitalizations per 100.000 inhabitants decreased 73% in that period, while the rate of non-congenital syphilis hospitalizations decreased 11%. 60% of non-congenital syphilis hospitalizations occurred in males, where hospitalizations rates increased 16%. In females, non-congenital syphilis hospitalizations rates decreased 35%. The rate of syphilis hospitalizations increased among patients aged over 45 years old, but decreased among younger patients. The highest syphilis hospitalizations rates were found in the coastal and more populous districts. The percentage of syphilis episodes presenting cardiovascular and neuropsychiatric comorbidities increased (158% and 126%, respectively), while the percentage of syphilis episodes presenting HIV co-infection decreased by one-third. HIV co-infection was particularly frequent among younger patients and with recent syphilis stages, while neuropsychiatric and cardiovascular comorbidities were more common among older patients and with late syphilis stages. In-hospital mortality rates rose 19%.

Conclusions: Between 2000 and 2010, syphilis hospitalizations rates decreased in Portugal. However, non-congenital syphilis hospitalizations increased among males and among the elderly. When compared to other European countries, Portugal appears to present an atypical pattern concerning syphilis epidemiology, although care should be taken when analysing hospitalizations and administrative data.

References:

- [1] Bremer, V. et al. (2012), *Syphilis on the rise again in Germany--results from surveillance data for 2011*, Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, 17 (29).
- [2] Jebbari, H. et al. (2011), *Variations in the epidemiology of primary, secondary and early latent syphilis, England and Wales: 1999 to 2008*, Sexually transmitted infections, 87(3):191-8.
- [3] Bernstein, K.T. et al. (2013), *Epidemiologic characteristics of an ongoing syphilis epidemic among men who have sex with men, San Francisco*, Sexually transmitted diseases, 40(1): 11-7

Study of food waste in primary schools and kindergartens

D. Familiar¹, A. Rocha¹.

¹ Faculdade de Ciências da Nutrição e Alimentação, Universidade do Porto, Portugal.

Nowadays there is a growing concern about the quality of school lunch. Studying the provision of meals regarding the production of food waste allows understanding if children are consuming a lunch that meets their nutritional needs. Determining the causes and determinants of waste may contribute to the improvement of service quality.

This research work aimed to evaluate the food waste during lunch at three Portuguese primary schools and kindergartens.

The leftovers and plate waste resulting from the school lunch were evaluated by meal components (meat / fish, the main component of carbohydrates and accompanying vegetables).

There was obtained an average of 15,4% of leftovers. According to Vaz [1], this value is considered unacceptable (acceptable limit lies on 3%). The average of plate waste obtained was 27,5% and according to Aragão [2], this figure is also considered unacceptable (acceptable limit lies on 10%).

The accompanying vegetables are the dish component determining the highest amount of waste.

Considering the social importance and nutritional benefits of school lunch, the results of food waste are a matter of concern. The amount of leftovers and plate waste obtained are considered unacceptable and reflect the need to improve the planning of meals and menus adequacy to consumer preferences.

References:

[1] Vaz, C. (2006), *Restaurantes: controlando custos e aumentando lucro*, LGE, Brasília.

[2] Aragão, M. (2005), *Controle da aceitação de refeições em uma unidade de alimentação institucional da cidade de Fortaleza – CE*, Universidade Estadual do Ceará, Fortaleza.

Hepatitis E virus in wild boar for human consumption

R.M.S. Oliveira¹, J.R. Mesquita^{1,2}, M.S.J. Nascimento¹

¹ Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal

² Agrarian Superior School of the Polytechnic Institute of Viseu, Portugal

Hepatitis E virus (HEV) is nowadays the major etiological agent of enteric-transmitted hepatitis worldwide and a public health concern. Zoonotic foodborne transmission plays an important role in industrialized countries, where cases of infection with genotype 3 HEV (HEV3) due to consumption of raw or undercooked pork, deer and wild boar have been reported [1]. Minimal information is presently available about the circulation in wild boar in Europe. However, the few studies available show prevalence rates in wild boar much lower than in domestic pigs [2].

The aim of the present work was to study the presence of HEV in stools of wild boar (*Sus scrofa*) confined population in Center of Portugal, bred for human consumption.

A total of 40 stool samples from wild boar were collected in February 2013. HEV RNA was extracted/purified with QIAamp Viral RNA Mini Kit (Qiagen, Germany) and detected by nested RT-PCR with the sets of primers described elsewhere [3]. The amplified products were separated by electrophoresis using a 0.8% agarose gel (SeaKem LE Agarose, Lonza, USA) and the amplicons with the expected size (330 bp) were purified (GRS PCR & Gel Band Purification Kit, GRiSP, Portugal) and sequenced (StabVida, Portugal). Sequences were edited and aligned with the BioEdit software package, version 2.1 (Ibis Biosciences, Carlsbad, USA). Phylogenetic analysis was performed using MEGA version 5.0 software.

Five stool samples of the 40 studied (12.5%) presented the expected amplified product. Phylogenetic analysis showed that all the amplicons clustered with HEV genotype 3, subgenotype 3a (Fig.1), confirming that a single strain was circulating within the wild boar breeding farm.

In conclusion, this study confirmed the circulation of HEV3 in wild boar of Portugal. Hence, a potential public health problem could arise from the consumption of raw or undercooked meat of infected wild boar.

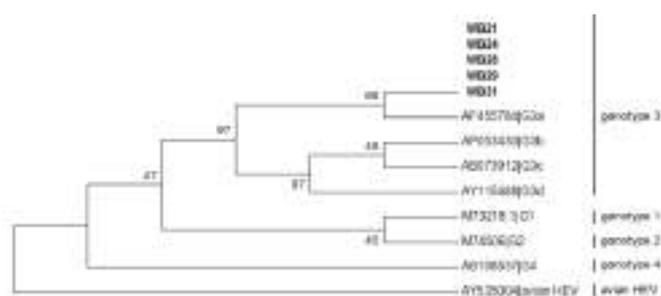


Fig 1. Phylogenetic analysis of HEV strains circulating in wild boar

[1] Kumar S (2013), *Hepatitis E virus: the current scenario*. Int J Infect Dis., 17(4): p. e228-33.

[2] de Deus N(2008), *Epidemiological study of hepatitis E virus infection in European wild boars (Sus scrofa) in Spain*. Vet Microbiol. 129(1-2): p. 163-70.

[3] Johne R (2010), *Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR*. J Gen Virol. 91(Pt 3): p. 750-8.



A3

PHYSICS I

V
PARALLEL
ORAL
SESSIONS

Spatial variations of the fine-structure constant in symmetron models

Marvin F. Silva^{1,2}, C.J.A.P Martins², Hans A. Winther³ and David F. Mota³

¹ Faculdade de Ciências, Universidade do Porto, Portugal

² Centro de Astrofísica, Universidade do Porto, Portugal

³ Institute of Theoretical Astrophysics, Norway

We investigate the variation of the fine-structure constant, α , in symmetron models using N-body simulations in which the full spatial distribution of α at different redshifts has been calculated.

In particular, we obtain simulated sky maps for this variation, and determine its power spectrum. We find that in high-density regions of space (such as deep inside dark matter halos) the value of α approaches the value measured on Earth. In the low-density outskirts of halos the scalar field value can approach the symmetry breaking value and leads to significantly different values of α .

If the scalar-photon coupling strength is of order unity we find that the variation of α inside dark matter halos can be of the same magnitude as the recent claims by Webb et al. [1] of a dipole variation. Importantly, our results also show that with low-redshift symmetry breaking these models exhibit some dependence of α on lookback time (as opposed to a pure spatial dipole) which could in principle be detected by sufficiently accurate spectroscopic measurements, such as those of ALMA and the ELT-HIRES.

References:

[1] J. A. King, J. K. Webb, M. T. Murphy, V. V. Flambaum, R. F. Carswell, M. B. Bainbridge, M. R. Wilczynska, and F. E. Koch, MNRAS 422, 3370 (Jun. 2012)

Cosmic Superstrings in the Planck Era

J.P.P. Vieira^{1,2} and C.J.A.P. Martins¹

¹ Centre of Astrophysics, University of Porto, Portugal.

² Department of Physics and Astronomy, Faculty of Sciences, University of Porto, Portugal.

Topological defects are some of the most fascinating astrophysical objects ever hypothesized. These are persistent concentrations of energy which are inevitably formed in phase transitions in the early Universe [1,2], and their detection and characterization would provide unique means of looking into that exciting era of cosmic history.

Cosmic strings have an array of surprising properties – for example, despite being practically one-dimensional (they are expected to be thinner than a proton), a realistic string has a length of cosmological scale, only a few meters of which should weigh as much as the Earth. Adding to that the fact that they are expected to be produced in a very broad class of cosmological scenarios [3], it is easy to understand why the study of these defects is so important in cosmology.

Understanding the cosmological evolution of realistic models of cosmic string networks is an open problem which we intend to tackle in this work. In particular, we mean to characterize specific cosmological implications of cosmic superstring networks - cosmic superstrings are basically superstrings (from string theory) with cosmological scales which are predicted to be formed at the end of an inflationary epoch.

In this talk, the theory of the formation and evolution of cosmic strings will be reviewed, and our first attempts to go beyond the simplest models available [4-6] will be presented. By the end of this talk, the audience should have a good understanding of the basic theory of cosmic (super)strings as well as a notion of how we are trying to solve some major problems in this area.

Acknowledgements:

This work is done in the context of a Stimulus Grant from the Calouste Gulbenkian Foundation (Ref. 132590), with additional support from the project PTDC/FIS/111725/2009 from FCT. C.J.M. is supported by an FCT Research Professorship, contract reference IF/00064/2012, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

References:

- [1] Vilenkin, A. and Shellard, E.P.S. (2000), *Cosmic Strings and Other Topological Defects*, Cambridge University Press.
- [2] Kibble, T.W.B. (1976), *Topology of Cosmic Domains and Strings*, J. Phys. A9 1387.
- [3] Achúcarro, A. and Martins, C.J.A.P. (2009), *Cosmic Strings*, in *Encyclopaedia of Complexity and Systems Science*, R. Meyers (Ed.), Springer, New York.
- [4] Martins, C.J.A.P. and Shellard, E.P.S. (2002), *Extending the velocity-dependent one-scale string evolution model*, Phys. Rev. D65, 043514.
- [5] Martins, C.J.A.P. and Shellard, E.P.S. (2006), *Fractal properties and small-scale structure of cosmic string networks*, Phys. Rev. D73, 043515.
- [6] Avgoustidis, A. and Shellard, E.P.S. (2008), *Velocity-Dependent Models for Non-Abelian/Entangled String Networks*, Phys. Rev. D78, 103510.

Astrophysical Constraints on the BSBM Model

P.Leal^{1,2}, L.Ventura^{1,2}, C. J. A. P. Martins¹

¹ Centro de Astrofísica da Universidade do Porto, Rua das Estrelas, 4150-762 Porto, Portugal.

² Departamento de Física e Astronomia, Faculdade de Ciências, Universidade do Porto, Portugal

Recent spectroscopic measurements of the fine-structure constant, obtained from low-density absorption clouds along the line of sight of quasars [1], suggest a possible spacetime variation at the parts per million level, over a time span of about a quarter of the current age of the universe.

These astrophysical measurements can be used to set constraints on cosmological scenarios which include dynamical scalar fields. One relatively simple example of such a model is the BSBM one [2], in which a scalar field coupled to the electromagnetic sector will lead to variations of the fine-structure constant, with otherwise little change on the overall cosmological dynamics.

In this work we use the data of [1] to obtain up-to-date constraints on the BSBM. In particular we discuss how the constraints on the scalar field coupling depend on the subset of the data of [1] being used, and specifically the differences between the data obtained with the HIRES spectrograph at the Keck telescope and that from the UVES spectrograph at the VLT.

We also do a similar analysis for a more recent dataset, which despite containing a smaller number of astrophysical measurements is arguably more reliable (since the data reduction procedures are expected to provide more robust estimates of systematic uncertainties).

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal), with additional support from grant PP-IJUP2011-212 (funded by U. Porto and Santander-Totta). C.J.M. is supported by an FCT Research Professorship, contract reference IF/00064/2012, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

References:

[1] Webb, J.K. *et al.*, Indications of a spatial variation of the fine-structure constant, *Phys. Rev. Lett.* 107, 191101 (2011).

[2] Barrow, J.D, Sandvik, H, Magueijo, J., Behavior of varying-alpha cosmologies, *Phys. Rev. D* 65, 063504 (2002)

Evolution of the Fine-structure Constant in the BSBM Model

L.Ventura^{1,2}, **P.Leal**^{1,2}, **C. J. A. P. Martins**¹

¹ Centro de Astrofísica da Universidade do Porto, Rua das Estrelas, 4150-762 Porto, Portugal.

² Departamento de Física e Astronomia, Faculdade de Ciências, Universidade do Porto, Portugal

One of the deepest questions in all of science is that of the universality of physical laws – whether the laws we’re familiar with in the laboratory also apply elsewhere in the universe. Observationally, this can be addressed by testing the stability (in space and/or in time) of nature’s dimensionless fundamental couplings.

Recent observational evidence [1], suggesting a possible spacetime variation of the fine-structure constant (a measure of the strength of the electromagnetic interaction) has provided further motivation to study models where these couplings do vary.

One of the simplest such models is the Bekenstein-Sandvik-Barrow-Magueijo (BSBM) model [2], which introduces a scalar field coupled to the electromagnetic sector in such a way that although it may in principle account for the variation of the fine structure constant, it can do so without significantly affecting the background dynamics of the universe. This allows it to satisfy ‘classical’ cosmological constraints, and the only new model phenomenology will be that associated with the evolution of the fine-structure constant itself.

In this work we will discuss the evolution of the fine-structure constant in the recent (low-redshift) universe in the framework of the BSBM model, describing the types of behavior that may be obtained and how they compare to purely phenomenological parametrizations.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal), with additional support from grant PP-IJUP2011-212 (funded by U. Porto and Santander-Totta). C.J.M. is supported by an FCT Research Professorship, contract reference IF/00064/2012, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

References:

[1] Webb, J.K. *et al.*, Indications of a spatial variation of the fine-structure constant, *Phys. Rev. Lett.* 107, 191101 (2011).

[2] Barrow, J.D, Sandvik, H, Magueijo, J., Behavior of varying-alpha cosmologies, *Phys. Rev. D* 65, 063504 (2002)

Cavity ring-down for remote sensing

D. J. Passos, S. O. Silva, M. J. Marques and O. Frazão

Department of Physics and Astronomy, Faculty of Sciences, University of Porto, Portugal.
Institute for Systems and Computer Engineering of Porto (INESC Porto), Portugal

The cavity ring down spectroscopy (CRDS) is a widely used technology for chemical and molecular analysis in real time [1]. The evolution of this technique led to the development of diverse configurations based on the same underlying principle, i.e., a resonant optical cavity with high reflectivity mirrors.

The development of a fiber optic based CRD, which uses a fiber loop as resonant cavity, has been gaining popularity in the community, for presenting a potential alternative to the usual CRD [2]. In this manner, the conceptual study of the CRD technique became a focus of attention in the area of optical spectroscopy, for the last decade.

Recently these ideas have been applied to optical fiber sensors of physical parameters (deformation [3], pressure [4], temperature [5]...). In this kind of sensing configuration, optical impulses are inserted in a loop of optical fiber, which includes a physical sensor, and the decay of the circulating pulse is monitored. High sensitivities can be attained in this way.

In this work, we present the potential of these configurations for remote sensing by using an Optical Time Domain Reflectometer (OTDR) to send optical impulses to a fiber cavity ring placed after a long extension of fiber (~20 km). To illustrate the sensitivity of the setup we placed and characterized a displacement intensity sensor in the loop.

The cavity ring-down technique is seen to hold some potential for remote sensing, through its implementation on optical fibers.

References:

- [1] G. Berden, R. Peeters, and G. Meijer, (2000), *Cavity ring-down spectroscopy: Experimental schemes and applications*, Int. Rev. Phys. Chem., vol. 19, pp. 565-607
- [2] G. Stewart, K. Atherton, and B. Culshaw, (2004), *Cavity-enhanced spectroscopy in fiber cavities*, Opt. Lett., vol. 29, no. 5, pp. 442-444
- [3] W. P. Tarsa D. M. Brzozowski, P. Rabinowitz, and K. K. Lehmann, (2004), *Cavity ring-down strain gauge*, Opt. Lett., vol. 29, no. 12, pp. 1339-1341
- [4] C. Wang and S. T. Scherrer, (2004), *Fiber ring-down pressure sensors*, Opt. Lett., vol. 29, no. 4, pp. 352-354
- [5] C. Wang, (2005), *Fiber ring-down temperature sensor*, Opt. Eng. Lett., vol. 44, no. 3, 030503

Evaporation of fluids in microstructured fibers

J. P. Moura^{1,2}, J. L. Santos^{1,2} and O. Frazão^{1,2}

¹ Department of Physics and Astronomy, Faculty of Sciences, University of Porto, Portugal

² Optoelectronics and Electronic Systems Unit, INESC TEC, Portugal

Due to the massive economic relevance of telecommunications, fiber optics is one of today's most prominent research areas and one of the most important technological achievements of the last century [1]. The development of fiber optics technology naturally led to different applications such as fiber lasers, supercontinuum light sources, particle scintillators and, in particular, fiber sensors. Optical fiber sensors are light, small, immune to electromagnetic interference and can easily be applied to remote and distributed sensing [2].

The need for tailoring specific fiber properties such as dispersion and birefringence resulted in the unprecedented ability to create a large variety of fiber designs at the micrometer scale – the so called *Microstructured Optical Fibers*. As an example, one can create microstructured fibers with holes along their length that allow the inflow of fluids near the fiber core. This way, light that propagates within the optical fiber can interact with the fluids, enabling their detection.

Recently, Preter et al. introduced a new technique in microstructured fibers for liquid sensing [3]. Using in-line microcavities in single-mode fibers, the authors realized the monitoring of the evaporation dynamics of volatile organic compounds as a means to identify them.

In this work the same concepts were applied to cavities in a suspended-core fiber tip. The time-dependent spectral response of a suspended-core fiber was coarsely monitored when its cavities were filled with acetone. At the same time the phenomenon was observed using an optical microscope. This procedure allows the measurement not only of intensity variations but also of spectral oscillations in terms of wavelength and visibility when the sensing head is exposed to acetone.

References:

[1] Agrawal, G. P (2002), *Fiber-Optic Communication Systems*, 3rd ed., John Wiley & Sons Inc, New York.

[2] Lee, B. (2003), *Review of the present status of optical fiber sensors*, *Optical Fiber Technology*, 9 (2), 57-79.

[3] Preter, E., Preložnik, B., Artel, V., Sukenik, C. N., Donlagic, D. and Zadok, A. (2013), *Monitoring the evaporation of fluids from fiber-optic micro-cell cavities*, *Sensors*, 13, 15261-15273.



A4

**PSYCHOLOGY-
CHOLOGY &
EDUCATION
SCIENCES IV**

**V
PARALLEL
ORAL
SESSIONS**

The forensic psychological assessment in child custody and parenting capacity cases: characterization of the expertise (GEAV as an analyzer).

J. Santos¹ and C. Manita²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

² Associate Professor of the Faculty of Psychology and Educational Sciences, University of Porto, Portugal; Director of the Office of Studies and Attendance of Batterers and Victims of the Faculty of Psychology and Educational Sciences.

The forensic psychological assessment in child custody and parenting capacity cases, in Portugal, is required, usually, when the child is at risk or there is a disagreement between the parents toward the exercise of parental responsibilities. This assessment focuses primarily on family dynamics, parenting capacity and psychological functioning of subjects evaluated.

This study aims to analyze the characteristics of forensic psychological assessment in these cases, particularly, at the level of requests made by the courts, the population assessed and expert answers. It aims also to contribute to an improvement of practices, through the further knowledge of the forensic psychological expertise.

To implement this study we used a mixed methodology, combining qualitative and quantitative methods. Initially, the content analysis allowed the exploitation of expertise, contributing to the creation of a grid of data collection and analysis, and these data were subsequently quantified, within a matrix of SPSS, and subjected to statistical analyzes. The sample of cases examined is composed of 50 forensic psychological expertise, available in the archives of the Office of Studies and Attendance of Batterers and Victims, in which child and parents were evaluated.

The data indicate that these forensic assessments were performed, on average, over three evaluation sessions and using different sources of information: query of procedural documents, clinical and family interviews and psychological tests. The dimensions more often referred in the expertise are: risk and protective factors associated to the minor, the parents and the family context; clinical symptoms and developmental dimensions of the assessed subjects; the overall level of development of the child; conflicts and family disruptions; the parenting capacity. When the expert concludes that there are no minimum competencies for the proper exercise of parenting, this conclusion appears statistically associated to the approval of physical punishment by parents, the presence of cognitive deficits in the parents, the lack of social support, negative economic conditions and the need for family supervision. On the other hand, the affective skills of parents and appropriate emotional and psychological functioning emerged strongly associated to the conclusion that there are minimum competencies for the exercise of parenting.

Scaffolding on Productivity and Quality of Life Narratives of Children in Institutional Care

S. Silva¹, M. Henriques¹, D. Teixeira¹, P. Saraiva¹ and E. Braga¹

¹ Department of Psychology, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Humans have been assumed to be *storytellers* over the ages. Through narratives, people construct meanings for their experiences, to know the world and to transform it. Thus, the human being uses language to signify their experiences, both to himself as to the others who share their stories with him. So, the narratives are one of the most fundamental aspects of human knowledge. In this study we will focus on the cognitive model-narrative, by which argues that the existence of a matrix narrative, allows the person moves away from his experience, enabling to construct different meanings.

This investigation has as its object of study the life stories of children in situations of institutional care, with and without adult support. The sample consists of 42 children, aged between 6 and 13 years old, and residing in seven institutions in the district of Porto. This study's main objectives are: to analyze the themes elicited by children in the construction of their life narratives; to map the development of production of narratives by children living in an institution; to evaluate the importance of scaffolding for building narrative; to analyze the relationship between productivity and quality of life narratives; to examine the existence of relations between cognitive skills and narrative production. For this purpose, we used three methods of analysis: content analysis, narrative productivity analysis and narrative quality analysis by Assessment System Matrix Narrative (S.A.M.N) [1].

A thematic analysis of the narratives of life allowed identifying 18 themes that stood out by the frequency which emerged in the speech of children or the pertinence in life stories of the participants. This analysis allowed us to realize that the *Institutionalization* theme is one of the most recurrent in the narratives of children living in institutions. The results showed that productivity was significantly higher in Narratives of Life Supported compared with Narrative Life Spontaneous and Short Autobiographical Narrative. We found a moderate association between the Productivity results and the SAMN results, which seems to indicate a relationship between quantity and quality in life narratives. No significant results were found for the existence of a relationship between cognitive skills and narrative production.

References:

[1] Gonçalves, O. & Henriques, M. (2006). *Sistema de avaliação da matriz narrativa*. Universidade do Minho, Braga.

Attachment, caregiving, emotional regulation and caregiver burden in partners of women with breast cancer: a comparative study

J. Teixeira¹ & P. M. Matos²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

² Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Confronting breast cancer is not only a threat to the psychoemotional wellbeing of the diseased, but it also requires the adjustment from the romantic partner. The partner becomes the main informal caregiver, facing a range of challenges and demands that can lead to his burden.

Through a quantitative and cross-sectional approach, the present study used a total of 126 participants, and aimed to test, whether *attachment*, *caregiving* and *emotional regulation* predicted the *caregiver burden*, among partners of women with breast cancer ($N = 63$) comparing with partners from the overall population ($N = 63$). The following instruments were used: *Experiences in Close Relationships*, the *Caregiving Questionnaire*, the *Cognitive Emotion Regulation Questionnaire* and the *Caregiver Reaction Assessment Scale*.

The results indicated that the two groups of participants don't differ in *caregiver burden*, however its predictors are distinct, with *attachment* being the main predictor in partners of women with breast cancer and *caregiving* in partners from the overall population. In addition, *emotional regulation* and *duration of the relationship* showed to be predictors of the *caregiver burden* in the experimental group. *Emotional regulation* was tested as a mediating variable in the association between *attachment* and *caregiver burden*, but the hypothesis was not confirmed. The dimension of *caregiver burden* with highest values was *self-esteem* (mean over 4, in a maximum of 5), demonstrating the positive meaning assigned by the participants to caregiving.

The main conclusion of the study is that, regardless of the reason for the caregiving, the burden of these men is significantly influenced by individual aspects such as the attachment style, the type of caregiving and the emotion regulation strategies. The results are expected to have implications for psychotherapeutic interventions for this population.

Body image in amputation: relationship with depression, anxiety, satisfaction with social support and self-esteem

M. Silva¹ and R. Barbosa²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal

² Center of Psychology, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Amputation refers to the loss of a part of the human body, implying a set of changes not only physical, as well as psychological and social. The impact of amputation in the experience of any individual is reflected into changes at the level of their body image usually turning the way they look at the body. Moreover, it seems to interfere markedly in psychosocial adjustment of individuals experiencing the phenomenon, with influence on their self-esteem, in the possible emergence of psycho-pathological symptoms and social functioning.

This empirical study, based on quantitative and qualitative analysis, has as main objective the understanding of the impact of amputation in body image, linking it to the psychosocial adjustment of individuals (at the level of psycho-pathological symptoms, such as depression and anxiety, global self-esteem and satisfaction with social support).

The sample consists of 46 individuals amputees aged between 22 and 84 years ($M = 50.52$; $SD = 16.13$).

The instruments used were the BIS, to evaluate the degree of body dissatisfaction, the BSI, in two of their dimensions, to measure the levels of depression and anxiety, the ESSS to understand the degree of satisfaction with social support, the QAEG to assess global self-esteem, and a sociodemographic, clinical and psychosocial questionnaire. We resorted to the probabilistic sampling process for convenience, applying the battery of instruments in person (questionnaire on paper) and via the Internet (online questionnaire).

The findings point to the existence of associations among the body dissatisfaction, the global self-esteem, the satisfaction with social support, the depression, the anxiety and the age. It was observed that the global self-esteem emerged as a significant predictor of the body dissatisfaction and the age as a marginally significant predictor. The content analysis, through the exploration of personal representations of individuals amputees about bodily experiences, revealed important meanings for understanding the impact of the phenomenon on how these participants deal with the loss of a part of their body. Finally are taken into account the implications of these results for clinical practice with this population.

KEY-WORDS: body image; amputation, depression, anxiety, self-esteem, social support.

Fostering Parent Involvement in Writing: Development and Empirical Test of an Intervention Program for Parents

A. Camacho¹ & R. Alves¹

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Learning to read and write efficiently is of the utmost importance in elementary school. Specifically, writing can be a difficult and demotivating activity for beginning writers. Writing is a cognitive demanding task that requires several processes [1] and students frequently conceive it as a rigid activity [2]. Many studies showed that parent involvement influences children's competence and motivation in reading, but little is known about parent involvement in writing [2-4]. The main purpose of the current study was to develop an intervention program to promote parent involvement in writing and test its efficacy.

Five Portuguese classes of second graders were assigned to two conditions: parent involvement and control. In the parent involvement condition, the first author conducted an intervention program with four sessions to train parents to better support and motivate their children towards writing. In the control condition, parents were assigned to a waiting list. In the intervention sessions, parents were taught about the importance of parent involvement and of praising the effort of children. Parents were also trained in an interaction sequence, so to make effective suggestions and praises on their children's texts. Furthermore, parents were invited to think over the importance of writing in their children's life through a writing exercise. Over three months, teachers of all classes asked their students to write four texts at home. In the experimental condition, parents interacted with the children in accordance to the prescribed sequence. Parents' perceptions and children's writing skills were assessed at a pretest and posttest evaluation.

The detailed impact of this program on children's and parents' outcomes is being extensively considered. Preliminary results suggest that the intervention program had a positive effect in increasing the quality of texts produced by children.

The findings of this study seem to indicate that parent involvement and scaffolding may foster children's writing skill. This study highlights that some relational aspects of writing, namely parents' suggestions and praises seem to have a measurable effect on students' writing quality.

References:

- [1] Hayes, J. R. and Flower, L. S. (1980), *Identifying the organization of writing processes*, in Gregg, L. W. and Steinberg, E. R. (Eds.) *Cognitive processes in writing*, Lawrence Erlbaum Associates, Hillsdale, New Jersey, pp. 3-29.
- [2] Hidi, S. and Boscolo, P. (2007), *Writing and Motivation*, Elsevier, Oxford.
- [3] Saint-Laurent, L. and Giasson, J. (2005), *Effects of a family literacy program adapting parental intervention to first graders' evolution of reading and writing abilities*, *Journal of Early Childhood Literacy*, 5(3), 253-278.
- [4] Wollman-Bonilla, J. E. (2001), *Family Involvement in Early Writing Instruction*, *Journal of Early Childhood Literacy*, 1 (2), 167-192.

Eating well and better with *Nutri Ventures*

C. Pinho¹, F. Amaral¹, R. Tavares¹, T. Castro¹, C. Grande¹, M. Serra Lemos¹, L. Lima¹

¹ Department of Developmental, Educational and Clinical Research with Children and Adolescents, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

In Portugal, the prevalence of being overweight or obese in children has grown in recent years[1,2]. So it becomes essential the implementation of health promotion and health education programs. This information, combined with the skills of a pediatric psychologist working in the field, led the authors of the present study to develop the project "Eating well and better with *Nutri Ventures*". The project included the viewing of episodes of *Nutri Ventures* series by children. Simultaneously were developed and implemented activities designed to increase the knowledge of healthy eating. Fourteen preschool children of five years old participated in the study. It was initially held a moment of evaluation and subsequently, after the implementation of the project, a second moment of reevaluation. The results were analysed quantitatively, revealing a greater knowledge on the part of children about healthy foods and, as well as greater food diversity. The project implemented expects to be an incentive for the development of other projects in order to promote healthy eating behaviors in children.

References:

- [1] Silva J., & Silva C. (2010). Programas de prevenção da obesidade infantil. *Cadernos de Estudos Mediáticos*, 7, 155-167.
- [2] Ministério da Saúde. (n.d.). Programa Integrado de Avaliação do Estado Nutricional, Hábitos Alimentares e Abordagem do Sobrepeso e Obesidade em Crianças do Ensino Básico.

Life cycle of an association, dynamics of participation and learning opportunities: CRACS (Coletividade Recreativa e de Ação Cultural de Sousela)

C. Cardoso¹, T. Medina²

¹ Master in Educational Sciences

² Associate professor of Faculty of Psychology and Educational Sciences of University of Porto, Portugal.

This research seeks to understand the life cycle of an association, namely the Recreation and Cultural Association of Sousela - CRACS (Coletividade Recreativa e de Ação Cultural de Sousela), and aims to contribute to the (re)constitution of its history, and reflect upon the dynamics of associative participation and the learning opportunities it promotes.

Being a case study, several techniques/methods have been used in the present research, namely the biographical narratives, through which it was possible to “hear” the story of the association in the last 40 years, told by its main characters. The history of the association has been divided into three phases according to the most relevant moments mentioned by the participants. The first phase, called the warehouse phase - “o tempo do barracão”, comprises the foundation of the association and its first years of existence, which are characterized by strong participation, a high number of activities, and a democratic and participatory associative model of management, according to the political period the country was going through, after 25 April 1974. The second phase starts with the construction of the new headquarters, a period in which the association gets into debt, and several members (including founding members, who were very active until then) decide to withdraw from the association due to many of the decisions being made. The third phase begins with a set of changes regarding the management of the association’s bar, reaching the present day, and it is defined as a period of less activity, even though the association remains alive, despite the challenges.

The analysis of participation in the association supports the following conclusions. Participation can be grouped into three levels, which are inseparable even though they are presented separately: 1) a global level, which comprises social and political changes and its consequences; 2) a collective level, which includes the skill to make the best collective decisions regarding the development of the association; 3) a personal level, which comprises personal motivations which lead individuals to participate or not.

The experiences taking place within associative participation can be considered as opportunities to learn, meaning that the importance of associations as relevant educational settings should be considered.



A5

**BIOLOGICAL
SCIENCES V**

V
PARALLEL
ORAL
SESSIONS

Effect of sunlight on cadmium containing nanoparticles (quantum dots) toxicity

B. F. Silva¹, M. N. Vieira^{1,2} and R. Pereira^{1,2}

¹ FCUP – Faculty of Sciences, University of Porto, Department of Biology, Rua do Campo Alegre, Edifício FC4, 4169-007 Porto, Portugal.

² CIIMAR – Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR) University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal.

Cadmium containing nanoparticles (NPs), known as quantum dots, are small, semiconductor NPs that are increasingly being applied in existent and emerging technologies, especially in biological applications due to their exceptional photophysical and functionalization properties [1]. However, they are very toxic compounds due to the highly reactive and toxic cadmium core [1,2]. The application of shell and surface capping to quantum dots has been used to improve their optical capabilities and reduce toxicity, though most existent studies focus on their cytotoxicity and the effect of UV light irradiation, as opposed to the effect on the ecosystems [3].

Therefore, this study aimed to determine the toxicity of three different NPs pre and post exposure to sunlight, in order to assess the effect of environmentally relevant irradiation levels in their toxicity, which will act after their release to the environment. A battery of ecotoxicological tests was performed with organisms that cover different functional and trophic levels (*Daphnia magna*, *Raphidocelis subcapitata*, *Chlorella vulgaris* and *Vibrio fischeri*) at defined concentrations in order to determine EC values.

The results showed that both quantum dots were very toxic to all tested species pre-exposure, while post-exposure tests caused an effective decrease in toxicity for CdSeS/ZnS and CdS 480, and an increase in CdS 380. The results of this study suggest that sunlight exposure has an effect in the aggregation and precipitation reactions of larger quantum dots, causing the degradation of functional groups and formation of larger bulks which are less prone to photo-oxidation due to their diminished surface area.

References:

- [1] Lopes, I., Ribeiro, R., Antunes, F. E., Rocha-Santos, T. A. P., Rasteiro, M. G., Soares, A. M. V. M., Gonçalves, F., Pereira, R., 2012. *Toxicity and genotoxicity of organic and inorganic nanoparticles to the bacteria Vibrio fischeri and Salmonella typhimurium*. *Ecotoxicology*, 21:637-648.
- [2] Derfus, A. M., Chan, W. C. W., Bhatia, S. N., 2004. *Probing the toxicity of semiconductor quantum dots*. *Nano Letters*, 4(1):11-18.
- [3] Moore, M. N., 2006. *Do nanoparticles present ecotoxicological risks for the health of the aquatic environment?* *Environment International*, 32:967-976.

Treatment of olive mill wastewater by phytoremediation with *Lemna minor* or biosorption with cork

L. Oliveira¹, M.T. Borges¹, M.N. Vieira^{1,2}, T. Rocha-Santos³, R. Pereira^{1,2}

¹Department of Biology, Faculty of Sciences, University of Porto, Portugal.

²Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal.

³ISEIT/Instituto Piaget Viseu, Viseu, Portugal & CESAM – Center of Environmental and Marine Studies, University of Aveiro, Aveiro, Portugal.

The olive oil industry is an important economic activity in the Mediterranean countries, producing a large amount of a residual effluent named olive mill wastewater (OMW), which represents a serious environmental problem [1]. The chemical composition of OMW is very variable and depends on several factors, but in general their main composition has a high organic load, low pH, intense smell and very dark brown color. The high complexity of its organic constituents, contribute to its resistance to biodegradation, causing negative environmental effects [1-2]. There is still no generally accepted method of treatment for OMW mainly due to technical and economic limitations.

This work aimed to find solutions to this environmental problem, using techniques of phytoremediation and biosorption. This work had two main objectives: 1) to test the potential of *Lemna minor* to remediate OMW and improve their physical and chemical properties; and, 2) to carry out a biosorption test with two types of cork granulates for evaluating the potential of this adsorbent for the treatment of olive mill wastewater.

The tests have shown that *L. minor* can adapt to the effluent and increase its biomass. This increase was higher for concentrations of 25% and 50% of OMW, for different contact periods. For higher concentrations after 14 and 21 days, there was a widespread loss of fronds pigmentation and tissue necrosis occurred. The improvements in some parameters, color and phenolic content, suggest that biomass must remain physiologically active during the tests, by renovation each 7 or 14 days. Although *L. minor* proved some potential for phytoremediation of OMW, it will be important in the future to perceive if the reduction in the toxicity is occurring in parallel with the improvement of chemical properties of the effluent.

The biosorption assay with cork demonstrated the need for a preliminary treatment of the cork granules. It was also found that differences between treatments depend on the exposure times of the cork. Chemical analysis of the effluent allowed us to calculate the removal rates of different organic constituents of OMW, for which very low reduction rates were recorded. In general the results obtained are insufficient and it is necessary to test new forms of cork contact with the effluent to improve its performance as an adsorbent.

References:

[1] McNamara C.J., Anastasiou C.C., O'Flaherty V., Mitchell R. (2008), Bioremediation of olive mill wastewater, *Int Biodeter Biodegr* 61: 127 – 134.

[2] Linares A., Caba J.M., Ligeró F., De la Rubia T., Martínez J. (2003), Detoxification of semisolid olive-mill wastes and pine-chip mixtures using *Phanerochaete flavidobalva*, *Chemosphere* 51: 887 – 891.

Ecotourism Potential in National Parks: a Case Study

F.Santarém¹, Rubim Almeida^{1,2} and P.Santos^{1,3}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² CIBIO – Research Center in Biodiversity and Genetic Resources, University of Porto.

³ CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, University of Porto.

Ecotourism is being referred by many authors as a potential tool to biodiversity conservation and rural communities' development ^[1]. However, it is also considered as a new form of impacting the ecosystems in Protected Areas ^[2]. Most of the studies only assess the social benefits of ecotourism to rural communities or its impacts in specific habitats or species. Very few combine biological, ecological and cultural data. There is a need to combine these different variables to allow scattering tourists through the year and to lessen the negative impacts caused by recreational activities ^[3]. The main goal of this study was to apply a novel approach that proves to be useful in ecotourism research, by providing and linking the aforementioned data.

Ecotourism Potential Value (EPV) of five hiking trails in Castro Laboreiro, a parish that belongs to the Peneda-Gerês National Park, was achieved by assessing eight different criteria: Number of Habitats (NH); Medium Value of Habitats (MVH); Landscape Diversity (LD); Species Richness (S); number of Natural Marks (NM); number of Anthropogenic Marks (AM); Vertebrate Conservation Status-Plant Range Distribution (VCS-PRD); and Number of Endemisms (NE). The three first were evaluated once; the others were evaluated by season. Two approaches were adopted, one where all criteria weighted equally and one where criteria weighted differently.

EPV was higher in two trails in all seasons. One is explainable by the highest S, NM and LD results and high results in AM, NE and VCS-PRD criteria. The second is explicable by the highest AM, NH and MVH results. It was also verified that when we opt for an approach where we weigh more some criteria than others, EPV of trails by season may vary from the results obtained when we weighed all criteria the equally.

By accomplish a spatial and temporal evaluation, it could be possible to answer where and when recreational activities should take place, scattering them through the year lessening the negative impacts of the mass tourism, especially in summer. This study provided relevant information that helps to manage ecotourism activities in protected areas, achieving one of the main goals of ecotourism: the sustainable development.

References:

[1] Stem, C.J., Lassoie, J.P., Lee, D.R. and Deshler, D.J. (2003) How 'Eco' is Ecotourism? A Comparative Case Study of Ecotourism in Costa Rica, *Journal of Sustainable Tourism*, 11 (4), 322-347.

[2] Zhou, Y., Buesching, C.D., Newman, C., Kaneko, Y., Xie, Z., Macdonald, D.W. (2013) Balancing the benefits of ecotourism and development: The effects of visitor trail-use on mammals in a Protected Area in rapidly developing China, *Biological Conservation*, 165, 18-24.

[3] Monteros, R., L.-E., de los (2002) Evaluating ecotourism in natural protected areas of La Paz Bay, Baja California Sur, México: ecotourism or nature-based tourism? *Biodiversity and Conservation*, 11, 1539-1550.

Metazoan parasites of *Trachurus picturatus*

A. Pereira¹ and A. Saraiva^{1,2}

¹ Department of Biology, Faculty of Science, University of Porto, Portugal.

² CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

A metazoan parasites research was carried out on 58 specimens of the blue jack mackerel, *Trachurus picturatus* (Teleostei: Carangidae) off Matosinhos. Nine parasite species were detected: the Monogenea *Gastrocotyle trachuri* (Prevalence=65,5%) and *Pseudaxine trachuri* (Prevalence=12,1), the Digenea *Monascus filiformis* (Prevalence=50,0%), *Tergestia sp.* (Prevalence = 22,4%) and a Hemiuridae gen. sp. (Prevalence=27,6), a Cestoda plerocercoid larvae (Prevalence=3,4%), the Acantocephala *Rhadinorhynchus sp.* (Prevalence=6,9%), the Nematoda *Anisakis sp.* (Prevalence=93,1%) and the Copepoda *Caligus sp.* (Prevalence=3,4%). The results obtained were compared with that one's conducted on fish of the genus *Trachurus* in other geographical areas.

Proline Dehydrogenase cDNAs characterization in *Solanum nigrum* L. and *Solanum lycopersicum* L.

T. Braga¹, P. Melo¹ and J. Teixeira¹

¹ BioISI – Biosystems & Integrative Sciences Institute, Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal.

Proline dehydrogenase (PDH; EC 1.5.99.8) is a mitochondrial flavoprotein that catalyzes the first of two reactions that convert proline into glutamate. Proline is a proteinogenic amino acid, essential for primary metabolism [1]. It is a compatible solute, providing protection to plants from stress by intervening in several biological responses, such as cellular osmotic adjustment, detoxification of reactive oxygen species (ROS), protection of membrane integrity and protein stabilization [2]. There are two genes reported as encoding PDH, *PDH1* and *PDH2* in *Arabidopsis* and *Nicotiana tabacum* [3-4]. The interest of this work lied in the possibility of enhancing the accumulation of proline in a stressful situation by silencing PDH thus blocking the proline catalytic pathway. Two related plant species were used, *Solanum nigrum* L. (black nightshade) and *Solanum lycopersicum* L. (tomato). PDH-encoding cDNA sequences from other *Solanaceae* available at the NCBI database were used to obtain a consensus sequence in order to design specific primers for *PDH1* and *PDH2* to be further used in RT-PCR reactions. Amplicons from both species were cloned into the pJET1.2 vector (CloneJET™ PCR Cloning Kit, ThermoScientific) and sequenced. BLAST analyses of the retrieved cDNA sequences revealed the successful cloning of partial PDH-encoding cDNAs for these two plant species. MEGA analyses of some known *Solanaceae* PDH1 and PDH2-encoding cDNA sequences showed that PDH1-related differ from those of PDH2, forming two distinct clusters, this way indicating that gene duplication occurred before speciation. The next step will be the silencing of PDH, using an antisense construct based on the DNA sequences obtained, and to observe if plants, with their proline catalytic pathway blocked, exhibit enhanced tolerance when subjected to a potential stressful situation, such as the exposure to heavy metals.

References:

- [1] Szabados, L. and Savouré, A. (2010), *Proline: a multifunctional amino acid*. Trends in Plant Science, 15 (2), 89-97.
- [2] Hayat, S., Hayat, Q., Alyemeni, M. N., Wani A. S., Pichtel, J. and Ahmad, A. (2012), *Role of Proline under changing environments: A review*. Plant Signaling and Behaviour, 7 (11), 1456-1466.
- [3] Ribarits, A., Abdullaev A., Tashpulatov, A., Richter A., Heberle-Bors, E., and Touraev A. (2007), *Two tobacco proline dehydrogenases are differentially regulated and play a role in early plant development*. Planta, 225, 1313–1324.
- [4] Funck, D., Eckard, S. and Muller, G. (2010), *Non-redundant functions of two proline dehydrogenase isoforms in Arabidopsis*. BMC Plant Biology, 10 (70).

Foliar application effects of 24-epibrassinolide in *Solanum nigrum* L. exposed to high levels of Ni

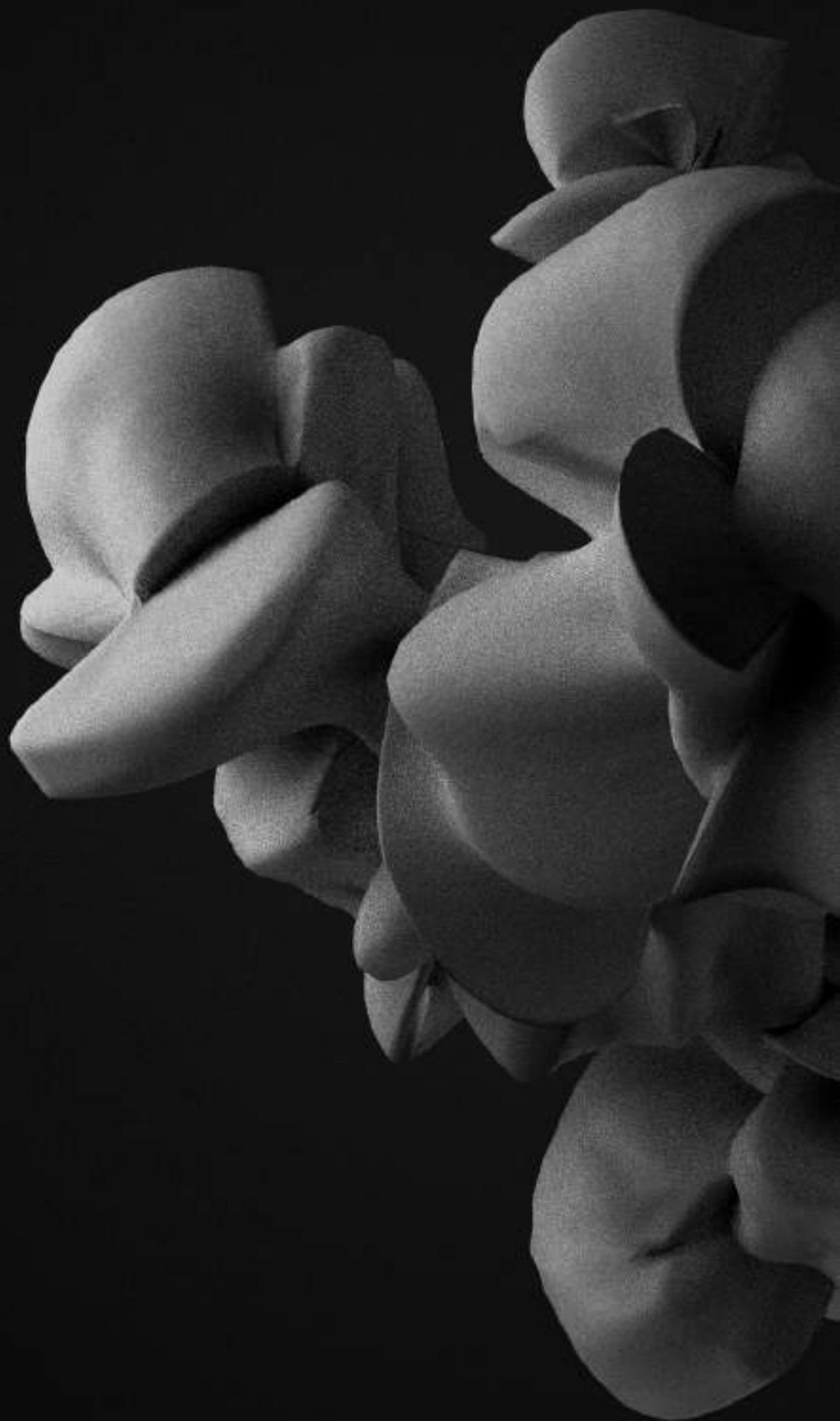
C. Soares, J. Teixeira and F. Fidalgo

Biosystems & Integrative Sciences Institute (BioISI), Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

Solanum nigrum L. proliferates in sediments with high levels of metal pollution and it is known for its ability to hyperaccumulate heavy metals. Nickel (Ni) is a metal of widespread distribution in the environment and is considered as a major pollutant all over the world due to its elevated quantities in soil [1], but it is also a vital micronutrient for plants. However, when present at high concentrations in the soil environment, Ni becomes phytotoxic. As brassinosteroids (BR), a novel class of plant steroidal hormones, have the ability to confer tolerance to plants exposed to several stresses, the objective of this study was to investigate whether exogenously applied BR induced Ni tolerance in *S. nigrum* plants. As Ni-induced phytotoxicity is associated with the generation of oxidative stress, in this work, parameters of oxidative stress were evaluated in order to understand the protective role of BRs' against Ni-induced stress. Preliminary tests were performed with 10 μM Ni, but due to low toxicity effects detected, a new treatment was performed with a higher concentration - 100 μM . After seedling, plants were grown hydroponically for 28 days under three conditions: control plants, watered with Hoagland solution (HS); Ni-treated plants, watered with HS with Ni; NiEBR-treated plants, watered with HS with Ni, but previously sprayed with 24-epibrassinolide 10 μM . Then, the plants were separated into shoots and roots, and biometric and oxidative stress parameters [lipid peroxidation (LP), proline and photosynthetic pigments] were evaluated. The application of 10 μM Ni lead to no symptoms of oxidative damage either at macroscopic or at the biochemical levels. For the 100 μM Ni-treated plants, in both shoots and roots, compared to the control plants, no significant differences in the PL were detected, but there was a decrease in roots of NiEBR-treated plants. For the proline levels, in shoots, there were no differences between the Ni-treated plants and the controls, but a decrease in the EBR-sprayed plants was found. In roots, significant differences were found among the three groups of plants. Regarding the photosynthetic pigments, there were only significant differences between the NiEBR-treated plants and the control ones. Regarding the biometric and fresh weight parameters, in the shoots, compared to the control plants, a decreased mass and height values were observed in the Ni-treated plants, but the pre-treatment with EBR reverted the negative effect of the metal. As for the roots, no differences were detected in their length, however, in terms of fresh weight, the same pattern of the shoots was observed. From macroscopic and pigment analysis, as well as growth rate, the results suggest that plants were under stress, caused by the high concentration of Ni, but that EBR could alleviate this toxicity. Although biochemical parameters don't reflect a serious oxidative stress, such may be due to the ability of *S. nigrum* to grow and deal with high heavy metals concentrations in substrate.

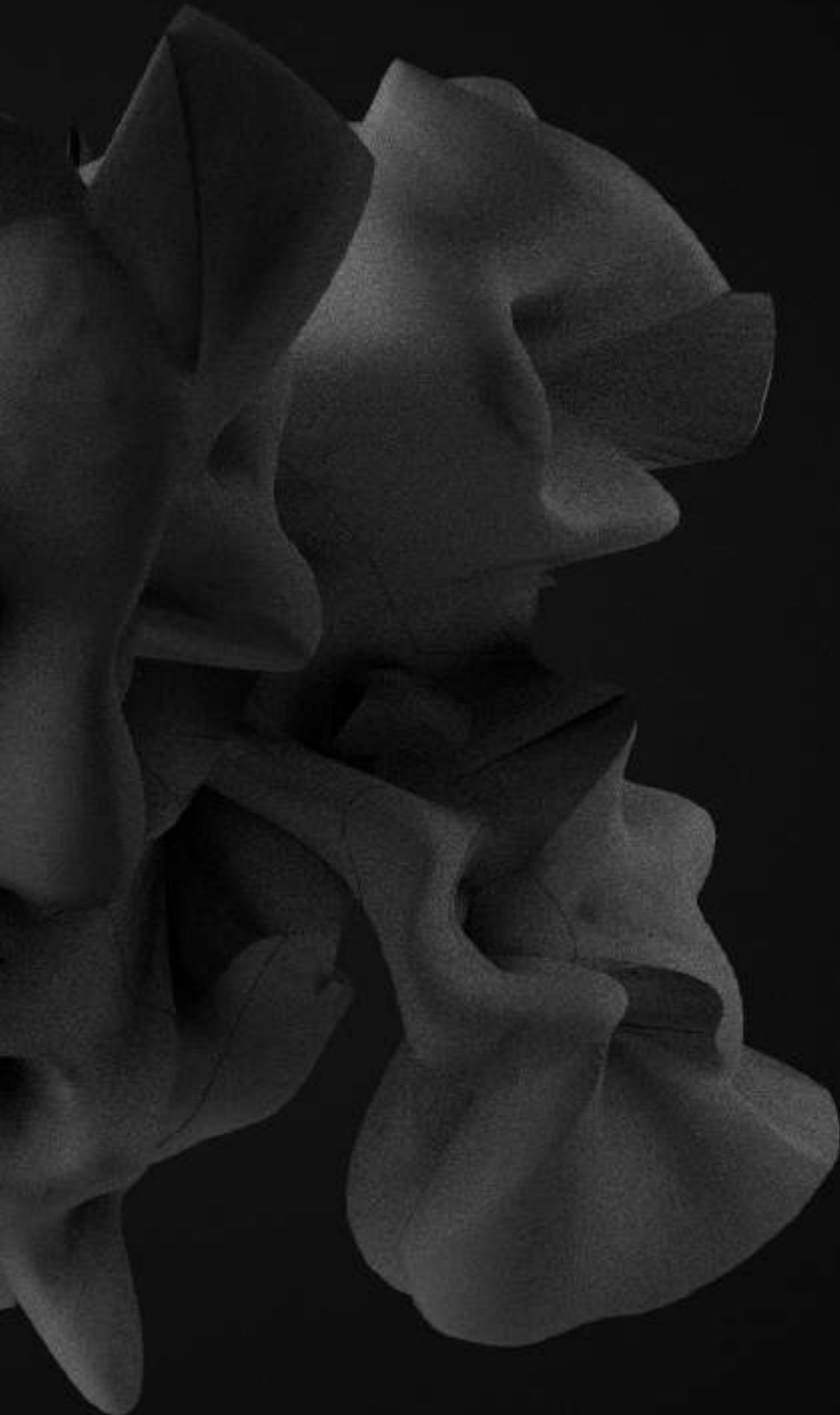
References:

- [1] Faryal R, Tahir F, Hameed A (2007). Effect of wastewater irrigation on soil along with its micro and macro flora. Pak. J. Bot. 39(1):193-204.



VI

PARALLEL
ORAL
SESSIONS



A1

BIOMEDICINE VI

VI
PARALLEL
ORAL
SESSIONS

Preparation and characterization of pH-sensitive liposomes for the treatment of Rheumatoid Arthritis

V. M. Gouveia, C. Nunes and S. Reis

REQUIMTE, Laboratório de Química Aplicada, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal.

Rheumatoid Arthritis (RA) is a chronic systemic inflammatory and autoimmune disease mainly characterized by the progressive inflammation of the synovial tissue of the body joints, destruction of cartilage and further bone erosion. Currently available treatment options include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs), disease modifying anti-rheumatic drugs (DMARDs) and also biological agents, either used as monotherapy or in combination therapy. However, all of these therapeutic agents are associated with severe side effects resultant from limited selectivity and widespread biodistribution of drug molecules into non-target tissues. Therefore, in order to overcome the drawbacks of conventional therapy, pH-sensitive liposomes came up as drug delivery systems, promising to improve the therapeutic efficiency of anti-arthritic agents, due their ability to mediate intracellular/specific and controlled delivery of drug molecules, while minimizing the dose of drug used, thus limiting adverse off-target unwanted effects. Although these liposomes are stable at physiological pH, they undergo rapid liposomal destabilization/degradation under mildly acidic conditions (pH 5.0) as presented in endosomes of target cells, hence releasing the loaded drug molecules.

The aim of the following work was to evaluate the *in vitro* therapeutic performance of the pH-sensitive liposomes loading a GC drug - Prednisolone Disodium Phosphate (PDP) -, through drug release studies mimicking both biological conditions at pH 7.4 and pH 5.0, as well as the liposomal stability during storage. Thus, pH-sensitive liposomes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) and cholesteryl hemisuccinate (CHEMS) in a molar ratio of 6.5:3.5 were prepared by thin film hydration technique followed by extrusion cycle and characterized in terms of hydrodynamic size, polydispersity, zeta potential, encapsulation efficiency and drug loading capacity.

The formulated pH-sensitive liposomes shown to be relatively uniform in size (110 ± 6 nm) with a low polydispersity index (0.065) and a negative zeta potential (-36 ± 5 mV). The average drug encapsulation efficiency and loading capacity were respectively (70 ± 2) % and (45 ± 1) %. The *in vitro* release profile of PDP from pH-sensitive liposomes exhibited a typical biphasic sustained release phenomena, namely initial burst PDP release and consequently slow release. Also, as expected, a higher release in biological conditions at pH 5.0 then at physiological pH 7.4 is observed. Thus showing the pH sensitivity of the liposomes as a trigger to controllably release the GC drug. Regarding all parameters of characterization, it was accomplished a stable liposomal formulation for a period of 1 month at 4°C. These results indicate the developed pH-sensitive liposomes as potential drug delivery systems to enhance the therapeutic efficiency of PDP in RA treatment.

Acknowledgments: We thank the financial support through the project PP-IJUP2011-279.

Addition of spent yeast extract on the production of cooked ham- Compositional and Sensory evaluation

**A. Alves¹, O. Viegas^{1,2}, G. Pancrazio², A. Lemos², O. Pinho² and
I.M.P.L.V.O. Ferreira¹**

¹ REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências químicas,
Faculdade de Farmácia, Universidade do Porto.

² Faculdade de Ciências da Nutrição e Alimentação, Universidade do Porto, Portugal.

Cooked ham is a very common brine-treated product consumed worldwide. The variations in raw material and the different manufacturing technologies that can be used for its production lead to great variability of the end product. Cooked ham manufacturers invest much effort to improve their products with new ingredients [1]. By-products from other food industries can a potential source of these new ingredients.

Saccharomyces yeast biomass is the second major by-product from brewing industry and it can be of value as a raw material with different applications for agro-industrial as a source of nutrients [2]. For this reason the present work was centered in the comparison of proximate composition and sensory evaluation of conventional cooked ham and cooked ham added of spent yeast extract (1% v/w). Different cooking times were used to produce cooked ham samples (1.5, 2, 2.5 and 3 hours).

Proximate composition, moisture, ash, protein and fat content were evaluated using AOAC methods. Total Na⁺ and K⁺ were quantified by flame photometry. Sensory evaluation was performed at the industry by a trained panel composed of 11 panelists.

Two-way ANOVA outcomes revealed significant effects of addition of spent yeast extract to ham on protein (F=208.739; p=0.000), ash (F=20.402; p=0.000) and Na⁺ (F=57.416; p=0.000). This effect is due to the protein content and intercellular composition of spent yeast extracts. Significant effects of cooking time were observed for moisture (F=4.548, p=0.005), protein (F=5.828; p=0.001), lipids (F=2.952, p=0.037); Na⁺ (F=29.844; p=0.000) and K⁺ (F=3.338; p=0.029). Sensory panel analysis indicates that cooked ham with the addition of spent yeast extract was significantly different from conventional ham. However, both cooked hams were accepted by panelists.

Authors acknowledge Primor team (Drs Fátima Carvalho, Paulo Soares, Monica Loureiro, Pedro Gomes) for cooked ham production and sensory analyses.

References:

[1] Li, C.B., Szczepaniak, S., Steen, L., Goemaere, O., Impens, S., Paelinck, H. and Zhou, G. (2011) *Effect of tumbling time and cooking temperature on quality attributes of cooked ham*, International Journal of Food Science and Technology, 46 (10), 2159-63.

[2] I.M.P.L.V.O. Ferreira, O. Pinho, E. Vieira, J.G. Tavela (2010) *Brewer's Saccharomyces yeast biomass: characteristics and potential applications*, Trends in Food Science & Technology, 21, 77-84.

Vortex-assisted liquid-liquid micro-extraction coupled to spectrophotometric propofol determination in biological matrices

Rúben Calais¹, Célia G. Amorim¹, Paula Pinto¹, Lúcia Saraiva¹, Maria C.B.S.M. Montenegro¹, Alberto N. Araújo¹

¹REQUIMTE/Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, R. Jorge Viterbo Ferreira, 228; 4050-313 Porto, Portugal

Propofol is a phenolic compound used in medicine as an intravenous anesthetic with fast onset and offset of action. A major problem related with propofol use is the absence of an adequate analytical method to monitor circulating levels during surgery. This difficulty arises from the low levels at which propofol exerts its physiological effects which imposes the use of large bench top chromatographic systems. In this project, a vortex assisted liquid-liquid micro-extraction is under development to pre-concentrate propofol and to improve separation in biological matrices. Ionic liquids are ionic medium resulting from combinations of organic cations and various anions and may be liquid at room temperature. They have been considered as green solvent and have been applied in organic synthesis and catalysis, etc. Its low vapor pressure, viscosity and miscibility with water and other organic solvents made it have great applications in many fields. In this context it is exploited the use 1-butyl-3-methylimidazolium hexafluorophosphate as extracting phase for liquid-liquid micro-extraction of propofol. The high enrichment factors enabled by the procedure will provide simple spectrophotometric determination of the propofol at the wavelength of 260nm.

Adult Scoliosis

H. Sousa¹, João Maia Gonçalves²

¹ Faculty of Medicine, University of Porto, Portugal.

² Department of Spine Surgery, Orthopaedics Service - Centro Hospitalar de Vila Nova de Gaia, Vila Nova de Gaia, Portugal

This non-systematic review discusses the actual knowledge on adult scoliosis, thinks on the growing importance of this pathology and points out the possible benefits of collaboration between medical, biomechanics and bioengineering professionals to the understanding, diagnosis, pre-surgical analysis, treatment and outcome for this condition.

Scoliosis is a complex three-dimensional rotational deformity occurring in the skeletally mature individual, with a curve measuring $>10^\circ$ according to the Cobb method. Adult scoliosis prevalence is more than 30% in persons aged more than 60 years, and considering the demographic shift towards an ageing society, we can expect an increase in its prevalence [1, 2].

Pathogenesis of adult scoliosis consists of a vicious cycle of asymmetric loading of the spine, asymmetric degeneration, and asymmetric deformity and is triggered by pre-existing age related degenerative changes and/or spine deformity [1].

Adult scoliosis can have a significant and measurable impact on quality of life, with considerable more associated symptoms than the more known and studied adolescent scoliosis. Diagnosis is usually made by a full history, physical exam and full-length standing posterior-anterior and lateral radiographic images, followed by an exhaustive clinic and imagiologic investigation [1, 2].

The objective of treatment is to improve the manifestations of the condition. Initial conservative management is indicated, however, conservative measures lack evidence support and efficiency in the long term and controversy remains over the role of surgical intervention; nevertheless surgical treatment is indicated in case of failure of conservative measures and requires careful patient selection, pre-operative assessment and pre-optimization to reduce the incidence of complications [2].

The knowledge of the spine biomechanics is a very important tool for many medical applications such as diagnosis, treatment and surgical interventions. The use of computational models, like finite element, for medical purposes is increasing, allowing biomechanical study of several spine pathologies with considerable advantages like providing data on some measures impossible to acquire by other methods, multiple testing, help to understand the pathogenesis and progression of specific conditions, surgical planning and treatment outcome prediction, all at a low cost [3].

References:

[1] Aebi M. (2005), The adult scoliosis,. *European spine journal*,14(10), 925-48.

[2] Silva, F.E. and Lenke, L.G. (2010), Adult degenerative scoliosis: evaluation and management, *Neurosurgical focus*, 28(3):E1, 1-10.

[3] Adams, M.A. and Dolan, P. (2005), (Perspective) Spine biomechanics, *Journal of Biomechanics*, 38, 1972–1983.

Role of PKG-related Pathways in the Diastolic Response to Acute Myocardial Stretch Under Ischemic Conditions

A. M. Leite-Moreira, M. Neiva-Sousa, J. Almeida-Coelho, J. S. Neves, R. Castro-Ferreira, R. Ladeiras-Lopes and A. F. Leite-Moreira

¹ Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto, Portugal.

Introduction: Acute myocardial stretch induces both systolic and diastolic adaptive responses. The mechanisms responsible for diastolic adaptation remain largely unknown. However, in the presence of ischemia, this response is not observed. Therefore, we aimed to evaluate the role of cGMP-dependent protein kinase (PKG) and associated signaling pathways in the diastolic adaptive response to acute myocardial stretch under ischemic conditions.

Methods: Rabbit papillary muscles (0.2Hz, 30°C) were acutely stretched from 92% to 100% of Lmax in a modified Krebs-Ringer solution (A) under basal conditions. Group B was stretched during ischemia and other protocols were performed in the ischemic setting in the presence of (C) 8-Bromo-cGMP (an agonist of PKG, 10⁻⁵ M, n=7), (D) B-type Natriuretic Peptide (BNP, 10⁻⁶ M, n=7), (E) S-Nitroso-N-acetylpenicillamine (SNAP, a nitric oxide donor, 10⁻⁵ M, n=9), (F) Sildenafil (phosphodiesterase 5 inhibitor, 10⁻⁶ M, n=7) and Sildenafil combined with either (G) BNP (n=8) or (H) SNAP (n=6) Immediate and delayed responses to muscle stretch were evaluated. Results are presented as mean±standard error of mean (P<0.05).

Results: Under basal conditions (group A), after immediate increase in myocardial passive tension (PT) induced by acute myocardial stretch, there was a significant and time-dependent decrease in PT of 46.2±1.8% in the 15 minutes following stretch. Under ischemic conditions (group B), diastolic response to acute stretch was completely abolished throughout the 15 minutes of ischemia (increase in PT of 3,5±8,1%). The presence of an agonist of PKG (group C) promoted a significant decrease in PT of 20,6±3,2% after stretch during ischemia, as did the addition of sildenafil (group F, decrease in PT of 14,9±5,3%). The presence of either BNP (group D) or SNAP (group E) did not significantly improve the diastolic adaptation to acute stretch (decrease in PT of 5,6±6,7% and 9,2±6,2%, respectively). The simultaneous addition of sildenafil with BNP (group G) or SNAP (group H) elicited a synergistic effect, with the fall in PT being significantly greater than those observed when either drug is added alone (decrease of 30,3±6,4% and 34,1±4,7%, respectively).

Conclusions: PKG hypoactivity may be associated with the dysfunctional diastolic adaptation during ischemia. According to these results, a likely mechanism for this impaired function is the depletion of intracellular cGMP stores, caused by either decreased synthesis or increased activity of phosphodiesterases under ischemic conditions.

Development of glyceryl behenate (Compritol® E ATO) matrix tablets for sustained release of thiamine hydrochloride

A. Fernandes, D. Gomes, L. Gaião, S. Fernandes, S. Oliveira, M. Estanqueiro, J. Conceição, J.M. Sousa Lobo

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Controlled release tablets are prepared with special excipients or specific process, which separately or in conjunction, modify the velocity, the place or the moment of drug release. Controlled release tablets include sustained release, retarded release and sequential release tablets. These systems present several advantages, such as, increase of the plasmatic concentrations of drugs with short half-life. To obtain controlled release, matrix systems can be used. Matrix systems can be defined as a system that controls the release of a drug molecularly dispersed in a support resistant to desegregation. In this work, glyceryl behenate was used as a lipidic matrix, in which the drug is release by diffusion or erosion [1, 2].

The aim of this study consists in the development and technological characterization of sustained release tablets containing glyceryl behenate and thiamine hydrochloride (vitamin B1) as a model of a very water soluble drug.

The developed formula composition is the following: thiamine hydrochloride (2%, w/w), behenate glyceryl (35%, w/w), lactose (20%, w/w), cellulose microcristalline (Avicel® PH 101) (34.5%, w/w), colloidal silicon dioxide (2%, w/w), talc (5%, w/w), zinc stearate (1.5%, w/w). The formula components were mixed on a Turbula Wab mixer for 15 minutes and directly compressed using a single-punch compression machine Korsch 9048-71 (Germany). Weight variation, hardness determination and friability were carried out [3]. Additionally, *in-vitro* drug release was evaluated in two tablets in a Sotax model AT7 dissolution apparatus. The dissolution medium was maintained at 37°C ± 0.5°C and stirred at 100 rpm. Samples (10 mL) were withdrawn at predetermined time intervals (15, 30, 45, 60 and 90 minutes), without volume reposition. Samples were filtered and assayed by spectrophotometry at 273 nm.

The obtained results showed that the produced batch presented uniformity of mass, a hardness average of 88.2 N and a mass loss of 0.76% in friability assay. At the end of 90 minutes, the maximum amount of drug released was 61.6%.

We can conclude that the obtained tablets showed good mechanical characteristics (hardness and friability), uniformity of weight and sustained release of thiamine hydrochloride.

References:

- [1] Pezzini, B.R., Silva, M.A.S., Ferraz, H.G. (2007). Formas farmacêuticas sólidas orais de libertação prolongada: sistemas monolíticos e multiparticulados. *Revista Brasileira de Ciências Farmacêuticas*, 43 (4), 491-502.
- [2] Lopes, C.M., Sousa Lobo, J.M. and Costa, P. (2005), Formas farmacêuticas de libertação modificada: polímeros hidrofílicos. *Revista Brasileira de Ciências Farmacêuticas*, 41 (2), 143-154.
- [3] Farmacopeia Portuguesa 9: Ministério da Saúde, INFARMED, I.P., 2009.

A2

ENGINEERING II

VI
PARALLEL
ORAL
SESSIONS

Hydraulic bulge test for stress-strain curve determination

Hugo Campos^{1(*)}, **Abel D. Santos**^{1,2}, **Bruno Martins**¹

¹ INEGI, Institute of Mech. Eng. and Ind. Management, University of Porto

² FEUP– Faculty of Engineering, University of Porto

Rua Dr. Roberto Frias 400, 4200-465 Porto, Portugal

(*)hcampos@inegi.up.pt, www.inegi.up.pt.

Relevant properties for mechanical characterization of sheet metal materials are usually obtained by using the widely accepted standard uniaxial tensile test. However the range of strain obtained from tensile test is limited and the bulge test represents an alternative to obtain ranges of deformation, higher than tensile test, thus permitting a better characterization for material behavior [1].

In this work it is presented a numerical study by finite element analysis to perform a sensitivity analysis for some influencing variables used in bulge measurements, thus giving some guidelines for the evaluation of the stress-strain curve from performed experimental results using a developed experimental mechanical system.

REFERENCES

- [1] Santos, A.D., P. Teixeira, and F. Barlat, *Flow Stress Determination Using Hydraulic Bulge Test and a Mechanical Measurement System*, IDDRG 2011

Dynamometric analysis of swimming starts: 3D assessment of moments and forces

Pedro Fonseca¹, Sofia Abreu¹, Mariana Marques², Rita Freitas², Miguel Medeiros², Karla de Jesus², Luís Mourão², Hélio Roeseler³, Ricardo Fernandes², Mário Vaz¹ and J. Paulo Vilas-Boas²

¹ Faculty of Engineering & LABIOMEPE, University of Porto, Porto, Portugal

² Faculty of Sports & LABIOMEPE, University of Porto, Porto, Portugal

³ CEFID, University of the State of Santa Catarina, Florianópolis, Brazil

Introduction: Swimming starts are an important part of swimming competitive events, however most of attention was devoted to the study of swimming techniques [1]. In 2010, the international governing body for swimming (FINA) decided some rule changes for ventral and dorsal starts, apparently without empirical and scientific bases, and unknowing their performance consequences. Since classifications in competitive swimming events have continuously been decided by less than a second, instrumented starting blocks have been optimized [2]. This study aimed at the development of a new dynamometric central to assess independent right and left upper and lower limbs forces and moments at individuals in their ventral and dorsal starting and turning techniques.

Method: In a first phase, a prototype structure was designed using SolidWorks 3D CAD Premium (Dassault Systèmes SolidWorks Corporation, Waltham, USA), and strain gauges were placed in order to obtain a 3D-6DOF waterproof force plate. The prototype was reproduced to obtain seven modular platforms, four of which geminated, to measure external right and left lower and upper limbs kinetics at individual and relay starts. Twenty-four waterproof strain gauges (Kyowa, Electronic Instruments, KFW-5-120-C1-5M2B, Tokyo, Japan) were used in all force plates to register deformations under load. The dynamometric central was projected to comply with the FINA facilities and starting rules. The linear static and model simulations were conducted using Ansys 12.1 (Ansys Workbench 12.1, Swanson Analysis Inc., Houston, PA, USA) for deformation and vibrations analysis. **Results:** The statistic simulations with 8000N revealed mean deformations of $\sim 350 \mu\epsilon$ and $\sim 500 \mu\epsilon$ on the vertical and anterior-posterior direction, respectively for the geminated force-plates, and $300 \mu\epsilon$ and $250 \mu\epsilon$ on the vertical and anterior-posterior direction, respectively for the non-geminated force plates. The modal analysis displayed natural frequencies of 300 Hz for the four geminated force plates, 200 Hz for the two underwater force plates and 180 Hz for the non-geminated force plate. **Conclusions:** Based on these preliminary results, the current dynamometric central might be considered the most modern and versatile tool for starts and turns analysis.

Acknowledgments: The authors would thank IJUP for funding this project through project number PP/IJUP2011/123.

References:

- [1] Vilas-Boas, J.P., Cruz, M.J., Sousa, F., Conceição, F., Fernandes, R.J., Carvalho, J. (2003). *Biomechanical Analysis of Ventral Swimming Starts: Comparison of the Grab with Two TrackStart Techniques*, in JC Chatard (Ed.), "Biomechanics and Medicine in Swimming IX", University of Saint Etienne, pp.249-253.
- [2] de Jesus, et al. (in press). *Backstroke start kinematic and kinetic changes due to different feet positioning*. J. Sports Sci..

Control of a Swimming Ergometer

A. Ferreira¹, S. Soares^{3,4}, J. P. Vilas-Boas^{3,4}, L. Machado^{3,4} and M. V. Correia^{1,2,4}

¹ Faculty of Engineering, University of Porto, Portugal.

² INESC-TEC Technology and Science, Porto, Portugal.

³ Centre of Research, Education, Innovation and Intervention in Sport (CIFID), Faculty of Sports, University of Porto, Portugal.

⁴ LABIOMEPE - Biomechanics Laboratory of the University of Porto, Portugal.

A swimming ergometer is an apparatus where an athlete can simulate freestyle swimming movements out of the water environment. It can be used to develop muscular strength, which is a key factor in swimming competition events, or to evaluate the athlete's physical condition or determine anaerobic performance in a simple and non-invasive way [1]. The swimming ergometer consists of two hand paddles attached to two ropes, which when pulled, induce rotation on an isokinetic semi-accommodated resistance device. During simulated swim the swimmer body adopts a position similar to freestyle swimming, pulling the hand paddles simultaneously or alternately [2].

The aim of this work was to develop the software to control a swimming ergometer prototype, built at the Biomechanics Laboratory the University of Porto, to be used to train swimmers and evaluate their physical condition and performance. The prototype is based on two electric motors and drive inverters which are responsible for applying resistance to the movement performed by the swimmer (Fig.1 left).



Fig. 1. Left: Swimming ergometer; Right: software control.

The software developed (Fig. 1 right) allows two training regimes, constant force or isokinetic with adjustable settings, and collects data signals of force, velocity, power and stroke rate for both arm movements separately. In a pilot test, it was possible to observe performance differences between individuals, which proves the suitability of using the ergometer to perform evaluation studies of swimmers [3].

References:

[1] Ribeiro S. (2007), *Avaliação indirecta da funcionalidade anaeróbia de nadadores de diferente estatuto maturacional com recurso a testes laboratoriais e de terreno*, Tese de doutoramento em Ciências do Desporto, Faculdade de Desporto da Universidade do Porto.

[2] Zamparo P., Swaine I. (2012), Mechanical and propelling efficiency in swimming derived from exercise using a laboratory-based whole-body swimming ergometer, *Journal of Applied Physiology*, 113 (4):584–594.

[3] Ferreira A. (2013), *Controlo de ergómetro simulador de natação*, Tese de Mestrado em Engenharia Biomédica, Faculdade de Engenharia da Universidade do Porto.

Development of Fail-Safe Control Methodologies for Multi-Motor Electric Vehicles: An Initial Feasibility Study

S. Almeida¹, A. Lopes¹, R. E. Araújo¹

¹ INESC TEC, Faculty of Engineering, University of Porto, Portugal.

Recently, the multi-motor electric vehicles (MMEVs) opened a new class of chassis control problems. In contrast to the vehicles based on internal combustion engines, the vehicles with electric motors give the possibility to independently control the driving/braking torque in each motor wheel [1,2]. As discussed in [3], the benefits of independently control the torque in each motor wheel are counteracted by the increasing probability of failure due to the higher complexity of the propulsion system. This motor faults, without the proper accommodations, may result in vehicle performance deterioration or even instability due to the loss of desired torque on a particular wheel. Hence, the study and development of mathematical vehicle models that describes the MMEV dynamics, taking in consideration the effects of fault occurrence, is important for the design of controllers that guarantees stability and performance of the EV.

In this context, our work proposes to apply the concepts of fault-tolerant control methods to an MMEV. To address this problem, we start revisiting the modeling of the vehicle dynamics focusing on a single-track model. In the next step of our study, we formulate the fault problem in terms of the state space model. We consider that in each motor of the vehicle can occur a fault represented as a perturbation of the desired torque value. The motivation for such formulation is that the fault gain matrix, which links the motor faults to the state variables of the vehicle, is always available no matter how the faults changes with time. Such mathematical description provides an important knowledge of the dynamical model limitations and behavior of the vehicle before and after motor driver faults occur. To mitigate the faults and to show the viability of an idea about the development of a fault-tolerant controller, it is essential to include a fault gain matrix.

A scheme that validates the proposed faulty mathematical model was developed using a co-simulation between Simulink[®] and CarSim[®] software. The study developed in this work permits a better understanding of the behavior of the MMEV in the presence of motor faults. Based on these ideas, we can start to isolate the faults and improve the advanced motion controller for the uCar MMEV prototype [4].

References:

- [1] A. Silveira, R. E. Araújo, and R. Castro, *Survey on fault tolerant control diagnosis and control systems applied to multi-motor electric vehicles*, in Technological Innovation for Sustainability (S. B. Heidelberg, ed.), pp. 359–366, 2011.
- [2] Y. Hori, *Future vehicle driven by electricity and control-research on four-wheel-motored UOT electric march II*, IEEE Transactions on Industrial Electronics, vol.51(5),pp.954-962, 2004.
- [3] N. Mutoh and Y. Nakano, *Dynamics of front-and-rear-wheel- independent-drive-type electric vehicles at the time of failure*, IEEE Transactions on Industrial Electronics, vol. 59, pp. 1488–1499, 2012.
- [4] R. de Castro, R. E. Araújo and D. Freitas, *Wheel slip control of EVs based on sliding mode technique with conditional integrators*, IEEE Transactions on Industrial Electronics, vol. 60, pp. 3256–3271, 2013.

Sheet metal mechanical characterization using biaxial test and numerical modelling

Tiago Vaz¹, Abel D. Santos¹

¹ Faculty of Engineering, University of Porto, Portugal.

Parameters for material characterization is an important topic in sheet metal forming simulation. For this purpose a methodology has been developed by using a biaxial tensile test of a cruciform specimen combined with finite element analysis. Abaqus code has been used and some tools have been developed in the Matlab numerical computing environment for post-processing and analysis. Scripts were developed in both programs allowing us to perform tasks quite efficiently and automatically.

Initially the geometry of the specimen has been optimized as well as the corresponding mesh to be used in numerical simulations. Analysis was performed directly from results obtained by numerical simulation where fundamental relations were determined to establish a method of inverse analysis. Based on the relationships determined from direct analysis, it was possible to build an heuristic algorithm [1] to determine the parameters of constitutive models. This algorithm is suggested to be used for materials characterized by Hill'48 yield criterion and the Swift hardening law, being perfectly adequate for steel materials. The algorithm tests were performed using two different materials, and the results obtained have a maximum error of 2% in materials characteristics.

Keywords: Numerical modeling, Material characterization, Biaxial tensile test

References

[1] Prates, P., 2010. Metodologia de Análise Inversa para Determinação Simultânea dos Parâmetros de Leis Constitutivas, com Recurso a um Provete Cruciforme. Dissertação de Mestrado. Faculdade de Ciências e Tecnologia, Universidade de Coimbra (in portuguese).

DINAM – testing a prototype dynamometer for handgrip strength

S. Teixeira¹, **R. Silva**¹, **B. Santos**², **T. F. Amaral**^{1,2}, **M.R. Quintas**² and **M. T. Restivo**²

¹ Faculty of Nutrition and Food Science, University of Porto, Portugal.

² IDMEC - Faculty of Engineering, University of Porto, Portugal.

Hand grip strength (HGS) is used as an indicator of nutritional status and of global health [1]. However, the dimensions and weight of dynamometers commonly used may pose difficulties for practical use in children, undernourished and frail individuals. A new dynamometer prototype was developed [2] and innovates by overcoming the former technical and ergonomic problems.

This study aims to assess its reliability and to quantify the impact of the measurement of arm positions and anthropometric characteristics on the discrepancy between assessments of HGS with the prototype and a reference dynamometer.

We compared on 31 volunteers (>18 years) HGS values obtained with the prototype to those gathered with a reference JAMAR[®] dynamometer. Data on lifestyle and anthropometric parameters was collected. HGS was assessed with two dynamometers in three different positions (flexion 90°, pending arm and arm on horizontal extension). Through multivariate linear regression analysis, the independent effect of anthropometric characteristics in the discrepancy between the two dynamometers was quantified.

Higher HGS values were obtained with JAMAR[®] than those obtained by the prototype in the three tested positions ($p < 0.002$) (mean = 3.8, SD = 4.6 KgF). Discrepancy in HGS values obtained by each of the dynamometers has been influenced by arm position ($p < 0.002$). Anthropometric indicators like triceps skinfold, ts , and relaxed arm girth, ag , were positively associated with discrepancy respectively ($\beta_{ts} = 0.63$, $p = 0.03$) and ($\beta_{ag} = 2.249$, $p = 0.036$). Flexed, fa , and tensed arm girth affected negatively ($\beta_{fa} = -2.304$, $p = 0.02$).

Although tested on a small sample of participants the present data shows that there are differences on HGS values with different measurement of arm positions and between the two dynamometers. These differences were affected by both ts and ag . Thus, although comparisons between the prototype and Jamar dynamometers characteristics have revealed good results at lab tests, the prototype exhibits discrepancies when compared with JAMAR in field use, which the team believes to be due to ergonomic reasons. Due to these reasons a new pre-prototype has been designed even at sensorization level. This pre-prototype is working and the final prototype will be produced in the near future.

[1] Stobaus, N K, Gonzalez, MC, Schulzke, JD, Pirlich, (2011), *M. Hand grip strength: outcome predictor and marker of nutritional status*, Clinical Nutrition 30(2):135-42.

[2] Andrade, T, Monteiro, L, Guerra, R, et al., *Development of a Handgrip Dynamometer for Nutritional Assessment*, 2nd Meeting of Young Researchers at UP, IJUP 2009.

A3

**CRIMINOLOGY,
ECONOMY
& LAW**

**VI
PARALLEL
ORAL
SESSIONS**

Firing distance estimation through the analysis of the gunshot residue deposit pattern around the bullet entrance hole using FT-NIR spectroscopy

F. Machado¹, C. Lima¹, L. Fernandes^{2,3}, J. Sarraguça¹, J. Lopes¹, A. Santos^{2,3} and A. Almeida¹

¹ REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

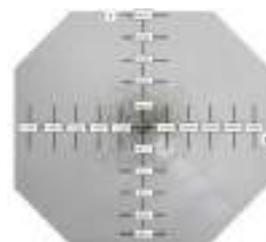
² CENCIFOR – Centro de Ciências Forenses, Portugal.

³ National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal.

The estimation of the firing distance (the distance from which a firearm was fired) is of utmost importance in the investigation of firearm-related cases. Besides the examination of clothing targets, cadavers, etc., at the incident scene, laboratory methods for firing distance estimation includes visual and microscopic examinations, color tests and instrumental analysis of the gunshot residues (GSR) deposits around the bullet entrance holes [1]. GSR has both organic and inorganic components. Its analysis has been based mostly on the metallic components Pb, Sb and Ba, the reason why instrumental techniques such as scanning electron microscopy/energy dispersive X-ray (SEM/EDX), graphite furnace atomic absorption spectrometry (GFAAS) and, more recently, inductively coupled plasma-mass spectrometry (ICP-MS) have assumed particular importance in this area [2].

In the present study⁴ we assessed an alternative approach for firing distance estimation, based on the analysis of the organic components of GSR using Fourier transform-near infrared (FT-NIR) spectroscopy. The advantages of a FT-NIR approach lie in the fact that it is essentially a non-destructive technique, minimal or no sample preparation is required, and measurements are less prone to contamination and less time consuming. Moreover, portable equipment is available, enabling an *in situ* analysis, at the incident scene, a very attractive feature from a practical point of view.

The models relating firing distance with spectral information were developed using samples obtained from a series of shots at different distances, from 20 to 80 cm, against a square white cloth (30x30 cm; 70% polyester/30% cotton) as target. The spectra were collected around the bullet entrance hole in 4 perpendicular directions and at 5 radial distances using a diffuse reflectance optical fiber probe. This approach was tested for two different types of ammunition using a 9 mm Glock pistol. Chemometrics techniques for multivariate analysis were applied to the spectral data. A PLS model was constructed for predicting the firing distance.



References:

- [1] Zeichner, A. (2012), *Shooting Distance: Estimation of*. Wiley Encyclopedia of Forensic Science.
- [2] Santos, A., et al. (2007), *Firing distance estimation through the analysis of the gunshot residue deposit pattern around the bullet entrance hole by inductively coupled plasma-mass spectrometry: an experimental study*, The American Journal of Forensic Medicine and Pathology, 28(1), 24-30.

⁴ Conducted by F. Machado and C. Lima under the Course Unit “Projeto I”, Master in Pharmaceutical Sciences, FFUP, 2013.

Psychoactive substances present in *Salvia divinorum* acquired in smartshops or in the Internet

E. M. Moreira¹, F. Carvalho¹, M. L. Bastos¹, P. Guedes de Pinho¹

¹REQUIMTE, Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

In order to circumvent the unavailability and illegality of general recreational drugs, the market of legal drugs quickly spread around the world, including Portugal. New psychoactive substances might be found in internet websites and smartshops, claiming to be legally commercialized and safe to be consumed. However, the lack of information on these products, as well as their inadequate control concerns legal authorities and scientific community.

The present study evaluates the composition of ten different samples of one of the most popular legal drugs, *Salvia divinorum*, with potencies labeled between 5x and 60x. Samples were acquired in smartshops and internet websites, and the main psychoactive compound salvinorin A, a neoclerodane diterpenoid acting as a kappa opioid receptor agonist, was identified and quantified, alongside with 3 other isomeric compounds, salvinorin B, C and D. Several solvents were tested about their efficacy of extraction, being acetonitrile the one that provided better results. The analysis was achieved using GC-MS methodology and concentration of salvinorin A ranged from 2,6 and 521,2 mg/g, contradicting the information mentioned on most labels and/or advanced by marketers. The referred concentrations may be considered very high, since it would be enough to weight small amounts from 0,4 mg to 75,8 mg (depending on each sample's concentration) to achieve hallucinogenic effects. The concentrations of the remaining compounds proved to be significantly lower than those of salvinorin A: 1,59-117,86 (mg/g) for salvinorin B, 0,03-11,87 (mg/g) for salvinorin C and <0,32 (mg/g) for salvinorin D.

Citizens' Perceptions of Private Security Agents

S. Moreira¹

¹ School of Criminology, Faculty of Law, University of Porto, Portugal.

The employment of private security agents in most European countries, as well as in Portugal, has increased rapidly in the past decade. While private security agents are more and more visible and come in contact with the general public, very little is known about the citizens' perceptions and level of satisfaction with private security agents.

The goal of this study is to assess citizens' level of satisfaction with private security agents. More specifically, we examine their perceptions about their professionalism and integrity; powers; regulation of their work; and, finally, the relationship between private security agents and police officers. To achieve these goals, a paper and pencil self-reported questionnaire was administered to a sample of 163 individuals from the Porto area.

Overall, results indicate that respondents expressed satisfaction and a predominantly positive or neutral perception about private security agents. In addition, we found that older individuals and female respondents tend to be more satisfied and to have a more positive perceptions of private security agents. We also found that respondents who contacted directly with private security agents report being more satisfied with them than those who did not contact. Findings and policy implications will be discussed.

Given the little knowledge in Portugal about citizens' perceptions of private security agents, this study may provide a basis for the development of other studies in the future in this field.

Marine Spatial Planning – contributions for (future) legal regime in Portugal

F. Noronha¹

¹ Attending Masters Degrees in Law at Faculty of Law, University of Porto, Portugal; Integrated Member of Marine Environmental Law Research Group at Interdisciplinary Center of Marine and Environmental Research (CIIMAR), University of Porto, Portugal.

Our work aims at making a theoretical contribution to the understanding of the concept of Marine Spatial Planning (MSP), whose centrality to a new holistic and integrated view of the uses and activities of the sea is nowadays recognized by the doctrine and by various international organizations and conventions, particularly for being a tool for implementing the ecosystem approach in the marine environment.

Based on the concept of Marine Protected Areas (MPA), MSP proposes a rational use of the maritime space by allocating sea areas to different uses and activities, balancing the imperatives of biodiversity protection with economic exploration. Simultaneously, MSP provides a legal framework for the use of marine space, especially through spatial plans under which uses and activities may be licensed.

While in some European countries (*e.g.*, Belgium, Germany, Netherlands), MSP is already well developed, Portugal is taking the first steps with the Bill No. 133/XII on MSP (framework law), currently under discussion in Parliament. At the same time, the European Union issued a Proposed Directive on MSP, according to which the Bill No. 133/XII should be aligned. We analyze both documents and present some critical suggestions.

As is known, Portugal has an enormous historical and cultural link to the sea, as it took Portuguese people to discover the world through the 15th century and beyond (the so called “Age of Discovery”). In the last decades, however, Portuguese governments have shown an alarming lack of interest in exploring the whole sea potential (economic, social and environmental potential). This is particularly disturbing when Portugal has the biggest Exclusive Economic Zone of all the European Union countries and has recently submitted a proposal on the extension of its Continental Shelf to the Commission on the Limits of the Continental Shelf (created by the United Nations Convention on the Law of the Sea).

Thus, a MSP framework law may be the turning point for this situation, since it establishes a legal scheme for all the activities that can be undertaken in the sea by which stakeholders may get the permits and concessions they need in order to develop their activities.

Universal Service, Competition and Regulation

P. Ferreira¹

¹ Department of Economics, Faculty of Economics, University of Porto, Portugal.

The Universal Service appears linked to the need to provide quality services to the people at affordable price. These should be regarded as essential to the harmonious development of society by their importance to ensure fairness and equal opportunities. Universal service is present in many sectors of economic activity, reaching many vital areas for Economic Growth, such as Telecommunications, Postal Services or Energy.

The main goal of universal service is to provide certain economically identifiable customers that otherwise would not be supplied, as the cost of providing that service is higher than the revenue that the provider company could get. However it's regarded by the government as "public service" and must supply to whomever they wish. According to the OECD (2010), historically the Universal Services are provided by state monopolies, as they generate large economies of scale. They include restrictions on entry and uniform price. There are many ways that can lead to a given provider go to be the supplier of Universal Service. One of the most common is the subsidies, which can take different types. One of the fastest penalizes competition is cross-subsidization, with all the encouragement that this generates for inefficient practices, including restrictions on the entry of new players in the market in which the company operates. Cross-subsidization has been the primary mechanism for financing the universal service but should be eliminated. To prevent its preservation, entry barriers should be reduced, progressing to systems that enable explicit subsidies, such as sector specific taxes, externalities in substitute goods' taxes or even the general taxes.

It has also seen the discontinuation of certain universal service obligations. This results from the progress of technology that allows unprofitable services start to be profitable. Thus, assuming that a service can be provided by the market, in the absence of any compensation, it should be liberalized. Mueller (1997) argues that competition, and not the existence of a single company, was largely responsible for the existence of universal service. This by itself can meet the entire demand. When there is liberalization of sectors previously covered by the Universal Service, the role of the Regulator is critical. This should ensure the quality of service, as well as the universality of access to it. It should also promote competition, and it shall identify the need for elimination of artificial barriers to entry, and any predatory behavior. In the case of reimbursement scheme, the Regulator function is a periodic review of costs submitted by the provider companies, so that no more than the actual loss resulting from the practice of universal service is to be paid by the Government.

I want to thank Professor António Brandão who granted me the opportunity to address this issue in the course of Economics and Regulatory Policy.

References:

- [1] Organisation for Economic Co-operation and Development (2010), *Universal Service Obligations*.
- [2] Mueller, M. (1997), *Universal Service: Competition, Interconnection, and Monopoly in the Making of the American Telephone System*, MIT Press and AEI Press, Washington, DC.

Incommunicability and deliberation: the shaping of the jurors conviction in Brazilian and French courts

Beatriz Bellintani¹, Ana Lúcia Pastore Schritzmeyer² (advisor)

¹ Faculty of Law, University of São Paulo, Brazil.

² Department of Anthropology, Faculty of Philosophy, Languages and Literature, and Human Sciences, University of São Paulo, Brazil.

This research aims to investigate the so called “rule of incommunicability” which guides the members of the jury's conduct during the trials in Brazil, as opposed to the deliberative nature of the French institution in order to analyze the political and ideological premises involved in the shaping of the decision of the jurors in both countries and how that reflects on the role of the Jury as a political institution.

As to the methods of work, I did during the first semester a bibliographical survey of all types of works produced on the subject in Brazil. This first stage of research provided the groundwork for the creation of questions that will compose an interview to be made with jurors from São Paulo as well as for the critical analysis of interviews with French jurors that have already been made by researchers of the University of Lille III [1-2]. In the next semester, the idea is to accomplish the previously described fieldwork in the criminal court of São Paulo (Brazil) to create a network of interviewees which will help to produce more material of study.

The initial research led me to discover the political strength of the Jury. Despite it being such an ancient institution it underwent several changes, such as the voting procedure and the crimes of its competence. Those transformations are directly related to the political contexts where they took place. However, some of the current characteristics of the Brazilian Jury persist for quite some time as it is directly related to the elements and principles of the liberalism and the constitutional democracy. [3-5]

The bibliographical research on the “incommunicability rule” points to the prevalence of individualistic values attached to the ideals of a liberal vision according to which the jurors must shape their opinion about the defendant's guilt listening only to their conscience and intimate convictions [6]. This perspective disregards the opinion as socially shaped and considers debate and deliberation as disadvantageous by implying that “true opinion” is only achieved by a meditative state, reason why the jurors are kept isolated and cannot express their thoughts on the trial. Having that in mind, the aim of the interviews is mainly try to understand the perception they have of their own roles and the rules that guides their conducts during trials.

References:

- [1] GIGLIO-JACQUEMOT, Armelle. Entretien de Christine. 24/03/2010
- [2] JELLAB, Aziz; GIGLIO-JACQUEMOT, Armelle. « Les jurés à l'épreuve des assises: description et portraits d'une expérience marquante » In Cahiers de la justice, 2012 . <http://vosdroits.service-public.fr/particuliers/F2193.xhtml> (accessed in 16/02/13)
- [3] BARBOSA, Ruy. O Juri sob todos os aspectos. Rio de Janeiro, 1950
- [4] FRANCO, Ary Azevedo. O Jury no Estado Novo. São Paulo, 1939
- [5] RANGEL, Paulo. Tribunal do júri: visão lingüística, histórica, social e jurídica. Rio de Janeiro: Lumen Juris, 2009
- [6] SCHRITZMEYER, Ana Lúcia Pastore. Controlando o poder de matar: uma leitura antropológica do Tribunal do Júri. Tese de doutorado, PPGAS-USP, 2002.

A4

**SPORT
SCIENCES I**

**VI
PARALLEL
ORAL
SESSIONS**

Motor Readiness and Physical Education Classes

C. Malafaya¹, T.N. Gomes¹, F.K. dos Santos^{1,2}, M. Souza^{1,2}, R. Chaves^{1,2}, D. Santos¹, C. Morêda¹, S.Pereira¹, A. Borges¹, R. Garganta¹ and J.Maia¹

¹ CIFI²D, Faculty of Sports, University of Porto, Porto, Portugal.

² CAPES Foundation, Ministry of Education of Brazil, Brasília – DF, Brazil.

The motor readiness is required to realize, with success, motor tasks. In the context of physical education classes, identifying students' motor readiness is relevant to adapt activities according to interindividual differences among students from the same grade, as well as among grades in the same school. Assuming that motor readiness is related to motor proficiency and physical fitness, the use of specific tests allows the assessment of the motor readiness.

The purposes of this study were (1) to use the discriminant analysis to verify differences in the expected motor readiness categories among school grades; and (2) to create a “physical aptitudegram” based on centiles distribution using quintile regression.

Sample comprised 2986 Portuguese youth, from grades 5th to 9th (aged 10 to 15 years) of both gender (1388 girls, 1598 boys), from Leça da Palmeira Basic School. Physical fitness was assessed using five tests from three different physical fitness test batteries (EUROFIT, AAHPERD, Fitnessgram): handgrip, standing broad jump, shuttle run, 50 yard dash, and 1 mile run/walk test. The statistical analysis involved, firstly, the discriminant analysis, which was computed in software SYSTAT 13 and allowed to detect children that were misclassified according to their primary group (ie, school grade); secondly, the quintile regression was used, according to sex and age, computed in the software STATA 13, where children were classified in five groups (P20, P40, P50, P60, P80), and those who belonged, at least, between P20 and P40 were classified as having a minimum motor readiness level.

Results showed an increment in the performance, in both sexes, over the school grades, with boys being more physically active than girls in all five tests. The discriminant analysis showed significant results for both boys (Wilks' $\Lambda=0.48$, $F=54.32$, $p<0.001$) and girls ($\Lambda=0.58$, $F=33.71$, $p<0.001$), with a low percentage of students (45%) appropriately classified in their motor readiness according to their grade. The quintile regression results showed a high heterogeneity and interindividual variability for each grade, in boys and girls, meaning different levels of motor readiness.

In conclusion, low physical fitness profile was observed in both sexes, with a low percentage of students (45% of boys, 37% of girls) being appropriately classified for their respective grade, which means that there is a high variability in motor readiness among children from the same grade, that can be considered by the physical education teachers when planning their classes. Moreover, the results of this study can be useful for physical education teachers, especially for those from Leça da Palmeira Basic School, because the reference values presented for physical fitness tests can be used to evaluate children's motor readiness according to grade (age) and sex.

The vision of the female coaches about their inclusion in leadership positions in sport

da Silva M.C.M.¹; Almeida, J.A.¹; Mourão, L.²; Silva, P³.

¹Mobility students in Universidade do Porto; Faculdade de Educação Física – Universidade Federal de Juiz de Fora, Brasil;

² Professor of Faculdade de Educação Física – Universidade Federal de Juiz de Fora, Brasil;

³CIAFEL, Faculdade de Desporto – Universidade do Porto

Sport has a clearly masculine history and many sports still reflect social values associated with masculinity. The struggle for inclusion in Sport by women, was always present, however, with limited visibility, there seems to be some concern of society about the inclusion of women in this field (Moura, 2010). It is even more restrict to women their act as coaches, this still is surrounded by male dominance, especially, in high performance teams, since the literature suggest that women have been marginalized and discriminated in leadership positions facing different barriers.

The present study aims to understanding the point of view of women coaches regarding their profession and the obstacles along their career

A qualitative approach employing in-depth individual interviews was made to a group of 13 women coaches high level competition of Brazil (mean age \pm 45 years) of several sports (swimming, soccer...). All participants agreed to participate and provided an informed consent. The guide for the interviews was structured around the under-representation of women coaches in Brazil. The interviews were taped with the participants' consent. The interviews were fully transcribed and content analysis technique and interpretation was applied. The categories of analysis were based on the structural influence, organizational and relational analysis, that research suggests that explain the underrepresentation of women coaches (Norman, 2010).

The results suggests a focus in relational analysis with 20 references, highlighting the women's double shift, the lack of opportunity, the male sexism followed by the organizational influences. The speeches were focused on issues related to barriers that women coaches have to face in their career and in particular financial support and recognition. The few references to structural influences refer to the concept of Kanter (1977) that the homologous reproduction seems to explain the continuity of a small number of women coaches since the environment who plays the role of coach is dominated by men and structured by their norms and values, women hardly will be indicated or accepted. We conclude that coaching is still a male domain, and the inclusion of women and their skills for this profession is difficult. The association of sports to masculinity and, in turn, the alleged connection of the exercise with masculinity authority (Connell, 1987) depart women out of leadership positions such as in the role of coach.

References

- [1] Moura, D.L. et al. (2010) *Esporte, mulheres e masculinidades*, Esporte e Sociedade, 13(5).
- [2] Norman, L. (2010), *Bearing the burden of doubt: female coaches experiences of gender relations*, Research Quarterly for Exercise and Sport, 81 (4), 506-517.
- [3] Kanter, R. M. (1977). *Men and women of the corporation*. . New York: Basic Books.
- [4]Connell, R. (1987). *Gender and Power: Society, the Person and Sexual Politics*. Stanford: Stanford University Press.

Correlates of Vigorous Physical Activity in Children. A Multilevel Analysis

A. Borges¹, T.N. Gomes¹, F.K. dos Santos^{1,2}, M. Souza^{1,2}, R. Chaves¹, D. Santos¹, S.Pereira¹, R. Garganta¹ and J.Maia¹

¹ CIFI²D, Faculty of Sports, University of Porto, Porto, Portugal.

² CAPES Foundation, Ministry of Education of Brazil, Brasilia – DF, Brazil.

It is known that children physical activity (PA) has declined. Since they spend most of their awake time at school, where they can be engaged in active activities (structured and non-structured), it seems that school characteristics may be important in promoting increases in children's PA.

The aim of this study was to identify school and student correlates responsible for inter-individual variability in vigorous PA (VPA) of Portuguese children.

The sample comprised 1075 Portuguese children (aged 6 to 10 years) of both gender (512 girls, 563 boys), from 24 primary schools from North of Portugal. PA was assessed with the Godin & Shephard questionnaire [1], and VPA was used. Body mass index (BMI) was computed using measured height and weight, and cardiorespiratory fitness was estimated with the 1 mile run/walk test using the procedures described by the Fitnessgram® battery test. School characteristics included school size (estimated by the number of students), school setting (rural, suburban and urban areas), characteristics of playground area (with or without playing facilities), frequency and duration of physical education classes, and qualification of teacher responsible for physical education classes. Multilevel modeling was used to examine subject and school characteristics related to the VPA. HLM 6.0 software was used, with significance level set at 5%.

Results showed that school effects explain about 27% of the total variance of VPA in children. Older children ($\beta=1.34$, $p=0.005$) and boys ($\beta=3.64$, $p=0.003$) had higher levels of VPA; no significant association ($p>0.05$) was found for BMI and cardiorespiratory fitness. At the school-level, school size had a significant effect on children's VPA, i.e., with increasing school size there was an increase in children's VPA ($\beta=0.11$, $p=0.020$); children from urban schools reported less VPA than those from rural schools ($\beta=-17.41$, $p<0.001$), but children with more than two physical education classes per week had more VPA. The qualification of the teacher responsible for physical education classes was also related to children's VPA, where children whose physical education classes were taught by a graduated in physical education had lower VPA levels as compared to those whose physical education classes were taught by their regular schoolteacher ($\beta=-18.33$, $p<0.001$).

In conclusion, results indicated that school environment is an important correlate of children's VPA, allowing children to be active and healthy. This is a relevant information that should be used by school principals, regular schoolteacher, and physical education teacher to develop curricula programs, interventions, and policies to promote PA classes and other unstructured PA (recess and lunchtime play).

References:

[1] Godin, G., Shephard, R.J. (1985). *A simple method to assess exercise behavior in the community*. Can J Appl Sport Sci. 10(3):141-6.

Pedal reaction time of Track & Field athletes: effect of sex and competition distance

Fortuna, J.¹; Oliveira, A. F.¹; Calçada, C. ¹; Teixeira, L.¹; O. Vasconcelos¹, P. Rodrigues^{1,2}

¹ Motor Control and Learning Laboratory, CIFI2D, faculty of Sport; University of Porto, Portugal

² CIIERT/Edutec, Instituto Piaget

The overall aim of our study was to analyze functional motor asymmetry (FMA) in pedal reaction time of Track and Field Athletes. Another purpose was to analyze the effect of sex and competition distance on that variable. Ten athletes of each sex participated in this study, which were divided into two distance groups (100m and 400m). The pedal reaction time for the preferred foot (PF) and for the non-preferred foot (NPF) was measured using the Nelson Foot Reaction (Nelson, 1979). Statistical procedures included descriptive statistics and the nonparametric Mann-Whitney test. The level of significance was set at $p \leq 0,05$.

The results showed that: i) 400m athletes presented worse results for the PF, NPF and MA than 100m athletes; ii) 400m female athletes had worse results with the NPF than 100m female athletes. 400m males athletes had worse results than 100m male athletes concerning the PF, NPF and MA.

Key Words: Track & Field, Sex, Simple Pedal Time Reaction, Motor Functional Asymmetry

References:

Nelson, J.K., 1979. Measurement of Physical Performance. Burgees Publishing Company. Minnesota.

CARDIORESPIRATORY RESPONSE OF AN ELITE ATHLETE OF TAEKWONDO DURING A SIMULATED COMBAT PILOT STUDY

V. F. Silva, A. Sousa, J. Ribeiro, R. J. Fernandes e S. Soares

Centre of Research, Education, Innovation and Intervention in Sport (CIFI₂D), Faculty of Sport, university of Porto, Porto, Portugal

The purpose of the study was to evaluate the cardiorespiratory response of an international level Taekwondo athlete during a simulated combat.

Two athletes of the Portuguese Taekwondo Federation (weight category: 68-80 kg) participated in a simulated combat. Just one of the athletes was evaluated, once the probability of occurrence of an injury due to the use of the research instruments, and as a consequence of the power of the upper and lower limbs attacks, is still unknown.

The athletes were equipped in accordance to the rules of the world competitions. The simulated combat consisted in two rounds of 2min each, with a rest interval of 1min. The oxygen consumption (VO₂) of the athlete was measured using a portable and telemetric gas analyser (K4 b², CosmedTM, Italy, Rome), programed to make the analyses breath by breath. The heart rate (HR) was monitored beat to beat through a heart rate monitor (Polar Systems, RS800, Electro[®], Kempele, Finland). The instruments were activated 5min before the combat starts and remained connected 7min after the combat finishes.

A descriptive analysis (mean±SD) was used to present the results. Normality of the data was tested with the *Shapiro-Wilk* test. The mean VO₂ and HR values were compared using a paired samples *t-test*. The level of significance was set at 5%.

The results for the 1st and 2nd rounds were a VO₂ peak (VO_{2peak}) and a HR peak (HR_{peak}) of 66.0/61.1 ml.kg⁻¹.min⁻¹ and 190/195 bpm, respectively. The mean values of VO₂ and HR can be observed in Table 1.

	1 st round	2 nd round	<i>t</i>	<i>p</i>
VO ₂	44.60±14.5	46.80±10.7	0,829	0,415
HR	173±24	184±12	4,232	0,001

Table 1. Mean (±SD) values of oxygen consumption (VO₂) and heart rate (HR) attained during the 1st and 2nd rounds of a simulated Taekwondo combat.

It was observed that the Taekwondo athlete kept the same VO₂ during the 1st and 2nd rounds, whereas the HR rose significantly in the 2nd round.

The results allowed concluding that in training periodization it is important to focus on anaerobic system because of the velocity of the combat. Additionally, focus in aerobic system is also important to withstand a long combat period.

Growth Charts for Portuguese School Children. A Study in Leça da Palmeira Basic School

A. Morêda¹, T.N. Gomes¹, F.K. dos Santos^{1,2}, M. Souza^{1,2}, R. Chaves^{1,2}, D. Santos¹, C. Malafaya¹, S.Pereira¹, A. Borges¹, R. Garganta¹ and J.Maia¹

¹ CIFI²D, Faculty of Sports, University of Porto, Porto, Portugal.

² CAPES Foundation, Ministry of Education of Brazil, Brasília – DF, Brazil.

Growth charts are important tools to describe and monitor children's growth and health, providing reference values for age and sex that can be used by pediatricians as well as by physical education and sports professionals. In spite of this relevance, there is no growth charts that represent the Portuguese youth population as a whole, nor local charts.

The purposes of this study were (1) to develop reference charts for height, weight, body mass index (BMI), waist circumference and fat mass from children at Leça da Palmeira Basic School; and (2) to compare their results with other Portuguese regional data and international reference studies.

Sample comprised 2836 Portuguese youth (aged 10 to 15 years) of both gender (1513 girls, 1323 boys), from Leça da Palmeira Basic School. Height, weight and waist circumference were measured according to International Society for the Advancement of Kinanthropometry protocol; body fat was estimated by the bio-impedance scale (TANITA BC-418 MA), and BMI was calculated using the standard formula. The percentile curves were built for each sex using the LMS method, using the LMSchartmaker Pro version 2.54 software. For further comparison, percentile 50 was used and compared with results from Azores, Vouzela, and mainland sample, as well as with international (CDC and NHAMES) studies.

Results showed that the physical growth of Leça da Palmeira children is similar to that observed in other studies, with a consistent trend of increasing with age in boys, while in girls there is a deceleration from 14 years. In general, children from Leça da Palmeira are heavier, had higher BMI and body fat than those children from national studies. However, boys showed the lower waist circumference until the age at 14, and girls had the lowest values after 12 years (when comparing with other Portuguese studies and CDC values).

In conclusion, this study provides relevant information related to growth, body composition and waist circumference from children from Leça da Palmeira, that can be useful to teachers, pediatricians, nutritionists, and coaches. Moreover, results provide local references for youth growth and body composition, which can be useful to understand growth patterns and health state for this population.

LOWER LIMBS DEXTERITY IN ELDERLY PEOPLE. EFFECT OF AGE AND GENDER

S. Oliveira¹, D. Oliveira¹, V. Herpich¹, O. Vasconcelos¹, P. Rodrigues^{2, 1}

¹Motor Control and Learning Laboratory, CIFI2D, Faculty of Sport, University of Porto, Portugal

²CIERT/Educec, Instituto Piaget

Aging is a biosocial growing phenomenon in the world's population, particularly in developed countries (Giannakouris, 2008). It involves several changes concerning various bodily structures and systems, with particular expression in the decrease of the sensorimotor abilities. The present study aimed to understand the effect of age and gender in the lower limbs dexterity (preferred foot and non-preferred foot) and in the pedal functional asymmetry. The sample was composed of 17 participants, 7 females and 10 males, aged between 71 and 84 years, divided into two groups, younger seniors (71 to 76 years old) and older seniors (77 to 84 years old). To evaluate pedal dexterity it was applied The Tapping Pedal Test described in the Battery Tests of FACDEX (1991). Statistical procedures included descriptive statistics and the nonparametric Mann-Whitney test. The level of significance was set at $p \leq 0.05$.

The main conclusions obtained in this study were the following: (i) Regarding the effect of age, with respect to the preferred foot, non-preferred foot and functional asymmetry, there were no statistically significant differences between the two groups; (ii) As for the effect of gender, according all the sample and in each group of age (younger and older seniors) there were, also, no significant differences regarding the preferred foot, non-preferred foot and functional motor asymmetry. It would be interesting to study, in future research, the effect of physical activity practice in elderly taking into account the same age range.

References:

[1] Giannakouris, K. (2008). *Ageing characterises the demographic perspectives of the European societies* [Versão electrónica]. Eurostat - Population and social conditions disponível em <http://ec.europa.eu/eurostat/>.

[2] FACDEX (1991). *Desenvolvimento somato-motor e factores de excelência desportiva na população escolar portuguesa*. Lisboa, Ministério da Educação e FCDEF - UP.

A5

AGRO FOOD I

VI
PARALLEL
ORAL
SESSIONS

Economic impact of food waste in a school centre

J. Ribeiro¹, A. Rocha¹

¹ Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto

The recent world food crisis, resulting from the euro zone financial crisis threatens the access of the small producers to the land, as well as food production and wealth generation strongly affecting families food security. Wealth is not uniformly distributed and a great part of the population remains in poverty situations and food insecurity. Children spend most of the time at schools and therefore it is in those places that they consume most of their daily meals. It is then of high importance to control the quality of food served and consumed namely by quantifying food waste at school canteen.

The aim of this study was to evaluate the economic impact of food waste from school meals in a school in the centre in the north of the country.

The work was carried out at school centre during 6 days and included 1684 meals. The produced food, leftovers and plate waste were measured. *Per capita* consumption, the cost of waste and the number of people who could be feed with the value corresponding to waste were calculated.

The results showed that were wasted 29% of the produced food, and that bread was the meal component more wasted. The average cost of the total waste was around 35€ per day and would be enough to feed 119 people.

The performance of the food service evaluated taking into account the waste feed is awful and produces unnecessary costs, so it is essential to define strategies to reduce food waste, such as staff training and awareness among users of the organization.

Sensory profiling of duck rice using conventional and rapid methods

C. Rocha ¹, A. Laranjeira², F. Machado³, R. C. Lima¹ and L. M. Cunha²

¹ Sense Test, Lda., Portugal

² REQUIMTE/DGAOT, Faculty of Sciences, University of Porto, Portugal

³ Ernesto Morgado, Portugal

Consumers take an active and crucial role in the development of new products, since they are the final acceptors of all development procedures. According to Cunha et al. “sensory aspects” are the most important determinants of individual food choice, for Portuguese consumers [1]. Sensory analysis emerges as a link between research and product development, and makes use of the senses (sight, smell, touch, taste and hearing) as a measuring instrument [2]. Descriptive analysis is one of the most powerful tools of sensory analysis, and has become a subject of interest among the research community [3]. In the last years, the rapid descriptive methods has gained popularity because they’re faster tools than conventional profile and use consumers to perform evaluations, while conventional profile requires a panel of trained judges [4]. The aim of this study is to trace a descriptive profile of pre-cooked duck rice, comparing profiles resulting from a conventional method (QDA) and rapid descriptive sensory methods (Check-All-That-Apply - CATA, *Flash Profile*, *Napping*[®] e *Ultra Flash Profile*). Eight samples of duck rice were used: six commercially available samples (two Industrial brands and four Own brands, divided in refrigerated and frozen) and two new products. Panel size ranged from 11 trained judges, for QDA, to 55 consumers familiar with sensory evaluation for CATA. The two new dehydrated samples of duck rice, that are new market proposals, are similar to the control product, and assume a close position to each other. They have similar recognized characteristics, either in the fast techniques or in the conventional profile. The results of rapid methods and QDA techniques are similar, so rapid methods are good tools of sensory profiling. However, they don’t replace the conventional profile since these methods are an important tool for product development and when a highly trained judge is needed.

Acknowledgements: This research was supported through project *NOVELTEC - Desenvolvimento de Novas Tecnologias de Suporte à Criação de Produtos Inovadores (Projeto Mobilizador n.º 13846)*, financed by AdI, with QREN and FEDER funds.



References:

- [1] Cunha, L.M., Lima, R.C. e Moura, A.P. (2012). *Motives underlying food choice: is consumerism an issue?* Beyond consumption, 2nd PERL International Conference, Book of Abstracts, TU-Berlin, p.36.
- [2] Drewnowski, A. (2002), *Taste, genetics and food choices* _ in From Genes to Culture, Anderson, H., Blundell, J. and Chiva, M. (eds.), Danone Institute, Belgium.
- [3] Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food: Principles and Practices*: Springer.
- [4] Valentin, D., Chollet, S., Lelièvre, M., & Abdi, H. (2012). *Quick and dirty but still pretty good: a review of new descriptive methods in food science*. International Journal of Food Science & Technology, 47(8), 1563-1578.

Amino acid characterization of wild duck (*Anas platyrhynchos*) meat

S. Janeiro¹, A. C. Vasconcelos¹, F. B. Pimentel¹, R. C. Alves^{1,2}, A.S.G. Costa¹, M.A.G. Quaresma³, M.B.P.P. Oliveira¹

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

³ Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal.

Consumer awareness of the relationship between nutrition and health lead to an increased concern in finding balanced nutritional foods with health benefits. Nowadays, meat consumption in the human diet represents about 40% of daily protein ingestion. Since hunting represents an important tradition in Portugal, the wild duck (*Anas platyrhynchos*) may be a viable option to conventional poultry birds [1,2]. As far as we know, there is no information about wild duck amino acid composition, hunted in Portugal. Thus, the aim of this study was to evaluate the amino acid profile of wild duck meat (breast and leg).

The amino acid characterization of the freeze-dried duck samples, breast ($n=3$) and legs ($n=3$), was performed after acidic hydrolysis, followed by derivatization with dansyl chloride. The compounds were analyzed by HPLC with fluorescence detection [3]. Each sample was analyzed in triplicate and results expressed in g of amino acid/ 100 g of freeze-dried sample.

The results show that lysine and leucine are the predominant amino acids, followed by isoleucine, threonine, phenylalanine, valine plus methionine, and histidine, in both types of samples. Referring to the non essential fraction, alanine, arginine and proline were the most abundant amino acids, followed by serine, aspartic and glutamic acids, glycine, tyrosine and ornithine. Although the general amino acid profiles were similar for breast and leg meat, higher contents of each amino acid were found in duck breast, except for glycine and proline.

These preliminary data contribute to a better knowledge of wild duck meat amino acid profile, showing that this type of meat could be a good source of animal protein due to its high biological value. Nevertheless, it is necessary to carry out additional analysis in order to improve knowledge about this species.

References:

- [1] Adzitey F. (2012), *Production potentials and the physicochemical composition of selected duck strains: a mini review*. Online Journal of Animal and Feed Research, 2(1), 89-94.
- [2] Cobos, A., Veiga, A., Diaz, O. (2000) *Chemical and fatty acid composition of meat and liver of wild ducks (*Anas platyrhynchos*)*. Food Chemistry, 68(1), 77-79
- [3] Pimentel, F. B., Alves, R. C., Costa, A. S. G., Torres, D., Almeida, M. F., and Oliveira, M. B. P. P. (2014), *Phenylketonuria: Protein content and amino acids profile of dishes for phenylketonuric patients. The relevance of phenylalanine*. Food Chemistry, 149(0), 144-150.

Acknowledgments: The authors thank to the company CAÇABRAVA for providing samples. R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

Wild and farmed duck breast meat: comparison of amino acid profiles by HPLC-FLD

**A. C. Vasconcelos¹, S. Janeiro¹, F. B. Pimentel¹, R. C. Alves^{1,2},
A. S. G. Costa¹, M. A. G. Quaresma³, M. B. P. P. Oliveira¹**

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

³ Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal.

Game birds arouse the interest of hunters for recreational activities and as a source of meat for human consumption. Generally, game bird meat is considered a good source of animal protein with low fat content, but rich in omega-3 and omega-6 fatty acids. However, information about the nutritional quality of this type of meat is scarce. From our knowledge, there is no reports about the amino acid composition of wild duck (*Anas platyrhynchos*) meat, in Portugal. Thus, this study involved the characterization and comparison of the amino acid profile of wild and farmed duck breast meats.

Freeze-dried samples ($n=3$ of each) were subjected to an acidic hydrolysis, followed by derivatization with dansyl chloride. Amino acids were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) with fluorescence detection (FLD), according to Pimentel *et al.*[1]. Each sample was analysed in triplicate and results were expressed in g of amino acid/ 100 g of freeze-dried sample.

The results showed that lysine and leucine are the main essential amino acids, in both types of samples (8.9 and 6.3 g/100 g, for wild samples, and 11.5 and 7.8 g/100 g, for farmed ones, respectively). Regarding the non essential amino acids, the predominant ones were alanine and arginine (4.1 and 3.8 g/100 g, for wild duck meat, and 4.6 and 5.2 g/100 g, for farmed ducks, correspondingly). However, serine was only detected in the wild group (3.1 g/100 g).

These early data show that, with a good sustainability of hunting activity, wild duck breast meat may be an alternative source of animal meat for human consumption. Consequently, knowledge about this species should be expanded and additional studies are required.

References:

1. Pimentel et al., *Phenylketonuria: protein content and amino acids profile of dishes for phenylketonuric patients. The relevance of phenylalanine.* Food Chemistry. (In Press). DOI: 10.1016/j.foodchem.2013.10.099.

Acknowledgments: The authors thank to the company CAÇABRAVA for providing samples. R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069-CIÊNCIA DO ALIMENTO).

Analysis of malt volatile compounds by headspace solid-phase microextraction coupled with GC-MS

M. Fernandes¹, S. Cunha¹, S. Meireles², T. Brandão² and I.M.P.L.V.O. Ferreira¹

¹REQUIMTE – Departamento de Ciências Químicas, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto

²UNICER, Bebidas de Portugal SGPS, SA, Leça do Balio

Barley is widely used to produce malt, although other cereals such as wheat can be malted. The process of malting is subdivided in three steps that involve the controlled germination, subsequent kilning and/or roasting process. Different types of malt, can be produced depending of the temperature to which it is subjected [1,2]. Different malts can be distinguished, namely pilsen, caramel, chocolate and wheat malt [3]. This study involved analysis of volatile compounds in these four types of malt. Solid-phase microextraction (SPME) technique was applied for extraction of sample volatiles and it was coupled with gas chromatography-mass spectrometry (GC-MS) for separation and identification of the compounds under study. The fiber and extraction conditions were optimized.

Two fibers were evaluated: carboxen-polydimethylsiloxane (CAR-PDMS) and divinylbenzene-carbox-polydimethylsiloxane (DVB-CAR-PDMS). The selected fiber was exposed to the sample headspace for 40 min at different temperatures: 50°, 60° and 70°. Analyses were performed with and without addition of sodium chloride solution (20% NaCl) with the same extraction temperatures.

CAR-PDMS fiber was more appropriate for extraction of a large number of compounds, without need of NaCl addition. The chosen method was reproducible using a temperature of 70° C, presenting less than 10% coefficient of variation. By analyzing the areas of chromatographic peaks it was observed that alcohols were predominant in samples of wheat and pilsen malt. The chemical family that exists in larger quantity in chocolate malt was furan compounds whereas in caramel malt were aldehydes. In the future we intend to quantify the key volatile compounds.

This work was supported by Project PP-IJUP2012-UNICER- 13.

References:

[1] Dong, L., Piao, Y., Zhang, X., Zhao, C., Hou, Y. and Shi, Z. (2013), *Analysis of volatile compounds from a malting process using headspace solid-phase micro-extraction and GC-MS*, Food Research International, 51 (2), 783-789.

[2] Huges, P. (2009). *Identification of taste – and aroma – active compounds of beer*, Beer in health and disease prevention, International center for brewing and destiling, Heriot-Watt University, Riccarton, Endinburgh, UK, 2009, pp. 227-238

[3] Vandecan, S.M., Saison, D., Schoupe, N., Delvaux, F. and Delvaux, F.R. (2010) *Optimisation of specialty malt volatile analysis by headspace solid-phase microextraction in combination with gas chromatography and mass spectrometry*. Analytica Chimica Acta, 671 (1-2), 55-60.

A6

PHYSICS II

VI
PARALLEL
ORAL
SESSIONS

Labview controlled Aerotech Stages

A. Gomes, V. Amorim, M.B. Marques, P. S. Marques, O. Frazão

Department of Physics and Astronomy, Faculty of Sciences, University of Porto, Portugal.

In this project, it is programmed a dual carriage, Fiber-Coupling Stage in order to fabricate Long Period Gratings and Fiber Tapers using Labview. The stage used was an Aerotech FiberCouple 130 Series controlled by an Aerotech A3200 Npq Drive Rack communicating over FireWire with the Windows PC, with the proper software installed. After understanding each type of production, the program was developed trying to maximize the results, taking into account the setup used. For Long Period Gratings (LPG) three specific parameters are implemented, namely the LPG period, LPG length and the velocity of fabrication. In this process both the translation stages work with the same velocity, in steps, and in the same direction. Relatively to the Fiber Tapers fabrication, it was divided in three sections, Long, Micro and Bottle Tapers. The first case, the two translation stage works with different velocity but with the same direction. Relatively to Micro Taper the velocity is the same but in opposite direction. Finally, the Bottle Taper, the velocity is low and we approach the translation stage.

In the future, works will be continued at INESC-PORTO where, along with the rest of the equipment, the implemented processes will be tested and refined.

Tracking the ultrafast carrier dynamics in graphene using pump-probe spectroscopy: preliminary femtosecond pulse measurements using the d-scan technique

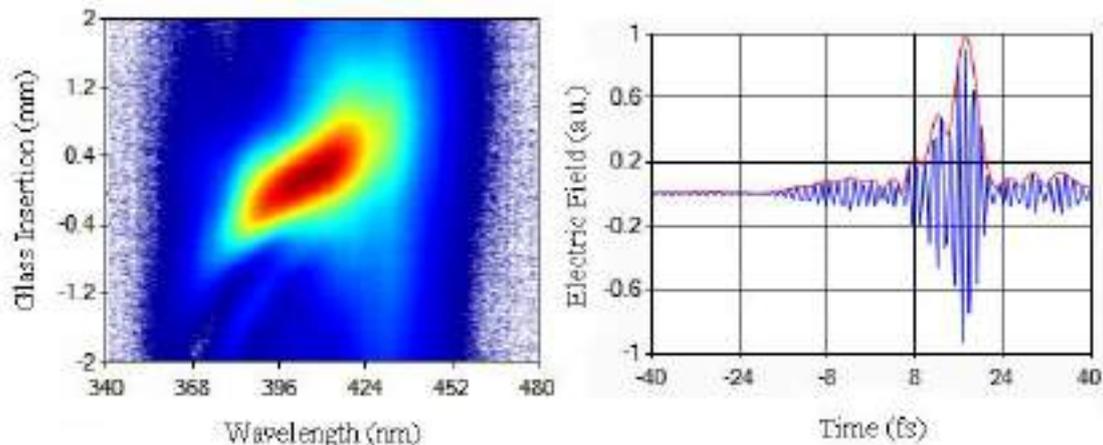
T. Magalhães^{1,2}, H. Crespo^{1,2}

¹ Department of Physics, Faculty of Science, University of Porto, Portugal.

² IFIMUP and IN - Institute of Nanoscience and Nanotechnology.

One of the major applications of ultrashort laser pulses is the time-resolved study of dynamical processes in various types of materials (e.g., semiconductors). These pulses can be used to perform excitation (pump) and probe microscopic processes at the femtosecond timescale. While the pump pulse is used to excite the sample into a non-equilibrium state, the probe pulse is used to monitor pump-induced changes as a function of the time delay between pump and probe, hence providing access to the ultrafast relaxation dynamics. One of the fundamental parameters in pump-probe spectroscopy is the temporal duration of the pump and probe pulses, since it determines the time resolution of the experiment. The ultimate goal of this work is to build a pump-probe setup that will enable measurements of the relaxation times in the excited electron population of graphene with unprecedented temporal resolutions.

To characterize a femtosecond pulse, one can use standard techniques like FROG or SPIDER. Recently, the new technique of d-scan was developed [1], which enables characterizing ultrashort laser pulses using iterative phase retrieval from dispersion scans (hence its name) obtained by measuring the generated second-harmonic signal as a function of dispersion. Two relevant advantages of this technique are its ease of alignment, robustness, and the fact that one does not have to split and temporally delay the pulse to be measured. We present d-scan measurements and reconstruction of an advanced few-cycle ultrafast laser oscillator. The figure below shows the measured SHG spectrum (left) and the retrieved electric field of the pulse in the time domain (right); pulse duration is 7.0 fs (FWHM), corresponding to only 2.5 optical cycles.



References:

[1] Miranda, M. Fordell, T. Arnold, C. L'Huillier, A. and Crespo, H. (2012), *Simultaneous compression and characterization of ultrashort laser pulses using chirped mirrors and glass wedge*, Opt. Express, 20(1): 688-697.

Frequency and Material Dependence of the Cooling Power in a Solid State Magnetic Refrigerator

R. Azevedo¹, D.J. Silva¹, J. Ventura¹, J. P. Araújo¹ and A.M. Pereira¹

¹ IFIMUP and IN-Institute of Nanoscience and Nanotechnology, Department of Physics and Astronomy, Faculty of Sciences, University of Porto, Portugal

Heat dissipation has recently been one of the major obstacles that the semiconductor industry has been facing. Solutions to this problem have so far relied on conventional heat sinks and fans, and to a lesser extent thermoelectric refrigeration which present low efficiency (~10 %). A viable alternative to these traditional methods has been proposed in the form of a solid state magnetic refrigerator [1]. These refrigerators are based on the magnetocaloric effect (MCE), in which the application or removal of an external magnetic field (H) on magnetocaloric materials (MCMs) causes its temperature to change in adiabatic processes, in order to compensate the reduction of magnetic entropy. The major advantage of the proposed refrigerator is that it does not require the use of fluids for the heat exchange processes, unlike the most common magnetic refrigerator technology.

In this work, we studied the dependence of the cooling power (CP) on the specific properties of the MCM and operating frequency. For that purpose, we used the same numerical method of Ref. [1]. Although gadolinium was the used standard MCM, we have varied some of its physical properties, e.g. specific heat (C_p), thermal conductivity (k), density (ρ) and adiabatic temperature variation (ΔT_{ad}). We concluded that increasing the k results in higher cooling power and optimum operating frequency. However, this frequency can be lowered by increasing any of the other parameters (C_p , ρ , ΔT_{ad}), with little to no effect on the CP maximum.

References:

[1] Silva, D.J., Bordalo, B.D., Pereira, A.M., Ventura, J., Araújo, J.P. (2012), *Solid state magnetic refrigerator*, Applied Energy, 93, pp. 570-574.

Effect of thermal treatments on Gd₅Si_{1.3}Ge_{2.7} thin films

A. L. Pires^{1,2}, J. H. Belo¹, I. Gomes¹, R. L. Hadimani^{3,4}, D.L. Schlager⁴, T.A. Lograsso⁴, Y. Ren⁵, X. Zhang⁵, D.C. Jiles^{3,4}, A. M. L. Lopes², A. M. Pereira¹, J. P. Araújo¹

¹ IFIMUP and IN - Departamento de Física e Astronomia da Faculdade de Ciências da Universidade do Porto, Rua campo Alegre, 687, 4769-007 Porto, Portugal.

² CFNUL - Centro de Física Nuclear da Universidade de Lisboa, Av. Prof. Gama Pinto, 2, 1649-003 Lisboa, Portugal.

³ Department of Electrical and Computer Engineering, Iowa State University, Ames, Iowa 50011, USA

⁴ Ames Laboratory, US Department of Energy, Iowa State University, Ames, Iowa 50011, USA

⁵X-ray Science Division, Argonne National Laboratory, Argonne, Illinois 60439, USA

The magnetic properties such as the giant magnetocaloric effect (MCE), the colossal magnetostriction and giant magnetoresistance have boosted the interest in the Gd₅(Si_xGe_{1-x})₄ family of compounds [1]. Nowadays, an interest in the study of MCE in materials with reduced dimensions has grown in order to produce miniaturized devices [2]. It is well known that heat treatment drastically influences the magnetic effects on these materials in their bulk form. In this work we devote our studies to the production of a thin film of Gd₅Si_{1.3}Ge_{2.7}, with special attention on the effect of heat treatment on its morphology, structure and magnetization properties. Femtosecond laser pulsed ablation was used for the preparation of a Gd₅Si_{1.3}Ge_{2.7} thin film on a SiO₂-covered Si substrate. Different annealing temperatures (300°C, 400°C and 500°C) were chosen for this study with a fixed annealing time (2h) and a fixed cooling procedure, which consisted in quenching into water. The SEM images show that all samples have the same granular-type morphology. Nevertheless, on the annealed samples the crystallographic structure presents drastic changes, namely by the decrease/disappearance of the Gd₅Ge₄ [O(II)] phase structure, at room temperature, when compared with the as-deposited sample. Simultaneously there is an increase of the peaks associated with the Gd₅Si₄ [O(I)] phase. Regarding the magnetic properties, the temperature dependence of the magnetization of the as-deposited sample shows two magnetic transitions: a first order transition (~194 K), associated with a structural transition between two different crystallographic phases - O(II) to O(I), and a second order phase transition (~247 K) of the O(I) phase. After annealing (300°C and 400°C) these two phases are converted in only one phase and the samples show only one magnetic transition (the second order nature). So we conclude that the annealing promotes the transformation of the O(II) phase into O(I) phase. This is also confirmed by the drastic decrease of ~58% of the MCE, from 58 mJK⁻¹cm⁻³ (as-deposited sample) to 25 mJK⁻¹cm⁻³ (sample annealed at 400°C). For similar annealings but with temperatures equal and above 500° C the magnetization reveals a typical paramagnetic behavior.

References:

[1] V. K. Pecharsky e K. A. Gschneidner, “Giant Magnetocaloric Effect in Gd₅(Si₂Ge₂),” *Physical Review Letters*, vol. 78, n.º 23, pp. 4494-4497, 1996.

[2] P. Lampen, N. Bingham, M. Phan, H. Kim e M. Osofsky, “Impact of reduced dimensionality on the magnetic and magnetocaloric response of La_{0.7}Ca_{0.3}MnO₃,” *Applied Physics Letters*, vol. 102, p. 062414, 2013.

Electrodeposition of Bi₂Te₃ Nanomaterials for Thermoelectric generators

M. Rosmaninho^{*1}, M.P. Proença¹, C.T. Sousa¹, J. Ventura¹, J.P. Araujo¹, A.M. Pereira¹

¹ IFIMUP and IN - Institute of Nanoscience and Nanotechnology, Departamento de Física e Astronomia, Faculdade de Ciências, Universidade do Porto

The fabrication of new power sources for portable, low energy, devices is one of the most challenging problems today. This limitation could be solved with the development of innovative micro/nanogenerators based on scavenging energy – the so called energy harvesting. Thermoelectric (TE) devices are rising as an alternative solution for all the wasted heat that most of the other energy sources, and even our own bodies, release [1]. TE nanomaterials have revealed new high records of power efficiency, measured in figure of merit (*ZT*) and overcome the *ZT* limit of 1, a value that had not been conquered for over several decades in bulk materials. Electrodeposition techniques are implemented with the ability to produce these TE nanomaterials in high quantities and with low costs.

In this work, two types of Bi₂Te₃ nanomaterials will be discussed: a) thin films deposited on top of flat substrates, and b) nanowire arrays grown inside nanoporous alumina templates.

The morphological, structural and crystallographic properties of the samples were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). From the XRD studies it was possible to observe that the electrodeposition of Bi₂Te₃ was successful, evidencing a crystalline nature. The SEM results revealed a cluster-like structure on the thin films, and the formation of nanowires inside the alumina templates. The Bi:Te atomic ratio was analysed by EDS, and was found to change with the electrodeposition applied potential. Tuning the Bi:Te ratio allows one to change the kind of semiconductor (p or n) the sample will present and thus the *ZT* value present.

By entering and dominating such an emerging science, this work attempts to bring a greater value to the knowledge of the scientific community on this topic, and to bring it closer to the industrial society.

References:

[1] L.E. Bell “Cooling, heating, generating power, and recovering waste heat with thermoelectric systems” *Science*, 321 (2008), 1457-1461.

Memristive Ag₂S Synapses: towards Artificial Neural Networks

C. Dias¹, L. Guerra¹, M. Proença¹, P. Aguiar², J. P. Araújo¹ and J. Ventura¹

¹ IFIMUP and IN-Institute of Nanoscience and Nanotechnology, and Department of Physics and Astronomy, Faculty of Sciences, University of Porto, Portugal.

² CMUP - Center for Mathematics of University of Porto, Portugal.

Present computer processing capabilities are becoming a restriction to meet modern technological needs. Therefore, approaches beyond the von Neumann computational architecture are imperative and the brain's operation and structure are truly attractive models [1]. Memristors are metal-insulator-metal nanostructures characterized by a nonlinear relationship between current history and voltage, which were shown to present properties resembling those of biological synapses [2] and can thus be used in neural networks capable of simulating the learning and adaptation characteristics of human brains [3].

We fabricated Ag₂S memristive samples using diversified methods (ion beam deposition, thermal annealing and electrodeposition), to optimize the processes and select the appropriate ones. The Ag₂S stoichiometry was characterized using X-ray Diffraction, Raman Spectroscopy, Fourier Transform Infrared Spectroscopy, Energy Dispersive Spectroscopy and Scanning Electron Microscopy, as well as electrical resistivity measurements. The Ag₂S thin films were fabricated either sandwiched between two electrodes or with contacts on top for transport measurements.

The expected electrical resistance switching associated with the Ag₂S system was obtained and different behaviors were observed in the devices depending on the measuring and fabrication conditions. In particular, types I and II memristive behaviors, bipolar and unipolar switching were observed. Furthermore, the dependence of the electrical resistance with time was studied and activity-dependent modifications were observed, indicating that learning can be obtained in these structures through stimulus repetition. These effects were attributed to the formation of metallic filaments due to electromigration inside the dielectric layer.

References:

- [1] Shimeng, Y., Wu, Y., and Jeyasingh, R (2011), *An electronic synapse device based on metal oxide resistive switching memory for neuromorphic computation*, in IEEE Transactions on Electron Devices, 58(8).
- [2] Chua, L. (1971), *Memristor-the missing circuit element*, in IEEE Transactions on Circuit Theory, 18(5).
- [3] Chang, T., Jo, S. H. and Lu, W. (2011), *Short-term memory to long-term memory transition in a nanoscale memristor*, in ACS nano, 5(9).

Piezoelectric Nanogenerators: Feeding the Future

F. Oliveira¹, M.P. Proença², J. P. Araújo², J. Ventura²

¹ Department of Physics and Astronomy, Faculty of Science, University of Porto, Portugal.

² IFIMUP and IN-Institute of Nanoscience and Nanotechnology, Department of Physics and Astronomy, Faculty of Science, University of Porto, Portugal

A nanogenerator (NG) is a nanometric device capable of converting energy from external sources (environment) into electric energy, feeding micro-devices and micro-systems [1]. In particular, a piezoelectric NG has the ability to convert mechanical energy into electrical one [2]. This mechanical energy can come from the direct deformation of a material, from fluid pressure or even from a vibration induced on the NG, thus enlarging the applicability of these devices. In recent years, a renewed interest started to emerge on zinc oxide (ZnO) nanogenerators for energy harvesting. In fact, zinc oxide has proved to be an efficient piezoelectric material and easy to grow in different nano-shapes, allowing novel NG configurations with higher efficiency and wider application possibilities.

In this work, two different ZnO geometries are presented: thin films electrodeposited on flat substrates; and nanowire (NW) arrays electrodeposited inside the nanopores of anodic aluminum oxide (AAO) templates. The electrodeposition processes were performed in an aqueous solution of zinc nitrate at room temperature and by applying a constant potential between the conductive substrate and a reference electrode. For the ZnO thin films, a glass substrate coated with a Cu contact was used as the conductive substrate. On the other hand, for the fabrication of the NW arrays, nanoporous AAO templates were produced by a two-step anodization process and then a Cu contact was sputtered on one of the sides of the template to serve as the conductive contact. Selected electrodeposited nanostructures were then annealed at 400°C for 4 h in air. The morphological, structural and crystallographic properties of the obtained samples were analyzed by scanning electron microscopy (SEM) and X-ray diffraction (XRD). The thin films' X-ray spectra revealed the characteristic peaks of ZnO, proving a successful electrodeposition on the substrates. Top view SEM images show that the size of the crystalline grains increase with the electrodeposition potential. Cross-section SEM images show ZnO deposited on the AAO templates, indicating the growth of 100 to 150 nm ZnO NWs.

Both nanofabrication techniques explored revealed a good performance and a high control allied to low costs and simple reproducibility, making the NG production a reality to the technology industry. Combining nanogenerators with the world of electronics and sensors (fire prevention, climacteric information, biological data, etc) and external energy sources, it is possible to permanently feed these systems without relying on batteries or similar energy powers.

References:

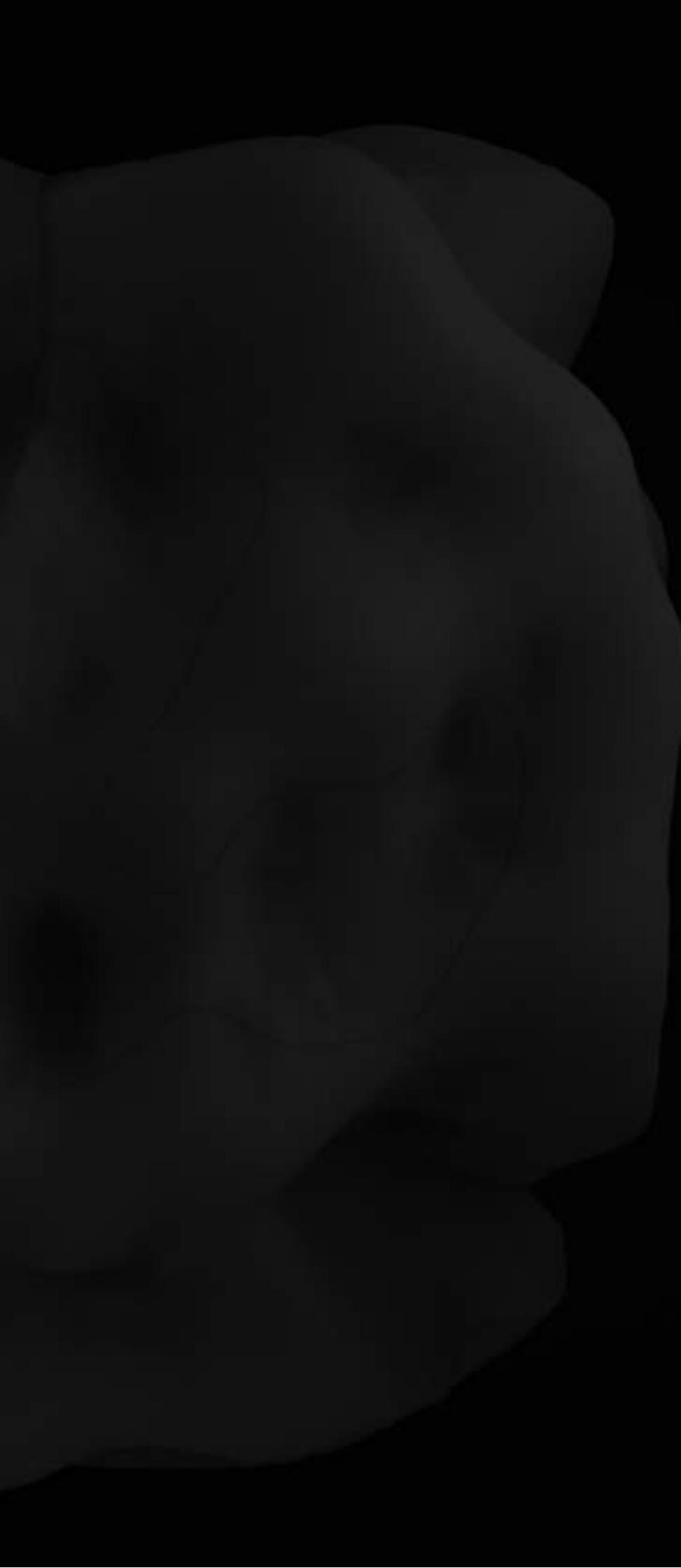
- [1] Z. L. Wang, G. Zhu, Y. Yang, S. Wang, and C. Pan, "Progress in nanogenerators for portable electronics," *Mater. Today*, vol. 15, no. 12, pp. 532–543, Dec. 2012.
- [2] Z. L. Wang and J. Song, "Piezoelectric nanogenerators based on zinc oxide nanowire arrays.," *Science*, vol. 312, no. 5771, pp. 242–6, Apr. 2006.





VII

PARALLEL
ORAL
SESSIONS



A1

BIOMEDICINE VII

VII
PARALLEL
ORAL
SESSIONS

MDM2 SNP309 TT homozygous are associated with increased risk of colorectal cancer development

L. Raeiro^{1,2}, J. Ribeiro^{2,3}, C. Pereira², P.Pimentel-Nunes⁴, R. Medeiros^{2,3}, M. Dinis-Ribeiro⁴, H. Sousa^{2,3}

¹ Faculty of Science, University of Porto, Portugal.

² Molecular Oncology Group, Portuguese Institute of Oncology of Porto FG – EPE, Porto, Portugal.

³ Virology Service, Portuguese Institute of Oncology of Porto FG-EPE, Porto, Portugal.

⁴ Gastroenterology Service, Portuguese Institute of Oncology of Porto FG-EPE, Porto, Portugal.

Colorectal cancer (CRC) is a severe malignancy worldwide, being in Portugal the second most frequently cancer with incidence rates of 40.6 and 24.1 per 100,000 each year in men and women, respectively. Despite the etiological association of dietary factors, several studies have described the role of genetic factors in the susceptibility to CRC development. MDM2 is an important protein in cellular regulation acting as the principal negative regulator of p53. In fact, the abolishment of the p53 cell-cycle regulation pathway by MDM2, contribute to cell proliferation and tumorigenesis.

We developed a retrospective case-control study to analyze a polymorphism in the promoter region of *MDM2* (SNP309 T>G) that has been associated with differential attenuation of the p53 pathway [1]. Our study was developed in 74 patients with CRC and 690 healthy individuals from the north region of Portugal. The genetic characterization of MDM2 SNP309 was performed by PCR followed by RFLP.

The genotype distribution of cases was: 46 (62.2%) TT, 23 (31.1%) TG and 5 (6.8%) GG; while for controls was 295 (42.8%) TT, 327 (47.7%) TG and 68 (9.9%) GG. Our study revealed that TT genotype was significantly more frequent in cases than controls, representing an over 2-fold increased risk for CRC development (OR=2.2; 95%CI 1.34-3.60; $p=0.001$). Moreover, the results showed that the increased risk was more evident in males (OR=2.41; 95%CI 1.29-4.53; $p=0.005$) than females (OR=1.85; 95%CI 0.82-4.14; $p=0.132$).

This is the first study to evaluate the role of the MDM2 SNP309 T>G polymorphism on the development of CRC in Portugal. Our results show a significant association of the TT genotype with CRC development, nevertheless this study was performed with a low number of cases and results need to be confirmed in a larger study. Moreover, a previous study in Portugal has showed that the GG genotype was significantly associated with the risk of nasopharyngeal carcinoma development [2] and therefore we may assume that there might be a biological difference for the differential association of the genotypes with cancer development.

References:

- [1] Bond, G.L., Hu, W., Bond, E.E., Robins, H., Lutzker, S.G., Arva, N.C., Bargonetti, J., Bartel, F., Taubert, H., Wuerl, P., Onel, K., Yip, L., Hwang, S.J., Strong, L.C., Lozano, G., Levine, A.J. (2004). A single nucleotide polymorphism in the *MDM2* promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 2004;119:591 – 602.
- [2] Sousa, H., [Pando, M.](#), [Breda, E.](#), [Catarino, R.](#), Medeiros, R. (2011). *Role of the MDM2 SNP309 polymorphism in the initiation and early age of onset of nasopharyngeal carcinoma*. *Mol Carcinog*. 2011 Feb;50(2):73-9

Let-7c expression in HPV-associated cervical lesions and cancer

M. Malta^{1,2}, **J. Ribeiro**^{2,3}, **P. Monteiro**⁴, **J. Loureiro**⁴, **R. Medeiros**^{2,3,5} and **H. Sousa**^{2,3}

¹Faculty of Science, University of Porto, Portugal.

²Molecular Oncology Group, Portuguese Institute of Oncology of Porto FG – EPE, Porto, Portugal.

³Virology Service, Portuguese Institute of Oncology of Porto FG – EPE, Porto, Portugal.

⁴Department of Pathology, Portuguese Institute of Oncology of Porto FG – EPE, Porto, Portugal.

⁵Portuguese League Against Cancer, Regional Center North, Portugal.

High-risk types of Human papillomavirus (HPV) are considered to be the main etiological factor for cervical cancer development, however, the majority of HPV infections are asymptomatic. As the outcome of HPV infection is variable, clinicians still demand for the identification of useful biomarkers of predictive/prognosis of HPV infection [1,2]. Recent studies have shown the importance of microRNAs as biomarkers in different cancers, and *let-7c*, a tumour suppressor microRNA, has been reported as underexpressed in several cancers, including cervical cancer [3,4].

In this study, we aimed to characterize *let-7c* expression in premalignant lesions and invasive cervical carcinomas. The relative expression of *let-7c* was determined by two-step qRT-PCR methodology in 79 women with different cervical cytologies: normal epithelium with HPV (n=21) and without HPV (n=17); LSIL (n=15); HSIL (n=15); CIS (n=7); ICC (n=4).

The data analysis revealed an increased expression of *let-7c* among normal cases with HPV infection ($2^{-\Delta\Delta C_t} = 2.24$) and a decreased expression in both premalignant lesions and invasive cervical cancer (LSIL: $2^{-\Delta\Delta C_t} = 0.91$; HSIL: $2^{-\Delta\Delta C_t} = 0.90$; CIS: $2^{-\Delta\Delta C_t} = 0.06$; and ICC: $2^{-\Delta\Delta C_t} = 0.51$).

Let-7c has been shown to be involved in several cellular pathways and therefore its levels could be associated with a differential activation of cellular responses to HPV infection and transformation. Our results showed that *let-7c* is overexpressed after HPV infection and under expressed in cervical lesions and cancer. Thus, considering the significantly variation of *let-7c* levels in different cervical cytologies, this microRNA should be further studied as a potential biomarker of HPV infection and cervical cancer development using a non-invasive methods.

References:

- [1] Hwang SJ, Shroyer KR. (2012), *Biomarkers of cervical dysplasia and carcinoma*. Journal of Oncology, 2012:507286.
- [2] Woodman CBJ, Collins SIY, Lawrence S. (2007), *The natural history of cervical HPV infection: unresolved issues*. Nature Review Cancer, 7: 11-2
- [3] Lui WO, Pourmand N, Patterson BK, Fire A. (2007), *Patterns of known and novel small RNAs in human cervical cancer*. Cancer Research, 67(13):6031-43.
- [4] Ma D, Zhang YY, Guo YL, Li ZJ, Geng L. (2012), *Profiling of microRNA-mRNA reveals roles of microRNAs in cervical cancer*. Chinese Medical Journal (English), 125(23):4270-6.

Personality, anxiety, depression and Atopic Dermatitis severity: a cross sectional study

L. Ferreira-Pinto¹, C. Leite¹, L. Delgado¹, A. Moreira^{1,2}, I. Lourinho³ and C. Lopes^{1,4}

¹ Laboratory of Immunology, Faculty of Medicine, University of Porto

² Immunoallergology Department, Centro Hospitalar São João, EPE, Porto

³ Centre for Medical Education, Faculty of Medicine, University of Porto

⁴ Allergy Unit, Hospital Pedro Hispano, ULS Matosinhos EPE, Portugal

Atopic Dermatitis (AD) is a multifactorial, immune mediated, chronic and relapsing skin disease, characterized by intense pruritus and eczematous lesions that contribute to significant emotional distress, sleep disturbance and impact on the Quality of Life (QoL) of both patients and their families. Psychological stress deregulates central and peripheral Hypothalamic-Pituitary-Adrenal axis leading to a possible immune imbalance and consequently an increase in skin pruritus. As there are few studies addressing the association of psychological parameters with AD severity, the aim of this study was to evaluate the association between anxiety and depression levels, personality traits and disease severity in adult patients with AD.

A total of 69 patients, over 16yo and previously diagnosed with AD were recruited between November, 2011 and June, 2012 to be clinically evaluated by a dermatologist and an immunoallergologist. Patients presenting severe comorbidities or other immune mediated skin diseases were excluded. Anxiety and depression levels were evaluated through the *Hospital Anxiety and Depression Scale* (HADS) – into normal, mild, moderate and severe. Personality traits were assessed through *NEO Five Factor Inventory* – evaluating neuroticism, extroversion, openness, agreeableness and consciousness. AD severity was evaluated through SCORAD and Quality of Life (QoL) through DLQI (*Dermatology Life Quality Index*). Statistical analysis was performed using SPSS 21.0®.

A total of 44 patients were enrolled – mean age (SD) of 31 (13) yo, 39% males. SCORAD mean (SD) was 45 (28) and DLQI mean (SD) was 8 (5). 34% of patients presented anxiety while only 14% presented depression. No correlation was found between anxiety and SCORAD while a positive correlation was suggested between depression and SCORAD ($R = 0.3$; $p=0.068$). As for personality traits, a one-way ANOVA mean comparison for each trait and SCORAD showed significant differences only for Consciousness ($p=0.039$). Concerning QoL, a linear regression model presented SCORAD as the strongest predictor.

As for other immune mediated skin diseases, future studies are required to undoubtedly prove the association between AD severity and several psychological parameters. Also, it is of vital importance to establish whether depression is a cause or a consequence of having a more severe phenotype of the disease. In a near future, psychotherapeutic interventions may present as an important instrument for disease control.

This work was supported within the scope of the Investigação Jovem na Universidade do Porto **PP_IJUP2011_91**.

Effects of epigenetic modulation upon mitochondrial dynamics and neurite outgrowth in developing neurons

P. Guedes-Dias^{1,2}, J. P. de Proença¹, M. R. Duchén² and J. M. A. Oliveira¹

¹ *REQUIMTE*, Dep. Drug Sciences, Pharmacology Lab, Fac. Pharmacy, University of Porto, PT ² Dep. Cell and Developmental Biology, University College London, UK

The epigenome comprises a series of heritable modifications controlling cell differentiation and development that do not result from direct gene sequence changes. Epigenetic deregulation associates with neurodegenerative diseases, and its pharmacological modulation may thus potentially attenuate neurodegeneration [1,2]. Here we tested the impact of epigenetic-modulatory drugs (lysine deacetylase inhibitors) upon mitochondrial dynamics and neurite outgrowth in developing neurons.

Primary rat cortical and striatal neurons were treated at 0 days *in vitro* (DIV) with single or combined 1 μ M entinostat (MS-275) and 1 μ M tubastatin A (HDAC-1 and -6 selective inhibitors, respectively), in parallel with respective untreated controls. Drug efficacy was confirmed by Western Blotting for histone and tubulin acetylation. Neurons were co-transfected with EGFP and mitoDsRed encoding plasmids at 2 DIV and imaged live at 3DIV. Neurite outgrowth (EGFP) was evaluated by Sholl analysis and Mitochondrial dynamics (mitoDsRed) by particle number, size and fractional occupancy analysis.

Entinostat and tubastatin increased histone H3K9 and α -tubulin K40 acetylation, respectively. Sholl analysis revealed higher enclosing field and total intersections in cortical vs. striatal neurons. Entinostat decreased both parameters in both neuronal populations. Average intersections were similar in cortical vs. striatal neurons at all treatments. Maximal intersection count and the distance of its first occurrence were highest for cortical neurons under control or tubastatin treatment. Consistently, these neurons exhibited a lower rate of branching decay (with distance from soma). In cortical neurons, tubastatin co-treatment partially rescued entinostat's effects on neurite size, total intersections and branching decay. Mitochondrial fractional occupation (MFO) was similar in cortical vs. striatal neurons, either in control conditions or following tubastatin treatment. Entinostat with/without tubastatin increased MFO, consistently with a modest increase in mitochondrial size and number per 100 μ m neurite.

In summary, sister primary cultures of cortical and striatal neurons differ in branching profile. 3DIV cortical neurons are larger and more complex than striatal counterparts. Entinostat approximates the branching of cortical neurons to that of control striatal neurons, but with increased mitochondrial fractional occupation. Tubastatin displays a trend for increased branching and for partly reversing entinostat-evoked phenotypes.

Acknowledgements:

Supported by SFRH/BD/72071/2010, PTDC/NEU-NMC/0237/2012 (Fundação para a Ciência e a Tecnologia), FCOMP-01-0124-FEDER-029649 (COMPETE), and PPII_ZEBRA (U.Porto & Santander-Totta)

References:

- [1] Narayan, P. and Dragunow, M. (2010), *Pharmacology of epigenetics in brain disorders*, British Journal of Pharmacology, 159, 285-303.
- [2] Guedes-Dias, P. and Oliveira, J.M.A. (2013), *Lysine deacetylases and mitochondrial dynamics in neurodegeneration*, Biochimica et Biophysica Acta, 1832, 1345-1359.

Search for early TTR-related biomarkers in a transgenic AD mouse model

L.M. Santos^{1,2,3}, C.A. Ribeiro^{1,3}, S.M. Oliveira⁴, M.J. Saraiva^{3,4}, I. Cardoso¹

¹ MiND, IBMC - Cellular and Molecular Biology Institute, Porto, Portugal.

² Department of Biochemistry, Faculty of Sciences, University of Porto, Portugal.

³ ICBAS - Abel Salazar Biomedical Sciences Institute, University of Porto, Portugal.

⁴ Molecular Neurobiology, IBMC- Cellular and Molecular Biology Institute, Porto, Portugal.

Transthyretin (TTR) has been shown to be neuroprotective in AD; however, its underlying mechanisms are still vastly unknown. Here we proposed to investigate sortilin (Sort1) and synaptophysin (Syp), suggested to be altered in AD, assessing its potential as a biomarker, and to evaluate TTR's effect on these prospective biomarkers. Experiments were performed in a transgenic AD mouse model bearing different TTR genetic backgrounds (two or one copies of the mouse TTR gene – AD/TTR^{+/+} and AD/TTR^{+/-}, respectively), previously described in our laboratory [1], using 3- and 7-months old mice. We show that Sort1 is decreased at both ages in AD/TTR^{+/-} mice, in relation to AD/TTR^{+/+}, suggesting that this protein could be used for early AD detection, even when β -amyloid (A β) deposits are absent, and follow-up of therapies. Still regarding Sort1, female gender showed a more accentuated decrease, compared to males; this is especially observed in older mice, thus showing the impact of aging in AD. As for Syp, its expression was increased in 3 months-old AD/TTR^{+/-} mice, compared to AD/TTR^{+/+}, contrary to 7 months-old mice that showed no significant differences, indicating an overlapping effect of aging over TTR reduction. Alterations observed for both Sort1 and Syp were not restored in AD/TTR^{+/-} mice treated with iododiflunisal (IDIF), known to stabilize TTR and shown to improve AD features, namely A β levels and deposition in the brain, and cognition in this mouse model [2]. This indicates that Sort1 and Syp are dependent on TTR quantity and that its stabilization was not sufficient to reverse the effects of the TTR reduction.

References:

[1] Oliveira, S.M., Ribeiro, C.A., Cardoso, I., and Saraiva, M.J. (2011) *Gender-dependent transthyretin modulation of brain amyloid-beta levels: evidence from a mouse model of Alzheimer's disease*. Journal of Alzheimer's disease: JAD 27, 429-439.

[2] Ribeiro, C.A., Oliveira, S.M., Guido, L.F., Magalhães, A., Valencia, G., Arsequell, G., Saraiva, M.J., and Cardoso, I. (2013) *Transthyretin stabilization by Iodo-diflunisal promotes A β peptide clearance, decreases its deposition and ameliorates cognitive deficits in an AD mouse model*. Journal of Alzheimer's Disease

Exercise preconditioning prevents left ventricular dysfunction and maladaptive remodeling secondary to pulmonary arterial hypertension in rats

C. Schmidt¹, A. Bovolini¹, G. Castro e Sousa¹, M. Hoffmann¹, D. Girardi¹, D. Moreira-Gonçalves¹, A. Leite-Moreira², T. Henriques-Coelho², J. Alberto Duarte¹

¹ Department of Biochemistry, Faculty of Sport, University of Porto, Portugal

² Department of Physiology and Cardiothoracic Surgery, Faculty of Medicine, University of Porto, Portugal

Exercise training can provide a cardioprotective phenotype that allows an improved response against several insults. Pulmonary arterial hypertension (PAH) directly affects the right ventricle but left ventricle dysfunction (LVD) was recently described in PAH patients, which is associated with left ventricle (LV) atrophy and/or neurohumoral activation. The objective of our study was to analyze the hypothetical cardioprotective effects of exercise preconditioning on LV in a rat model of PAH induced by monocrotaline (MCT).

The study was designed with 115 male Wistar rats, submitted for three interventions, showed here with three phases. The first phase was done with 60 rats were randomly separated in sedentary (SED; 4 weeks sedentary) and trained groups (EX; running sessions of 60 min/day, 5 days/week, at 25 m/min, during 4 weeks). After, animals were injected with MCT (60mg/kg; SED+MCT and EX+MCT) or the same volume of vehicle (SED+V and EX+V). Afterwards, all animals remained sedentary for additional 4 weeks. Next, animals were submitted to LV hemodynamic evaluation in baseline and isovolumic conditions, and LV samples were prepared for light microscopy analysis (cardiomyocyte cross sectional area and collagen deposition) and endothelin (ET-1). In a second phase, we developed the survival study with 40 animals submitted to the respective experimental protocols (SED+V, n=5; SED+MCT, n=15; EX+V, n=5; EX+MCT, n=15). The third phase was performed with 15 animals to assess their exercise tolerance.

We found in baseline conditions, systolic (peak systolic pressure and dP/dtmax) and diastolic function (dP/dtmin and Tau) were compromised in SED+MCT but not in EX+MCT (P<0.05). Under isovolumic conditions, SED+MCT showed additional deterioration in the same parameters, but these alterations were prevented in EX+MCT (P<0.05). This improved hemodynamic profile was paralleled with prevention of cardiomyocytes atrophy and fibrosis, and with normalization of ET-1 mRNA levels (P<0.05). Exercise preconditioning also enhanced exercise tolerance and positively impacted survival. Of note, these improvements were observed 4 weeks after the cessation of exercise training, highlighting that the protective phenotype promoted by exercise training is maintained for several days.



A2

ENGINEERING III

VII
PARALLEL
ORAL
SESSIONS

Ultra-Pure H₂ Production through Water-Gas Shift Reaction in a Packed-Bed Membrane Reactor

J. Silva¹, A. Helmi², F. Gallucci² and M. van Sint Annaland²

¹ Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

² Multiphase Reactors Group (SMR), Department of Chemical Engineering and Chemistry, Eindhoven University of Technology (TU/e), Eindhoven, The Netherlands.

Hydrogen as an energy carrier has been commonly thought to play an important role in the future of fuel cells [1]. Polymer electrolyte membrane fuel cells (PEMFCs) can generate and deliver electric power in a wide range. However, PEMFCs applied to road vehicles still present some technological limitations associated to CO sensitivity of the anode catalyst. At the moment the water-gas shift (WGS) reaction ($\text{CO} + \text{H}_2\text{O} \leftrightarrow \text{H}_2 + \text{CO}_2$) may be the most promising process to reduce the CO content in the H₂ streams. This technology combined with a membrane reactor would allow the attainment of highly pure H₂ that would be suitable for the use in PEMFCs, since the selective H₂ removal by means of a membrane would shift the equilibrium of the WGS reaction. In this work a highly permeable and H₂ selective Pd-Ag membrane and a highly active Pt-based catalyst were integrated into a packed bed membrane reactor (PBMR) and the WGS reaction was performed. A parametric study of the system was done.

The performance of the PBMR was analysed at different operating conditions in terms of CO conversion and H₂ recovery: temperature (300-400 °C), gas hourly spacial velocity (GHSV) (14736-20696 L_N·kg_{cat.}⁻¹·h⁻¹) and steam/CO ratio (4.7-6.5). The retentate pressure was kept at 3.5 bar while the permeate was kept under a sweep gas (N₂) flow rate of 345 mL_N·min⁻¹. A typical reformat feed was used.

For the case in which the temperature was varied, the performance of the PBMR was compared to the one of a traditional packed bed reactor (PBR). It was observed that for all temperatures the conversions obtained in the PBMR were higher than the ones attained with the PBR (close to the equilibrium). For all the other conditions studied, the conversions were also always higher than the equilibrium ones.

It was concluded that among the conditions tested, the ones that provided the best performance of the PBMR were: 400 °C, 14736 L_N·kg_{cat.}⁻¹·h⁻¹ and a steam/CO ratio of 6.5. Also, the performance of the PBMR could be further improved by using lower GHSVs and/or, more importantly, by using higher driving forces. Finally, it was verified that the H₂ stream produced is acceptably pure to be directly fed to a PEMFC.

Acknowledgements:

Joel Silva gratefully acknowledges TU/e and SMR group for receiving him for his Masters thesis project under the ERASMUS program and for making this project possible to happen.

References:

[1] Patil, C.S. (2005), *Membrane reactor technology for ultrapure hydrogen production*, PhD thesis, University of Twente.

Sustainability Evaluation of Biodiesel from Microalgae *Chlamydomonas sp* grown in Brewery Wastewater

T. M. Mata¹, J. Santos¹, R. Morais¹, A. Fraga¹, S. Meireles³, A. M. Mendes¹, N. S. Caetano^{1,2}, and A. A. Martins⁴

¹ LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto (FEUP), R. Dr. Roberto Frias S/N, 4200-465 Porto.

² Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto (IPP), R. Dr. António Bernardino de Almeida S/N, 4200-072 Porto, Portugal

³ UNICER, Bebidas S.A., Via Norte - Leça do Balio, Apartado 1044, 4466-955 S. Mamede de Infesta, Portugal

⁴ Dep. of Environmental Engineering, Faculty of Natural Sciences, Engineering and Technology (FCNET), Oporto Lusophone University, R. Dr. Augusto Rosa, 24, 4000-098 Porto, Portugal

Currently fossil fuels are cheaper and readily available, but they are non-renewable and their real costs are on their long-term environmental impacts and on sustainable development. Microalgae are seen as one of the best options for sustainable biofuels. However, environmental impacts caused by freshwater consumption, nutrients supply, carbon dioxide handling, among others, lead to constraints when designing and implementing microalgae production systems. To make these processes economically viable and environmentally sound, special efforts should be put on using cheap sources of carbon and nutrients or inexpensive culture systems [1]. Mixotrophic growth can potentially result in higher biomass productivities, and may become economically viable by using low-cost carbon sources such as industrial by-products, or even residual streams containing nutrients such as nitrogen and carbon [2]. Hence, this study performs a sustainability evaluation of biodiesel from microalga *Chlamydomonas sp.* grown in 20 % (v/v) of brewery's wastewater, supplemented with pentose sugars (xylose, arabinose or ribose resulting from the hydrolysis of brewer's spent grains (BSG)). The pentose addition aims to balance the C/N ratio of carbon poor wastewaters, while using difficult to ferment sugars resulting from BSG valorization [3]. The life cycle steps considered for the sustainability evaluation are: microalgae cultivation, biomass processing and lipids extraction at the brewery site, and its conversion to biodiesel at a dedicated external biofuel's plant. Three sustainability indicators (LCEE, FER and GW) are considered and calculated using experimental data complemented with literature data. Results show that biomass processing, especially cell harvesting, microalgae cultivation, and lipids extraction are the main process bottlenecks. The addition of each pentose influences differently, the biomass and lipid productivity. In particular, xylose is preferable in terms of lipid productivity, but from a sustainability point of view, ribose is better, though the difference from xylose is not significant. Nevertheless, culture without pentose addition presents the best sustainability results.

References:

- [1] Mata, T.M., Martins, A.A., Caetano, N.S. (2010), *Microalgae for biodiesel production and other applications*. *Renew. Sust. Energ. Rev.*, 14(1), 217-32.
- [2] Mata, T.M., Melo, A.C., Simões, M., Caetano, N.S. (2012), *Parametric Study of a Brewery Effluent Treatment by Microalgae *Scenedesmus obliquus**. *Bioresour. Technol.*, 107, 151-158.
- [3] Caetano, N.S., Moura, R.F., Meireles, S., Mendes, A.M., Mata, T.M., 2013, *Bioethanol from Brewer's Spent Grains: Acid Pretreatment Optimization*. *Chemical Engineering Transactions*, 35, 1021-1026, DOI: 10.3303/CET1335170.

Feasibility of using a TiO₂-contained in a paint coat to inactivate microorganisms in an aqueous system

P. I. Calvo, A. Mendes and O. C. Nunes

LEPABE – Faculdade de Engenharia, Universidade do Porto, rua Dr. Roberto Frias, 4200-465 Porto, Portugal

The world's population is expected to increase by 40-50 % within the next 50 years. Consequently, the increased water demand exacerbates the scarcity of clean water [3]. Microbiologically, drinking water is achieved by a chemical disinfectant, which is usually a powerful oxidizing agent such as chlorine, chlorine dioxide or ozone [2]. These popular disinfection methods often use aggressive chemicals and are not able to stay effective for long periods of time [1]. Given the risk that infections may be transmitted by contaminated drinking water, it is important to develop new disinfection systems. Advanced Oxidation Processes (AOPs), such as TiO₂-photocatalysis, is a promising water-disinfection treatment because its bactericidal activity [3]. In the presence of UV-A and oxygen, TiO₂ produces highly reactive free radicals like hydroxyl and superoxide radicals, OH· and O₂·⁻ respectively. In this work, a novel continuous photocatalytic system based on a porous grid coated with a photocatalytic paint incorporating 9 wt. % of TiO₂ was tested. Two wire mesh materials were tested, plastic and stainless steel. Photoinactivation assays were performed with cell suspensions of a Gram-negative bacterial strain (*Escherichia coli* DSM 1103) an initial density of 1 x 10⁶ cells mL⁻¹. The suspension recirculated through the mesh with a flow rate of 2 mL·s⁻¹ for 40 min.

Viability losses of 54.4 % and 39.9 % were obtained for the 2 x 2 mm² and 3 x 3 mm² stainless steel meshes, respectively. The effect of the surface area is important because of the amount of TiO₂ available determines the formation of hydroxyl and superoxide radicals needed for inactivating bacteria. The mesh material is another important parameter; for the same porosity (2 x 2 mm²), viability losses of 54.4 % and 38.4 % were obtained for meshes of stainless steel and plastic, respectively.

Acknowledgments:

To MICIT (Ministry of Science and Technology of Costa Rica) and CONICIT (National Council for Scientific and Technological Research), institutions that provided financial support for the Master's.

References:

- [1] Meyer, B. and B. Cookson, (2010) *Does microbial resistance or adaptation to biocides create a hazard in infection prevention and control?* Journal of Hospital Infection. **76**(3): p. 200-205.
- [2] Sohn, J., et al., (2004) *Disinfectant decay and disinfection by-products formation model development: chlorination and ozonation by-products.* Water Research. **38**(10): p. 2461-2478.
- [3] Teh, C.M. and A.R. Mohamed. (2011), *Roles of titanium dioxide and ion-doped titanium dioxide on photocatalytic degradation of organic pollutants (phenolic compounds and dyes) in aqueous solutions: A review.* Journal of Alloys and Compounds. 509(5): p. 1648-1660

Cultivation of *Chlamydomonas sp.* in brewery wastewater supplemented with pentose sugars

N. S. Caetano^{1,2}, J. Santos¹, R. Morais¹, A. Fraga¹, S. Meireles³, A. M. Mendes¹, A. A. Martins⁴ and T. M. Mata¹

¹ LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto (FEUP), R. Dr. Roberto Frias S/N, 4200-465 Porto

² Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto (IPP), R. Dr. António Bernardino de Almeida S/N, 4200-072 Porto, Portugal

³ UNICER, Bebidas S.A., Via Norte - Leça do Balio, Apartado 1044, 4466-955 S. Mamede de Infesta, Portugal

⁴ Dep. of Environmental Engineering, Faculty of Natural Sciences, Engineering and Technology (FCNET), Oporto Lusophone University, R. Dr. Augusto Rosa, 24, 4000-098 Porto, Portugal

Microalgae are usually photosynthetic and unicellular microorganisms that can be found in various habitats, either in marine or freshwater, in wastewaters or even in the air or soil. Although most microalgae grow mainly by performing photosynthesis, some species are heterotrophic or mixotrophic [1], and may use other energy sources. These organisms contain reserves of starch, lipids, chlorophyll, pigments and other high value compounds, which can be induced by stress conditions. Wastewaters, as the brewery wastewater (BW), is an interesting alternative and low cost grow medium for microalgae cultivation. It contains most of the nutrients needed, allowing for the combination of microalgae cultivation with wastewater treatment, this way contributing to increase the sustainability of both processes.

In this work *Chlamydomonas sp.* (Csp) was cultivated in BW with and without sugar addition (i.e. ribose, arabinose and xylose at 0.25 g/L and 0.5 g/L). The purpose was to evaluate the feasibility of using BW as a source of macro and micronutrients for Csp growth. Also, the addition of pentose sugars aimed to increase the biomass productivity by using non-fermentable sugars (obtained from the brewers' spent grains) as carbon source, with the final intention of producing oil as feedstock for biodiesel.

Results show that xylose addition promoted algal biomass productivity at c.a. 0.70 g/L (dry mass), obtained after 10 days. Microalgae were harvested by centrifugation and lipids were extracted using the Bligh and Dyer method [2]. The culture obtained without sugar addition had the highest lipid productivity (3.36 mg oil/L.day), followed by the culture with ribose addition (with 2.61 mg oil/L.day). The oil was esterified [3] and analyzed by GC. The maximum methyl ester content (22.8 % w/w) was obtained in the sample of biodiesel produced from the culture in medium with 20 % BW without pentose addition; therefore pentose addition reduced the algal lipids productivity.

Acknowledgment: Financial support from UNICER, project PP-IJUP2011-UNICER-107.

[1] Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins A. (2008), *Microalgal triacylglycerols as feedstock for biofuel production: perspectives and advances*, Plant J., 54, 621-639.

[2] Bligh, G., Dyer, W. (1959), *A rapid method for total lipid extraction and purification*. Can. J. Biochem. Physiol., 37(8), 911–917.

[3] Lepage, G., Roy, C. (1984), *Improved recovery of fatty acid through direct transesterification without prior extraction or purification*, J. of Lipid Research, 25, 1391-1396.

Treatment of a dye-containing effluent by heterogeneous Fenton-like oxidation in a continuous stirred tank reactor

Samuel Alexandre S. Queirós¹ and Luis M. Madeira²

¹ Faculty of Engineering, University of Porto, Portugal.

² LEPABE - Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

Practically all sectors of the industry use dyes. Their use generates huge amounts of effluents, which are highly coloured, highly toxic, present high chemical oxygen demand, and can't be treated by traditional processes. The Fenton reaction has proved its effectiveness for removing these pollutants [1]. The homogeneous Fenton process is based on the use of a catalyst, normally iron (Fe) in aqueous solution, which decomposes hydrogen peroxide to generate highly reactive hydroxyl radicals that attack the organic compounds present in the wastewater. The use of the homogeneous Fenton process in a Continuous Stirred Tank Reactor (CSTR) was described with success [2]. However, the homogeneous Fenton approach has many disadvantages, such as the need of about 50-80 ppm of iron ions in solution, well above the 2 ppm defined in the EU directives, as well the recovery of the catalyst, which is very expensive [1]. To overcome these facts, some attempts have been made to incorporate the iron in different types of supports, such as zeolites, activated carbons, among others, i.e., to perform a heterogeneous Fenton's process [2]. The aim of this work was to test and prove the concept of using the heterogeneous Fenton's reagent in a CSTR, and evaluate the influence of some parameters on the azo-dye Orange II (OII) degradation. The selected catalyst was a commercial zeolite – Alsi-Penta Fe/ZSM-5.

Dye removal was quantified by using a UV-Vis spectrophotometer and performing measurements in the reactor outlet stream at the dye maximum absorbance wavelength of 486 nm. Samples were taken to measure the Total Organic Carbon (TOC) and the iron content in the effluent. All runs were performed in duplicate (steady-state dye conversion was $\pm 10\%$).

A detailed parametric study was carried out, analyzing the effect of the main operating conditions while keeping the others constant; in all runs the feed dye concentration was set at 0.1 mmol/L. The parameters studied were the temperature (in the range 10 – 70 °C), the pH (1.5 – 4.0), the feed hydrogen peroxide concentration (1.75 – 20.0 mmol/L), the contact time – W/Q (10 – 200 mg.min/mL) and the size of the catalyst particles ($0.25 < d_{p1} < 0.60$ and $0.60 < d_{p2} < 0.80$ mm).

The catalyst stability was proven by 5 consecutive experiments under the same conditions (absolute deviation among runs $\pm 6\%$). For each parameter analyzed in the parametric study, the run that showed the best results was selected, and at the end a run was carried out with all parameters optimized; the results showed removals of 96% and 41% for color and TOC, respectively, and an iron content below legislated standards ($0.338 < 2 \text{ mg}_{\text{Fe}}/\text{L}$).

References:

- [1] - Duarte, F. M. (2013). *Treatment of textile effluents by Fenton-like oxidation processes with carbon-based catalysts*. PhD thesis, Faculty of Engineering of University of Porto.
- [2] - Franco, J. H. (2008). *Homogeneous and Heterogeneous Oxidation of the Azo-Dye Orange II with Fenton's reagent-Based Processes*. PhD thesis, Faculty of Engineering of University of Porto.

Brewers' spent grains characterization and its potential as raw material for bioenergy

N. S. Caetano^{1,2}, R. Morais¹, A. Fraga¹, R. F. Moura¹, S. Meireles³, A. M. Mendes¹, A. A. Martins⁴ and T. M. Mata¹

¹ LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto (FEUP), R. Dr. Roberto Frias S/N, 4200-465 Porto.

² Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto (IPP), R. Dr. António Bernardino de Almeida S/N, 4200-072 Porto, Portugal

³ UNICER, Bebidas S.A., Via Norte - Leça do Balio, Apartado 1044, 4466-955 S. Mamede de Infesta, Portugal

⁴ Dep. of Environmental Engineering, Faculty of Natural Sciences, Engineering and Technology (FCNET), Oporto Lusophone University, R. Dr. Augusto Rosa, 24, 4000-098 Porto, Portugal

Brewers' spent grains (BSG) are the main byproduct of the brewing industry. This is a lignocellulosic material consisting of husk and outer shell of the cereals remaining after the mashing process. This byproduct is rich in cellulose and hemicelluloses that can be converted into simple sugars and those, fermented to ethanol [1]. On a dry weight basis (dwt), BSG contain about 40-50 % polysaccharides (consisting of 15-18 % cellulose, 24-31 % hemicellulose and 2-3 % starch) and 30 % or more, of proteins [2]. As it results from the mashing step, it contains about 70 % water, thus preventing its long-term storage due to rapid microbial spoilage.

The BSG composition may vary, depending on the formulation used in the brewing process. In this study, it was performed the characterization of different samples of BSG regarding its moisture, ashes, cellulose, lignin and fat content. Results show that their corresponding values are respectively, 69.9-71.5 %, 3.6-4.1 %, 14.5-14.7 %, 35.7-37.5 % and 12.5-13.1 % (on a dwt basis, except for moisture that is on a volume per weight basis). These results support the conclusion obtained in previous work [1, 3] within the UNICER projects, that using BSG for bioethanol production is attractive, as there is the potential for obtaining 720 g sugars from 1 kg of dry BSG, of which 22.8 % is glucose that can be easily fermented to ethanol.

The heating value of BSG was also evaluated to be 20.1 MJ/kg (dwt) [3] meaning that it can also be used as fuel for combustion, if the water content can be lowered to less than 40%.

Acknowledgment: Financial support from UNICER, projects PP-IJUP2011-UNICER-333 and PP-IJUP2012-UNICER- 6 is acknowledged.

References:

[1] Caetano N.S., Moura R.F., Melo A.C., Silva V.F.M., Meireles S., Mata T.M. (2013) Bioethanol from Brewer's Spent Grain: Pretreatment, Hydrolysis and Fermentation. IJUP'13 - Investigação Jovem na U.Porto, 13 - 15 February 2013, Porto, Portugal, pp.80.

[2] Macheiner D., Adamitsh B.F., Kawer F., Hampel W.A. (2003), *Pretreatment and Hydrolysis of Brewers' Spent Grains*, Eng. Life Sci., 3, 401-405.

[3] Caetano N.S., Moura R.F., Meireles S., Mendes A.M., Mata T.M., 2013. *Bioethanol from Brewer's Spent Grains: Acid Pretreatment Optimization*. Chemical Engineering Transactions, 35, 1021-1026.

A3

PUBLIC HEALTH & EPIDEMIOLOGY II

VII
PARALLEL
ORAL
SESSIONS

PREVALENCE OF HUMAN PAPILLOMAVIRUS IN TISSUE SAMPLES FROM HEAD AND NECK CANCER PATIENTS

M. Esteves^{1*}, J. Silva^{1,2*}, E. Maltez¹, A. Oliveira¹, J. Ribeiro^{1,3}, R. Medeiros^{1,3}, E. Monteiro⁴, H. Sousa^{1,3}

¹ Molecular Oncology Group – CI, Instituto Português de Oncologia do Porto FG - EPE

² Department of Biochemistry, Faculty of Sciences, University of Porto, Portugal

³ Virology Service, Instituto Português de Oncologia do Porto FG - EPE

⁴ Otorhinolaryngology Service, Instituto Português de Oncologia do Porto FG - EPE

* these two students participated equally in this study

Head and neck squamous cell carcinoma (HNSCC), which includes several malignancies from the oral cavity, pharynx, nasopharynx and larynx, is a serious public health problem with almost 43.000 new cases diagnosed worldwide per year [1]. Besides tobacco smoke and alcohol, viruses have been associated with its development, in particular Human Papillomavirus (HPV).

The aim of this study was to characterize the prevalence of HPV in tissue samples of HNSCC patients. This study was performed with 42 patients (2 female and 40 males) diagnosed with HNSCC and mean age 54.8 (\pm 10.2) years old (range 34-87). DNA was extracted from formalin-fixed paraffin-embedded tumour samples and the detection of HPV DNA was performed by PCR using MY09/11 consensus primers [2]. HPV positive cases were genotyped by restriction fragment length polymorphism (RFLP) [2] and confirmed by HPV-16 specific PCR.

The overall frequency of HPV infection was 7.1% (3/42), being 2 from the oropharynx (tonsil and tongue) and one from the larynx (vocal cord). Regarding gender, 2 cases were from male patients and 1 from a female. Age stratification revealed that 2 cases were detected in patients under 55 years old and the other in one patient with over 62 years old. The 3 HPV positive cases were from current smokers and 2 of them were heavy drinkers. Moreover, the genotyping was only possible for 2/3 HPV positive cases, being both of them HPV16.

This is the first study in Portugal to evaluate the presence of HPV in head and neck cancer (HNC) patients. Despite the low prevalence of HPV in HNSCC our results are similar to those described in other populations, where HPV prevalence ranges from 5-20% [3]. In addition, we have found that 2/3 cases were positive for HPV16 and, in fact, literature refers that at least 90% of HPV-associated HNSCCs are HPV16 [3]. Despite preliminary, these data are important for future approaches on HPV-associated HNSCCs, where the viral activity should be studied as predictive/prognostic marker.

References:

[1] Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C. and Parkin, D. M. (2010), *Estimates of worldwide burden of cancer in 2008: Globocan 2008*, Int J Cancer, 127, 2893-917.

[2] Nobre, R. J., De Almeida, L. P. & Martins, T. C. (2008), *Complete genotyping of mucosal human papillomavirus using a restriction fragment length polymorphism analysis and an original typing algorithm*, J Clin Virol, 42, 13-21.

[3] Psyrris, A. and Dimaio, D. (2008), *Human papillomavirus in cervical and head-and-neck cancer*, Nat Clin Pract Oncol, 5, 24-31.

Oral health and related behaviours: comparing Turkish and Portuguese dental students population

Diogo Ribeiro¹, Benedita Sampaio-Maia², M.L. Pereira¹

¹ Preventive Oral Health Department of Dentistry Faculty University of Porto, Portugal.

² Microbiology Department of Dentistry Faculty University of Porto, Portugal

Introduction: Today's dentistry students will provide, in the future, dental services and eventually, will be responsible for public oral health education.^[1] The improvement of personal oral health among dental students has shown to be linked to their dental education experience as well as their evolution during the dental program: from first to final year and from pre-clinical to clinical years.^[2] This study was aimed to evaluate the oral health status and related behaviours among dentistry students of the 2nd and the 5th academic year of the Faculdade Medicina Dentária da Universidade do Porto (FMDUP, Portugal) and EGE Üniversitesi Dis Hekimliği Fakültesi (EUDHF, Turkey).

Methodology: This cross-sectional study included the participation of 259 students (120 from EUDHF, 139 from FMDUP). A 15 items questionnaire was designed to evaluate students' oral health attitudes and behaviors. Oral health status was assessed, by an oral examination for Decayed, Missing and Filled Teeth Index (DMFT-I), recorded according visual and tactile parameters of World Health Organization (WHO) criteria,^[3] and Simplified Oral Health Index (OHI-S), using Greene and Vermillion defined criteria for Debris and Calculus Index.^[4] Dental fissure sealants were recorded. Data analysis was performed using the Statistical Program for the Social Sciences (SPSS® v.21.0), considering a significance level of 0.05.

Results: Portuguese and Turkish students presented similar median values for OHI-S [0.67(0; 2.67) vs 0.67(0;2.83); P=0.240], decreasing in portuguese students' population throughout their educational years [0.83(0; 2.67) to 0.50(0; 2.50); P<0.001]. In both countries, students did not differ regarding DMFT-I [4(0; 19) vs 4(0;12), P = 0.944], but Portuguese students presented an inferior score of decayed teeth [0(0; 7) vs1(0;7); P=0.018]. When considered dental program evolution, an increased amount of filled teeth was observed in Portuguese students [2(0; 18) to 4(0; 12), P<0.001] and a decrease in decayed teeth in Turkish students [1(0; 7) to 0(0; 4); P< 0.001]. The prevalence of fissure dental sealants was higher in Portuguese students [0(0;15) vs 0 (0;7); P<0.001], but presenting an increasing tendency from 2nd to 5th academic year in both countries. Overall students from both countries reported appropriate oral health related attitudes and behaviours.

Conclusion: Portuguese and Turkish dental students from both years show good oral health status. In addition, the oral health of these students is significantly improved over the progression of their academic life (from 2nd to 5th academic year), suggesting that dental educational enhances awareness about personal oral health.

References

- [1] Sharda AJ, Shetty S. *A comparative study of oral health knowledge, attitude and behaviour of first and final year dental students of Udaipur city, Rajasthan, India.* International journal of dental hygiene. 2008;6(4):347-53.
- [2] Cortes FJ, Nevot C, Ramon JM, Cuenca E. *The evolution of dental health in dental students at the University of Barcelona.* Journal of dental education. 2002;66(10):1203-8.
- [3] Organization WH. *Oral Health Surveys - Basic Methods.* 4 ed1997.
- [4] Shivakumar M. *Preventive and Community Dentistry: Clinical Record Book,* 2006

Plate waste and his causes in lunch at Hospital Center of Alto Ave

A. Cruz¹, M. Fonseca²

¹ Faculty of Food and Nutrition Sciences, University of Porto, Portugal.

² Nutrition, Dietetic and Alimentation Service, Hospital Center of Alto Ave, Portugal.

Plate waste in hospitals has been associated with malnutrition. Furthermore, malnutrition may increase the length of stay in hospital, the use of hospital resources and could be a cause of mortality. Several reasons have been associated with high plate waste levels in hospitals, some of them very complex, such as the appetite variations.

The aim of this study was to determine plate waste and evaluate the reasons for plate waste in lunch at Hospital Center of Alto Ave.

The sample included 468 patients, from all hospital wards. Plate waste was determined using a 5-point scale of visual estimation to different meal components - carbohydrate component, protein component, vegetable component, bread component, soup component and fruit component. Patients were inquired about the reasons for the plate waste and the need of feeding assistance. Information about age, gender, type of diet, hospital staying and the hospital admission cause were also collected.

Of the 468 meals evaluated, 4.7% of trays remained intact (all meal components remained on the tray) and also 9.3% of the plate (excluding the bread component, the fruit component and the soup component). Desserts were the component with less waste, 75.7% of desserts were totally consumed. On the other hand, vegetables were the component with the high waste values, 33.2% of vegetables remained intact. These values are beyond the maximum of 10% for hospital plate waste recommended by the Nutrition Health Service. Regarding reasons for plate waste, loss of appetite, portions too large and food aversion were the most referred by patients.

The obstetric ward was the ward with less plate waste and the surgery ward was the one with high plate waste. Regarding the need of feeding assistance, 79% of participants did not need assistance. This study found a difference between familiar feeding assistance and hospital feeding assistance regarding to the soup and modified consistency diet, showing low plate waste for patients with familiar feeding assistance.

Our results showed high plate waste levels on this hospital. Some measures like review the food portions established and the meal requisition system, improve the food quality, establish the “protected meal time” concept, increase the number of feeding assistants educating them to the importance of feeding assistance and always realize a nutritional screening on the hospitalized patients in order to prevent situations of malnutrition, are measures that should be taken into account considering the reasons pointed out by the patients and the relations founded.

Molecular characterization of carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates from a Portuguese Hospital

C. Rocha¹, J. Botelho¹, M. Brilhante¹, H. Ramos², C. Sousa¹, F. Grosso¹ and L. Peixe¹.

¹ REQUIMTE. Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Portugal.

² Hospital Geral de Santo António, Porto, Portugal.

Pseudomonas aeruginosa is an opportunistic pathogen with high intrinsic resistance to several antimicrobial agents and carbapenems are commonly used as the last treatment resort. However, carbapenem-resistant *P. aeruginosa* (CRPA) are increasingly reported worldwide in result of decreased permeability, multidrug efflux, and/or production of metallo- β -lactamases (MBLs) which are usually encoded on transferable genetic elements. In this study we aimed to understand the relevance of MBL production among CRPA isolates obtained in a Portuguese clinical setting.

A total of 28 CRPA clinical isolates obtained in a Portuguese Hospital (2006) were included in this study. Antimicrobial susceptibility was performed by disc diffusion method. MBL production was search by Blue-carba and EDTA synergistic test. MBL genes and their association with integrons were searched by PCR and confirmed by sequencing. Clonality was studied by SpeI-PFGE.

Isolates were resistant to imipenem and meropenem, but presented variable susceptibility to ceftazidime, cefepime, aztreonam, ciprofloxacin and aminoglycosides (with amikacin being the most active agent). Two isolates, resistant to all antibiotics tested, presented the MBL VIM-2 encoded in 2.8 kb class 1 integrons. Overall, isolates were characterized by high clonal diversity, being identified 11 different PFGE-types.

Although the percentage of VIM-2 producing isolates was relatively low (7,1%), the possibility of MBL acquisition among other nosocomial gram-negative pathogens, highlight the importance of an early detection of MBL-carrying organisms, not only for the rapid implementation of infection control policies, but also for the selection of appropriate antimicrobial agents.

Demographic characterization of the deaths occurring in Portuguese Public Hospitals between 2000 and 2010

M.M. Estevinho¹, L. Ferreira¹, A. Silva¹, C. Ferreira¹, L. Ferreira-Pinto¹, A. Costa-Pereira¹, A. Freitas¹

¹Department of Health Information and Decision Sciences, Faculty of Medicine, University of Porto, Portugal

The place in which people die is of great importance, not just because individual choice is highly valued in contemporary society, but also for the planning of healthcare strategies. Place of death is influenced by personal and demographic factors, like cultural aspects, socio-economic status and education; by environmental and illness related factors [1]. It has also been reported that the health service-related factors, among others the availability of hospital beds play a key role [2].

Even though some studies have been carried out focusing the place of death's preferences, mainly for cancer patients, very few studies provide broad demographics and service use data for large populations. As such, this cross-sectional retrospective study aims to provide a baseline and reliable characterization of the deaths occurring in Portuguese public hospitals, between 2000 and 2010.

Data regarding in-hospital mortality was obtained from a national database of hospital admissions, while data from general population was collected from the National Institute of Statistics. The statistical analysis was performed using SPSS 21.0®.

A total of 1,155,151 people died in Portugal between 2000 and 2010, 41.4% of whom at a Public Hospital. The percentage of man who died at hospital was higher than the percentage of women (45.5%). From the in-hospital deaths, 31.5% were of people between 80 and 89 years old. 72.8% of the people who died with 15 or less years old died at hospital, whereas older people tend to die gradually more outside the hospital.

The region Lisboa was the one in which more people died at hospital, followed by Norte and Centro. Cardiovascular disease was the main cause of death, accounting for 35% of the cases. However, this disease accounted only for 13.1% of the in-hospital deaths, having respiratory problems a higher rate (26.3%). Concerning the in-hospital mortality, 87.3% of the admissions were medical and 92.1% were not programmed.

Based on the results of the present study, a better allocation of the end-of-life care settings may be achieved, with strong social and economic impacts.

Acknowledgments: The authors wish to thank ACSS for providing access to the data.

References:

- [1] Yanai, T., Hirase, S., Yamamoto, N., Ninchoji, T., Kubokawa, I., Mori, T., Hayakawa, A., Takeshima, Y., Iijima, K., Matsuo, M. (2012), *Place of death of pediatric cancer patients in a single institute during 7 years*, Kobe Journal of Medical Sciences, 27(2), 33-40.
- [2] Broad, J.B., Gott, M., Kim, H., Boy, M., Chen, H., Connolly, M.J. (2013), *Where do people die? An international comparison of the percentage of deaths occurring in hospital and residential aged care settings in 45 populations, using published and available statistics*, International Journal of Public Health, 58(2), 257-267.

Determinants of food choice at work: exploratory study in food service

Faria N.¹, Póinhos. R.¹, Mendes. S.² Rocha. Ada¹,

¹ Faculdade de Ciências da Nutrição e Alimentação, Universidade do Porto, Portugal.

² Instituto Técnico de Alimentação Humana, Portugal.

Introduction: The increasing number of meals taken away from home, associated to the fact that food behaviors are in the etiology of chronic non-communicable diseases, as obesity, the role of Food Services has taken an increased importance. The concern is not only about the quality of meals served, but also about employees' health status. The studies already performed have reported a large number of overweighted women, which has been associated with changes in eating habits after the beginning of working in this sector.

Objectives: Evaluate the weight status of women employed in the food production sector through BMI determination and comparison with Portuguese population representative data. Determine the factors influencing workers' food choice and to compare them with those identified for the Portuguese population.

Methodology: 300 employees of food production units' from the North area, with concession by a private catering company, participated in this study, including the hospital sector, academic sector and business sector. It was performed an individual interview with each collaborator, based on a guide. Each collaborator was weighed and measured. Of 300 employees, 96.3% (n = 289) were female, the only sex included in the analysis of the results. 34.6% (n = 100) worked in the hospital sector, 31.8% (n = 92) in the business sector and 33.6% (n = 97) in the academic sector.

Results: Most reported diseases were obesity (43.9%), constipation (21.1%), hypercholesterolemia and hypertension (19.4% each). About 60% of the collaborators were overweighted, being the average BMI 26.8 kg/m². The factors that influenced more their food choices were "trying to make a healthy diet", "food Flavor" and "food quality and freshness".

Conclusion: Most of the collaborators of food production units evaluated were overweighted, presenting a BMI higher than the average of the female population in the northern region. The data seem to indicate that changes of eating habits that happened at the beginning of activity in the Food Service, and does not seem to justify the present overweight. The pathologies reported more frequently by the collaborators differ from the ones more reported by the Portuguese population, as well as the factors that influence more food choices which can be an indicator that working in this sector of activity affects the determinants of dietary intake and workers' health status.

Perceptions of risk connected to the fish production and consumption

Gisèle M. Ntanda¹, Ana Pinto de Moura², Rui Costa Lima³, and Luís M. Cunha^{1*}

¹ REQUIMTE, DGAOT, Faculty of Sciences, University of Porto, Portugal.

² REQUIMTE, DCeT, University of Aberta, Portugal.

³ Sense Test, V.N. Gaia, Portugal.

* [Corresponding author: lmcunha@fc.up.pt](mailto:lmcunha@fc.up.pt)

Despite the perceived health benefits of fish consumption for human health, consumers may be uncertain about its associated hazards. Fish obtained through aquaculture production system has become a valuable alternative by increasing fish availability into the market, at more attractive prices, and helping to save the wild fish stocks from overexploitation [1]. The aim of this study is twofold: to evaluate the structure of fish consumption and to evaluate the risk perception related to wild and farmed fish consumption; the latter, through the use of the Portuguese version of the Perceived Food Risk Index (PFRI) [2]. A convenience sample of 141 individuals from the Great Oporto region was recruited through Sense Test consumer database, using the following criteria: regular seafood consumers, more than 18 years old and without allergy to fish. Questionnaires were answered through face-to-face interview, from April to May 2013. Consumers were asked to choose the five most relevant hazards out of a list of thirteen. Risk perception was evaluated over ten risk characteristics, for each of the following hazards: i) fish with heavy metals (mercury); ii) fish with dioxins; iii) fish contaminated with polychlorinated biphenyls (PCBs); iv) fish with reduced level of freshness; v) residues of antibiotics and v) residues of pesticides. Additionally, subjects were asked to rate the probability of each hazard occurring in both wild fish and in farmed fish. Sixty-eight percent of the respondents were female, with an average of 37 (\pm 12.1) years old. Over 85 % of the respondents had reported consuming fish more than 3 times during the week prior to the study. Heavy metals, lack of freshness, pesticide residues, pathogenic bacteria, and antibiotic residues are the five principal hazards chosen by consumers. Following the PFRI, results shown that all hazards present a high level of “dread” and a low level of “knowledge”, being less extreme for risk associated with lack of freshness. Results also revealed that, consumers perceive a lower probability of occurrence of all the six hazards in aquaculture fish in comparison with wild fish. Concluding, this work adds to knowledge about the perceptions of risk connected to fish production and consumption, namely that consumers perceive farmed fish as safer than wild fish.

References:

[1] Verbeke, W., Sioen, I., Brunsø, K., Henauw, S., & Camp, J. (2007). Consumer perception versus scientific evidence of farmed and wild fish: Exploratory insights from Belgium. *Aquaculture International*, 15(2), 121-136.

[2] Cunha, L. M., de Moura, A. P., Lopes, Z., do Céu Santos, M., and Silva, I. (2010). Public perceptions of food-related hazards: an application to Portuguese consumers. *British Food Journal*, 112(5), 522-543.

Acknowledgments: This work was carried out by Project PROAMBIENTE for enhancing SMEs organizational and management structure to enter foreign markets. PROAMBIENTE has the financial support of *Quadro de Referência Estratégico Nacional – QREN* and *Programa Operacional Regional do Norte – ON2*, supported by the European fund for regional development - FEDER. Authors Cunha and Moura also acknowledge support by *Fundação para a Ciência e a Tecnologia* through grant no. PEst-C/EQB/LA0006/2013.



A4

**SPORT
SCIENCES II**

**VII
PARALLEL
ORAL
SESSIONS**

THE EFFECT OF SPORT (KARATE AND FOOTBALL) OF FOOT DEXTERITY IN UNDER 15 MALE

F. Magalhães¹, J. Esteves¹, P. Oliveira¹, P. Rodrigues^{1,2}, O. Vasconcelos¹

¹Motor Control and Learning Laboratory, CIFI2D, Faculty of Sport, University of Porto, Portugal

²CIERT/Edutec, Institute Piaget

Foot dexterity means the skill that the individual expresses on the level of the lower limbs. Thus, being Karate and Soccer two sports where the lower limbs are of particular importance, this study aims to assess the effect of the modalities (Football and Karate) in foot dexterity in under 15 male. In this sense, we aim to verify the differences in foot dexterity between Karate and Football athletes. A Foot Preference Questionnaire was used for evaluation of lateral preference (Coren, 1993). The Foot Dexterity was assessed using the Foot Tapping test (FACDEX, 1990) which evaluates eye-foot coordination and speed of movement of the lower limb. The sample was formed by 6 Karate athletes with an age average of 14.33 ± 0.52 and 6 Football with an age average of 14 years old. All of these athletes have between 4 to 5 years of practice in the respective sport, with a frequency of 3 weekly workouts. The results say that there are statistically significant differences at the level of the Preferred Member where we can conclude that Karate athletes are more proficient at the level of the Preferred Member than Football athletes.

Key-Words: FOOT DEXTERITY, KARATE, FOOTBAL, ASYMMETRY.

References:

- [1] Rebelo, A. N., & Oliveira, J. (2006). Relação entre a velocidade, a agilidade e a potência muscular de futebolistas profissionais. *Revista Portuguesa de Ciências do Desporto*, 6(3), 342-348.
- [2] Schmidt, R. A. & Wrisberg, C.A. (2000). *Aprendizagem e performance motora: uma abordagem baseada no problema*. Porto Alegre: Artmed

The Effect of Training Session on Simple Reaction Time in Children Pedal Practitioners Athletics

D. Lucena^{1,2}, F. Silva^{1,2}, G. Scheffer^{1,2}, J. Machado^{1,2}, P. Rodrigues^{2,3} and O. Vasconcelos²

¹Bolsista CAPES (edital 008/2012) Programa de Licenciaturas Internacionais - PLI - 2012/2014
CAPES

²Motor Control and Learning Laboratory, CIFI2D, Faculty of Sport, University of Porto,
Portugal

³CIERT/Edutec, Instituto Piaget

The ability to react to certain stimuli quickly and effectively has become increasingly important in sport (Miyamoto & Meira Jr, 2004; Vagheti, Roesler & Andrade, 2007). Sports training induce effects on motor capacity. As the training session (TS) is the basic unit of sport training, we seek to determine the relevance of simple reaction time (SRT) in young track & field athletes after a training session with the preferred foot (PF), non-preferred foot (NPF) and functional motor asymmetry (FMA). The Nelson Foot Reaction Test (Nelson, 1979) was used. to analyzed the foot reaction time of 8 track & field athletes (7 females and 1 male) with a mean age of 13.38 ± 1.13 years. The sample was divided in two groups (Group 1: Less than 2 years of practice, Group 2: 2 to 5 years of practice). The test was applied before (moment1) and after (moment2) the training session. Statistical procedures included descriptive statistics and the nonparametric Wilcoxon test. The level of significance was set at $p \leq 0.05$. The results showed no significant differences between moments for all variables (PF, NPF and FMA). Regarding the time of practice, we also observed that there were no statistical differences between groups (Group 1 and Group 2) for all variables. It is believed that by controlling the intensity of the training session and increase the total sample may increase the reliability of the study.

KEYWORDS: Track & Field – Training Session – Pedal Simple Reaction time –

References:

- [1] Miyamoto R. J. & Meira Jr. C. M., (2004). Tempo de reação e tempo das provas de 50 e 100 metros rasos do atletismo em federados e não federados. Revista Portuguesa de Ciências do Desporto, 2004, vol. 4, nº 3 [42–48]
- [2] Vagheti C.A.O., Roesler H., Andrade A., 2007. Tempo de reação simples auditivo e visual em surfistas com diferentes níveis de habilidade: comparação entre atletas profissionais, amadores e praticantes. Rev Bras Med Esporte.13(2):81-5.
- [3] Nelson, J.K., 1979. Measurement of Physical Performance. Burgess Publishing Company. Minnesota.

Motor memory: a study with young swimming athletes

S. Lapa¹, A. Viana¹, J. Fernandes¹, J. Pinto¹, O. Vasconcelos¹, P. Rodrigues^{1,2}

¹Motor Control and Learning Laboratory, CIFI2D, Faculty of Sport, University of Porto, Portugal

²CIERT/Educec, Instituto Piaget

Memory is the ability that human beings have to receive, separate and organize information that comes from different sources (Godinho, Mendes, Melo, Barreiros, 1999). In this study we investigated the motor memory using the linear positioning device in 30 young (9-11 yrs old) athletes swimmers from the club F.C. Porto with 6 to 7 weekly hours of training. The sample was randomly divided in two groups with different practice conditions: with and without feedback. Each group had 6 male and 9 female athletes. The relation with the variables years of practice, sex, and attempts were also explored. Statistical procedures included the Mann-Whitney test, the Spearman test and the Friedman test. Mean and the standard deviation values are also presented.

Results showed a significant statistical difference between males and females in the without feedback condition. However, it was found no significant differences for sex, attempts and years of practice.

According to our study we conclude that the swimmers did not show significant differences when looking at the wall on turning back or when they do not.

Keywords: Motor Memory, young swimming athletes, the linear positioning device.

References

Godinho, M., Mendes, R., Melo, F. & Barreiros, J. (1999). *Controlo Motor e Aprendizagem: Fundamentos e Aplicações*. Lisboa: Edições FMH.

THE INFLUENCE OF THE PRACTICE OF PHYSICAL ACTIVITY ON ELDERLY'S BALANCE

A. Oliveira¹, I. Barbosa¹, S. Calado¹, T. Bernardo¹, P. Rodrigues^{1,2}, O. Vasconcelos¹

¹Motor Control and Learning Laboratory, CIFI2D, Faculty of Sport, University of Porto, Portugal

²CIERT/EduTec, Instituto Piaget

Aging is an inevitable process, which involves regression of skills at all levels, adversely affecting the functionality and quality of life of the elderly. The increasing aging of the Portuguese population is, in terms of public health, an increasingly heavy burden, due to the increase of average life expectancy, which is necessary to create solutions with the greatest speed and efficiency. All this is due to the fragility of the elderly and there are situations such as falls which indicate lack of balance are considered the leading cause of death in this population. "There is however a growing recognition of the benefits of formal and non-formal physical activity on healthy and successful aging" (Fisher, 2005).

The purpose of this study is to determine the effect of physical activity, years of practice, the age and number of hours per week on static balance on elderly. This study comprises a sample of 29 elderly women aged between 65 and 85 years, selected from senior classes of Faculdade de Desporto da Universidade do Porto (FADEUP). For data collection we used a sociodemographic questionnaire and the Tinetti scale for the assessment of static balance.

For statistical analysis we used SPSS 20, descriptive statistics to determine the average, standard deviation, minimum and maximum and the Mann - Whitney test in order to compare the years of practice, age and weekly hours of physical activity to their balance. The level of significance was set at $p \leq 0.05$.

The results showed no significant statistical differences on the effect of years of practice, age and influence of weekly hours on the balance of the elderly.

Key-Words: Balance, Elderly, Physical Activity and Practitioners.

References:

[1] Fisher, B. (2005). "Será possível envelhecer com saúde?". Consult. Dezembro 2013, disponível em www.luzimarteixeira.com.br/wp-content/.../08/tapoioidosos-e-af5.doc

Effect of years of practice and different positions on visual memory in male junior volleyball players

A. Ramos¹, M. Loureiro¹, M. Hurst¹, N. Abrantes¹, O. Vasconcelos¹, P. Rodrigues^{1,2}

¹ Motor Control and Learning Laboratory, CIFI2D, Faculty of Sport, University of Porto, Portugal

² CIIERT/Edutec, Instituto Piaget

Once volleyball is a sport that depends to a large extent on the visual system, because of its influence in decision making, it is important to study the player's ability to store information that is visually acquired. This research was carried out with the purpose of understanding if this ability develops with the years of practice, or if it is a naturally occurring feature, and also if different positions can influence or not this skill. The sample comprised 12 male junior volleyball athletes aged between 17 and 18 years old and were divided into two groups according to the years they had been practicing this sport: one group with 6 or less years of practice (6 elements), and another with more than 6 years (6 elements); they were also divided according to their positions (receiving and non-receiving players).

Two visual memory protocols (the Menvis-A test and a specific visual memory test) were applied. Statistical procedures included descriptive statistics and the nonparametric Mann-Whitney test. The level of significance was set at $p \leq 0.05$.

The results showed that, within this chronological age, the visual memory of the players seem not to be influenced by the years of practice and by the different positioning on the field.

Key Words: volleyball; visual memory; years of practice.

References: Almeida M. & Faria L. (2003). *Stress no contexto desportivo: estudo exploratório na divisão A1 masculina e feminina de voleibol*. Tese de Mestrado da Faculdade de Desporto da Universidade do Porto. Porto: edição do autor; Costa L. (2005). *Factores de eficácia no processamento de informação em crianças e jovens com dificuldades de aprendizagem*. Tese de Mestrado na Faculdade de Ciências do Desporto da Universidade do Porto. Porto: edição do autor.

Relationship between Tactical Skills and Young Basketball Players with height stature

¹D.B. R. Junior, ²L. F. Campos, ²M.V. de Paula, ³ M.J.C. Silva

¹Faculty of Educação Física e Desportos, University Federal de Juiz de Fora, ² Faculty of Educação Física e Desportos, University Federal de Juiz de Fora, ³ University Coimbra.

When considering basketball as a invasive game, dynamic in alternation between actions with the ball and without the ball, consequently between offensive and defensive phases become important to note, beyond the body dimensions, the specific skills and functional skills, also the components inherent tactical skills, in order to categorize and even better distinguish young practitioners more parameters show themselves to specialization sports. In the selection process of young basketball players, high stature seems to be one of the determination factors. Will the basketball players who have high school level height are those who have better knowledge of tactical skills? The aim of this study was to observed the relationship between youth basketball players with height stature, normal stature and their level of knowledge in tactical skills. We evaluated 224 student-athletes aged between, 12 and 17 years, participants of basketball games in National high School Games in 2010 and participants of basketball games in Minas Gerais State High School Games in 2011. For the study was applied Tactical Skills Inventory for the team sports (TACSIS) and measured body height. The classification criteria were used Center for Disease Control and Prevention (CDC), to classify as normal stature subjects between 50TH and 84th percentiles were selected, to classify as height stature subjects was included subjects with percentile in CDC classification above 85th. In all variables, we used the mean and standard deviations as descriptive statistics in order to observe differences between group's levels of Skills Tactics. For independent samples, the "t" test was used, assuming a significance level of 95% ($p \leq 0,05$). Significant differences between age sub-range of tactical skills "Knowledge about the actions of others" and percentage of height were identified. The difference between the percentages of stature found shows that the groups are well characterized within the classification used by the CDC. Regarding the sub-scale, it can be said that individuals height stature have greater knowledge of defensive actions since this sub-scale is related to the same. Individuals tall and normestaturais this age and this level of competitive participation have similar levels of tactical skills in 3 of 4 subscales TACSIS, thus showing a very large assimilation of these individuals when it comes to tactical skills, is thus necessitating further studies with this audience.

[1]Elferink-Gemser, M. T.; Visscher, C.; Richart, H.; Lemmink, K. A. P. M. Development of the tactical skills inventory for sports. *Perceptual Motor Skills*. (99): 883-895. 2004.

[2]RIBEIRO JUNIOR, D. B.; Elferink-Gemser, M. T.; FIGUEIREDO, A. J.; COELHO E SILVA, M. J.; Tradução e validação "Tactical Skills Inventory for Sports" para o português do Brasil e Validação para jovens basquetebolistas brasileiros. No prelo. 2013.

Accuracy throwing on different launching distances of Boccia athletes

B. Souza^{1,3}, **C. Cahua**¹, **M. Oliveira**^{1,3}, **P. Rodrigues**^{1,2}, **O. Vasconcelos**¹

¹ Motor Control and Learning Laboratory, CIFI2D, Faculty of Sport University of Porto, Portugal

² CIIERT/EduTec, Instituto Piaget

³ CAPES Foundation, Ministry of Education of Brazil, Brasília – DF, Brazil

The visual-motor coordination is the ability to coordinate vision with the movements of the body and its parts, which is of fundamental importance for Boccia athletes to succeed in this sport. This study investigate the effect of hand-eye coordination of BC2 class Boccia athletes in the most characteristic launching distances, which are 3m, 5m, 7m and 9m to a mini-goal device. At each distance 6 trials were performed by four male athletes aged 33-44 years. ". Statistical procedures included descriptive statistics, nonparametric Wilcoxon test and Kruskal - Wallis. The level of significance was set at $p \leq 0.05$. The only significant difference was found when mean distances in general were compared. This research concluded that increasing distance interferes negatively with the accuracy of release, that is, the shortest distance the greater precision.

Keywords: VISUAL MOTOR COORDINATION, BOCCIA, CEREBRAL PALSY, CLASS BC2.

A5

AGRO FOOD II

VII
PARALLEL
ORAL
SESSIONS

Exploiting DNA markers for saffron (*Crocus sativus* L.) authentication

Caterina Villa¹, Joana Costa¹, M. Beatriz P.P. Oliveira¹, Isabel Mafra^{1*}

¹ REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

*E-mail: isabel.mafra@ff.up.pt

Saffron is the most expensive spice in the world, which is obtained from the red-dried stigmas of the flowered *Crocus sativus* L. It is used for flavoring and coloring in food preparation and also in traditional and modern medicine. As a consequence of its high market value, saffron has been frequently associated to an unrivaled degree of adulteration performed in both ground and whole stigma with the most diverse materials and strategies. Thus, the detection of adulterants becomes a very important issue for the evaluation of the product value, to ascertain the unfair competition and assure the consumer protection against fraudulent practices. Different methods have been developed to assess quality and authenticity of saffron based on standardized methods to assess chemical and sensorial parameters. However, saffron authentication relies mostly on microscopic observation of morphological traits, which is time-consuming, depends on experienced personal and is susceptible of subjective interpretation [1]. Nowadays, DNA-based methods have proved to be the most suitable for the analysis of highly processed food and spices [2].

The aim of this work was to develop molecular markers for further evaluation of the authenticity of saffron. DNA was extracted from different leaf samples of *C. sativus* L. and other *Crocus* spp. by the CTAB method, as described by Mafra *et al.* [3]. DNA yields and purity were evaluated by UV spectrophotometry in a micro-volume plate accessory. All extracts were amplified by polymerase chain reaction (PCR) targeting three different loci, namely ITS1, ITS2 and *matK* and a SCAR marker. A real-time PCR assay using EvaGreen dye combined with High Resolution Melting analysis was carried out in order to discriminate different species of *Crocus* genus. Fragments obtained by the amplification of ITS1 were sequenced. The *primers* targeting ITS1 and *matK* loci were specific for the genus *Crocus*, while the *primers* targeting ITS2 locus and the RAPD marker were specific for the species *C. sativus* L. This study revealed that genomic tools provide easy, fast and reliable methods for saffron authentication.

Acknowledgments: This work was supported by FCT grant no. PEst-C/EQB/LA0006/2013, University of Porto “Projectos Pluridisciplinares” IJUP2011-290 and COST Action FA1101 “Saffron Omics”. The authors are grateful for the kind supply of samples from Bank of Plant Germplasm of Cuenca.

References:

- [1] Marieschi, M., Torelli, A., Bruni, R. (2013). *Quality control of saffron (Crocus sativus L.): development of SCAR markers for the detection of plant adulterants used as bulking agents*, Journal of Agricultural and Food Chemistry, 60 (44), 10998-11004.
- [2] Galimberti, A., De Mattia, F., Losa, A., Bruni, I., Federici, S., Casiraghi, M., Martellos, S. and Labra, M. (2013), *DNA barcoding as a new tool for food traceability*, Food Research International, 50 (1), 55-63.
- [3] Mafra, I., Silva, S.A., Moreira, E.J.M.O., Silva, C.S.F., Oliveira, M.B.P.P. (2008), *Comparative study of DNA extraction methods for soybean derived food products*, Food Control, 19 (12), 1183-1190.

Development of a new species-specific PCR assay for the detection of horse meat

L. Meira^{1,2}, S. Carneiro¹, F. Ramos², M. B. P. P. Oliveira¹, I. Mafra^{1*}

¹REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, ²Faculty of Pharmacy, University of Coimbra, Portugal. *E-mail: isabel.mafra@ff.up.pt

Nowadays, identification of species in food it is of major importance to evaluate food authenticity, with special emphasis on meat products. The consumer has the right of an informed choice, which may be a reflection of lifestyles, religious practices or health problems. Therefore, a correct and truthful labelling is crucial to inform the consumer about the identity and quality of food products they are buying [1]. Analytical techniques with high specificity and sensitivity are needed to verify labelling compliance. The methods based on DNA analysis have proved to be promising tools of high accuracy, emerging as an alternative to protein analysis. The high stability of DNA when compared with the proteins, its presence in most of biological tissues and the possibility to specifically amplify short sequences in millions of times by the polymerase chain reaction (PCR) technique are major advantages [2].

The aim of this work was to develop a new species-specific PCR assay for the detection of horse DNA in meat products. For method development and optimisation, a set of binary mixtures to be used as references was prepared by adding known amounts of horsemeat to cow meat in the range of 0.0001 to 25%. In order to assess the effect of heat treatment, a second set of mixtures was autoclaved. DNA was extracted using the Wizard method. Yield and purity of extracts were assessed by UV spectrophotometry using a micro-volume plate accessory. For the detection of horse species, new primers were specifically designed on a mitochondrial sequence of *cytb* gene to amplify a 141 bp fragment of *Equus caballus*. The optimised PCR results showed a relative limit of detection of 10 mg/kg (0.001%) and 1000 mg/kg (0.1%) in raw and thermally treated binary mixtures, respectively. In terms of absolute detection, the method allowed a sensitivity down to 10 pg of horse DNA for both raw and autoclaved meats.

It can be concluded that a new PCR method was successfully developed, providing an effective tool to detect horse meat adulteration in meat products.

Acknowledgments: This work was supported by FCT grant no. PEst-C/EQB/LA0006/2013 and University of Porto "Projectos Pluridisciplinares" IJUP2011-149.

References:

- [1] Mafra, I., Ferreira, I. M.P.L.V.O., Oliveira, M.B.P.P. (2008), *Food authentication by PCR-based methods*, Eur Food Res Technol, 227, 649-665.
- [2] Soares, S., Amaral, J.S., Mafra, I., Oliveira, M.B.P.P. (2010), *Quantitative detection of poultry meat adulteration with pork by a duplex PCR assay*, Meat Science, 85, 531-536.

Detection of horse meat adulteration in processed foods using the PCR technique

S. Carneiro¹, L. Meira^{1,2}, F. Ramos², M. B. P. P. Oliveira¹, I. Mafra^{1*}

¹REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, ²Faculty of Pharmacy, University of Coimbra, Portugal. *E-mail: isabel.mafra@ff.up.pt

Global incidences of food adulteration are increasing resulting on disruption of international food trade by frequent disputes over food safety and quality requirements. Concomitantly, to combat fraud and ensure safety and quality, food quality control has been increasing too. Concerning meat products, the focus of adulteration is the partial or total replacement of high valued commercial species by low-value ones. This results in commercial gain, which may cause serious damage on moral values and on public health. Recently, “the horse meat scandal” arose following the detection of horse meat in beef meat containing foods [1]. However, the presence of horse meat itself does not represent a health risk, unless it has illegally gone into the food chain, containing the veterinary drug phenylbutazone. This drug is not allowed for human consumption because it can cause rare cases of a serious blood disorder like aplastic anemia. Since it is difficult to identify what triggers the anemia, it is not possible to define a safe level of residue in meat. Consequently, horse meat of animals that were medicated with this drug cannot be used as food [2].

Therefore, this work aimed at detecting the fraudulent addition of horse meat in processed meat products by the use of polymerase chain reaction (PCR) technique. Following the previous development (Abstract IJUP2014 – “Development of a new species-specific PCR assay for the detection of horse meat”), the identification of horse meat adulterations in a range of processed meat products labelled as containing minced beef meat (lasagne, meat balls, dumplings, croquets, hamburgers, sausages, etc.) was intended. The samples were acquired prior (#33) and after (#34) the “horse meat scandal”, in 2010-2012 and 2013, respectively. DNA was extracted using the Wizard method. Yield and purity of extracts were assessed by UV spectrophotometry using a micro-volume plate accessory. The results showed the successful extraction of amplifiable DNA from all the beef-containing foods. The PCR amplification revealed the presence of horse DNA in 2 samples (Fig. 1) acquired before the outbreak of horse meat adulteration becoming known in the EU. This highlights the importance of fraud detection and control of labelling compliance of foods, demonstrating the usefulness of PCR as a rapid and sensitive tool for routine analysis to identify meat species in foods.

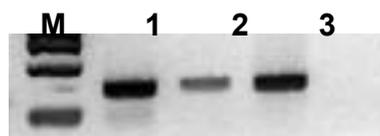


Fig. 1. Agarose gel electrophoresis of PCR products of *cytb* gene of horse. M, DNA marker; 1, positive control; 2, hamburger and 3, sausage samples; 4, negative control.

Acknowledgments: This work was supported by FCT grant no. PEst-C/EQB/LA0006/2013 and University of Porto “Projectos Pluridisciplinares” IJUP2011-149.

References:

- [1] Premanandh, J. (2013), *Horse meat scandal – A wake-up call for regulatory authorities*. Food Control, 34, 568-569.
- [2] European Food Safety Authority, European Medicines Agency (2013), *On the presence of residues of phenylbutazone in horse meat*, EFSA Journal, 11(4):3190.

Effect of juice treatment, urea addition and double distilling on ethyl carbamate formation in sugar cane spirit

G. C. Silvello¹, A. M. Bortoletto^{1,2} and A. R. Alcarde^{1,3}

¹ Department of Agri-Food Industry, Food and Nutrition, “Luiz de Queiroz” College of Agriculture, University of São Paulo, Brazil.

² PhD Student of Food Science and Technology.

³ Adviser Professor.

Ethyl carbamate (EC) is a potential carcinogenic compound with unknown formation pathways. The most likely reaction, though, is among ethanol and nitrogenous precursors as urea (Battaglia et al., 1990) [1] which is widely applied for yeast supplementation in industrial process. This project aimed to verify the influence of urea in increasing EC in cachaça and also to undergo the technique of double distillation to reduce EC level.

Sugar cane juices steady at 18°Brix had three different treatments: filtration only (fresh juice); boiling for 10 minutes and filtration (treated juice); contamination by environment microorganism and filtration (contaminated juice). All treatments were divided in two sub-treatments with addition of urea (0,1g.L⁻¹) or not. The fermented juices were distilled in a laboratory distiller with copper inside, separating two fractions: Head+Heart until 43%(v/v) ethanol and Tail until none ethanol for the first one, and Head+Heart until 65%(v/v) and Tail until 12%(v/v). Analysis of ethyl carbamate in each sample were done using a chromatograph Shimadzu GCMS-2010 (Alcarde et al., 2012) [1].

The contaminated juice with urea displayed spirits with the highest level of EC (285µg.L⁻¹), over the international safety limit of 150µg.L⁻¹. But the second distilling decreased it to 130µg.L⁻¹. The treated juice presented intermediate levels of EC, in accordance to studies of Zimmerli e Schlatter (1991) [2] concerning bread and winemaking relating heat treatment and EC formation.

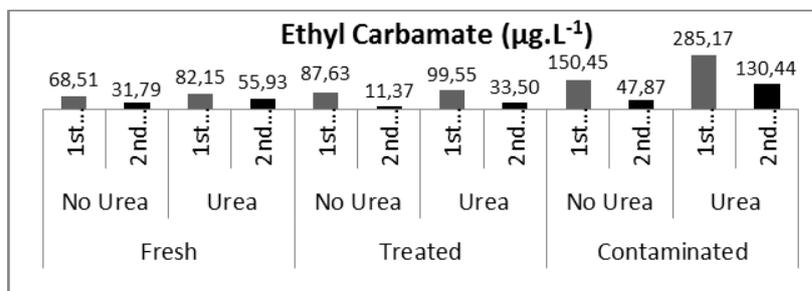


Fig. 1. Levels of EC compared for each treatment and sub treatment

This project showed the damage to the spirits by addition of urea, especially the contaminated juice. Fresh and heat-treated juices have lower EC level. The double distilling reduced EC levels more than 50% of the original spirit and it has proven to be an efficient and simple technique to reduce the level of the contaminant in the spirit.

[1] Alcarde, A. R.; Souza, L. M.; Bortoletto, A. M. *Ethyl Carbamate Kinetics in Double Distillation of Sugar Cane Spirit*. Journal of the Institute of Brewing, v. 118, p. 27-31, 2012.

[2] Zimmerli, B., Schlatter, J. *Ethyl carbamate: Analytical methodology, occurrence, formation, biological activity and risk assessment*. Mutation Research. v.259, p. 325–350, 1991.

Reducing the salt content in meat rissoles and its acceptability by the consumer.

J. Delgado¹, A. Lemos¹, R. Poínhos¹, O. Pinho¹

¹ Faculdade de Ciências da Nutrição e Alimentação, Universidade do Porto, Porto, Portugal.

² REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.

Studies have shown that a high salt intake is related to the development of cardiovascular and kidney diseases [1]. Currently, the Portuguese population has an average sodium consumption above twice the recommendation of the WHO, and processed foods are major contributors (75 to 80%) to sodium [2]. Salt reduction based mainly on voluntary agreements with the food industry is considered one of the most cost effective interventions to reduce salt consumption [3]. This work aims to study the effects of salt content reduction in the composition and acceptance of meat rissoles, a processed product.

The study was conducted in three phases: the first was the measurement of sodium and potassium by flame photometry and the second and third stages were two different types of sensory tests to test the consumer acceptability on the same samples. We performed a hedonic test on 40 subjects and also a triangular test to a group of 10 panelists.

A total of 12 groups were analysed, each including 20 samples. The groups differed regarding their content in salt (sodium chloride) and addition of herbs. Three types of pastry (“total salt pastry” vs. “half salt pastry” vs. “unsalted pastry”) were combined with four different kinds of filling (“total salt filling” vs. half salt filling” vs. “no salt filling” vs. “no salt filling with addition of herbs”). “Total salt” corresponded to a salt content similar to the traditional recipe of meat rissoles.

In the group of traditional recipe the maximum average of salt level was 1.28 g/100g while in the groups with no salt added the minimum average was 0.20 g/100g. The average potassium level was 0.15 g/100g and there were no significant variances between the groups. We found that generally consumers prefer the rissoles with 50 % salt reduction (“half salt pastry” and “half salt filling”). We also found that more than a half of the panelists preferred groups with salt reduction. The groups of samples containing herbs to mask the lack of salt had little acceptance by the consumers.

This study’s results may lead to a salt content reduction in this kind of food product, as well as to encourage the establishment of nutrition policies of salt reduction combined with the food industry.

[1] He, F. J. and G. A. Macgregor (2010), *Reducing population salt intake worldwide: from evidence to implementation*. Prog Cardiovasc Dis. 52: 363-382.

[2] Brown, I. J., I. Tzoulaki, V. Candeias and P. Elliott (2009), *Salt intakes around the world: implications for public health*. Int J Epidemiol 38(3): 791-813.

[3] Asaria P, Chisholm D, Mathers C, Ezzati M, R. B.(2007), *Chronic disease prevention: health effects and financial cost of strategies to reduce salt intake and control tobacco use*. Lancet. 370:2044-53.

How to develop a food product

S. Teixeira¹, L. Amaro¹, G. Campos¹, A. Mendes¹, A. Teixeira¹, J. Esteves², C. Lopes³ and D. Torres¹

¹ Faculty of Nutrition and Food Science, University of Porto, Portugal.

² Faculty of Engineering, University of Porto, Portugal.

³ Cerealis – Produtos Alimentares, S.A., Maia, Portugal.

In modern lifestyle meals away from home are no longer an option for leisure, but a requirement. Thus, the quality of the meals is largely conditioned by the options offered by the market. The food industry technology has evolved significantly with the introduction of new methodologies in order to keep up with the changes imposed by new patterns of life. However healthy food supply has still to be improved around the globe in view of consumers demand.

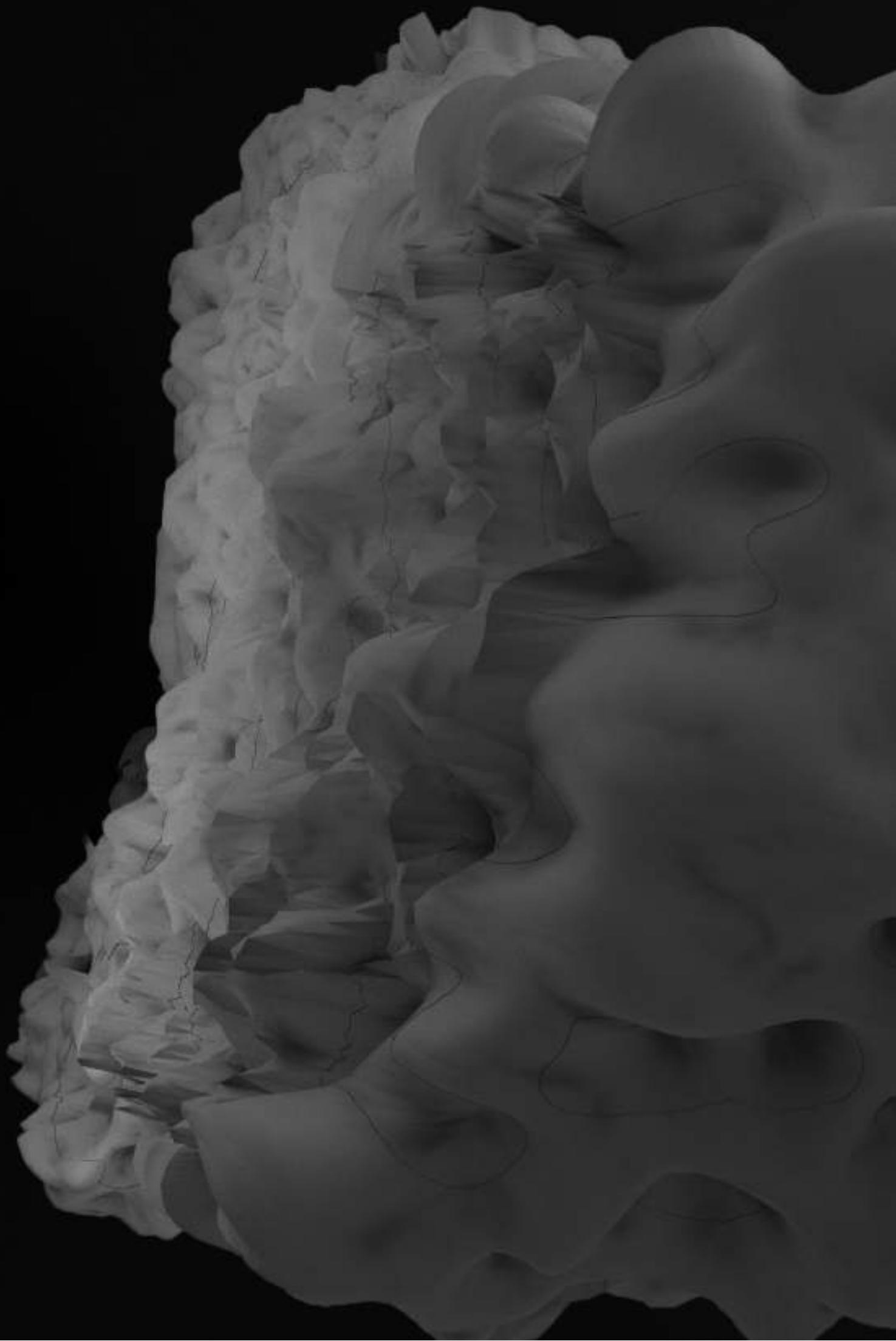
The aim of this project was to establish a process for the development of a tasty and nutritionally balanced cereal snack taking into consideration the existing industrial equipment in order to decrease investment costs and speed up time to market of the product. Several stages were identified: product concept; product formulation and processing; packaging development; storage tests; manufacture and marketing development. The first two stages were covered throughout the entire project and will be presented here.

The product concept. The cereal snack was to be nutritional balanced and aligned with the Portuguese food based dietary guidelines. Because cereals combine perfectly with dairies and fruits, a dairy or a fruit filling will be included in a cereal envelope by coextrusion to produce small cereal bars that can be packed together in variable number in order to target different ages (and different energy requirements). Dairy and fruit fillings should be pumpable at low temperature (20-40 °C), with a viscosity between 10-20 Pa.s at a shear rate between 1-100 s⁻¹, and should also have low water activity (a_w) < 0.55, to increase product shelf-life.

Dairy and fruit fillings formulation and processing. To miniaturize the production process, formulations were prepared in a Thermomix TM31 kitchen appliance (Vorwerk) with temperature and shear rate control. Viscosity was measured using a Carri-Med CSL 500K rheometer; a_w was evaluated with AquaLab Series 3 (Decagon); and nutritional facts were evaluated using the Food Composition Table of the National Institute of Health Dr. Ricardo Jorge. For sensory analysis, an affective test made by a convenient untrained panel was performed. Several simple ingredients were tested when combined with strawberry or milk powder to produce a fruit based or a dairy based filling, respectively. Potential formulations for dairy and fruit fillings were selected if having a_w < 0.55 and a good sensory evaluation. When measured, at different shear rates and at different temperatures (20, 40 and 60 °C), the viscosity was between 10 and 20 Pa.s, at typical coextrusion shear rate, when fillings were heated at temperatures higher than 40 °C or 20 °C for dairy or fruit filling, respectively.

The formulated dairy and fruit fillings showed sensorial and technological features that make them able to pursue the outlined product development process.

Supported by IJUP/CEREALIS project PP-IJUP2011-CEREALIS-345.



VIII

PARALLEL
ORAL
SESSIONS





A1

BIOMEDICINE VIII

VIII
PARALLEL
ORAL
SESSIONS

Discovery of a new small molecule inhibitor of the p53-MDM2 interaction

J. Soares¹, N. A. L. Pereira², L. Raimundo¹, M. Leão¹, C. Bessa¹, G. Queiroz³, A. Inga⁴, C. Pereira¹, M. M. M. Santos² and L. Saraiva¹

^{1,3}REQUIMTE, ¹Laboratory of Microbiology, Department of Biological Sciences, ³Laboratory of Pharmacology, Department of Medicine Biology, Faculty of Pharmacy, University of Porto, Portugal

²Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Portugal

⁴CIBIO, Centre for Integrative Biology, Laboratory of Transcriptional Networks, University of Trento, Italy

The p53 tumour suppressor protein is a powerful growth suppressive and pro-apoptotic protein that plays a central role in protection from tumour development. In tumours with a wild-type (wt) p53, the activity of this protein can be inhibited by overexpression of the endogenous negative regulator MDM2. In this case, inhibitors of the p53-MDM2 interaction have been considered an attractive therapeutic strategy against cancer [1]. In this work, yeast-based assays, consisting of *Saccharomyces cerevisiae* cells co-expressing human wt p53 and MDM2, were used to screen for inhibitors of the p53-MDM2 interaction. In our previous work, it was shown that, like in human cells, inhibitors of the p53-MDM2 interaction (such as Nutlin-3a) reduce the impact of MDM2 on p53 activity, restoring the p53 activity in yeast [2]. Using this approach, NAP78, synthesized by the Santos' research group, was identified as a new inhibitor of the p53-MDM2 interaction. The molecular mechanism of NAP78 was validated in human tumour cell lines derived from colon carcinoma with (HCT116 p53^{+/+}) and without (HCT116 p53^{-/-}) p53. In HCT116 p53^{+/+} cells, the potent growth inhibitory effect of NAP78 was associated to the induction of cell cycle arrest and apoptosis. It was also observed an increase of the levels of p53 and of proteins encoded by p53 target genes, such as MDM2, p21, PUMA and Bax, in HCT116 p53^{+/+} but not in HCT116 p53^{-/-} tumour cells. In conclusion, using a yeast-based p53-MDM2 screening assay, a new small molecule was identified as inhibitor of the p53-MDM2 interaction. NAP78 can be explored as possible anticancer agent and as a lead compound to the synthesis of more potent and selective inhibitors of the p53-MDM2 interaction.

Acknowledges: Supported by FCT through REQUIMTE (PEst-C/EQB/LA0006/2011) and iMed.UL (PEst-OE/SAU/UI4013/2011), FEDER funds through the COMPETE program under the projects PTDC/QUI-QUI/111664/2009, and FCOMP-01-0124-FEDER-015752 (ref. PTDC/SAU-FAR/110848/2009) J. Soares (SFRH/BD/78971/2011), N. A. L. Pereira (PTDC/QUI-QUI/111664/2009), M. Leão (SFRH/BD/64184/2009), C. Bessa (SFRH/BD/87109/2012) are recipients of FCT fellowships.

References:

[1] Patil, S.P. (2013), *FOLICation: Engineering approved drugs as potential p53-MDM2 interaction inhibitors for cancer therapy*, Med Hypotheses, 81(6), 1104-1107.

[2] Leão, M., Pereira, C., Bisio, A., Ciribilli, Y., Paiva, A.M., Machado, N., Palmeira, A., Fernandes, M.X., Sousa, E., Pinto, M., Inga, A. and Saraiva, L. (2013), *Discovery of a new small-molecule inhibitor of p53-MDM2 interaction using a yeast-based approach*, Biochem Pharmacol, 85(9), 1234-1245.

Cardiac damage induced by mitoxantrone: a time and age-dependent effect involving oxidative stress

J. L. Dores-Sousa¹, J. A. Duarte², V. Seabra³, M. L. Lourdes¹, F. Carvalho¹, V. M. Costa¹

¹ REQUIMTE (Rede de Química e Tecnologia), Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto; ² CIAFEL, Faculdade de Desporto, Universidade do Porto; ³ Departamento de Ciências Farmacêuticas, CESPU, Instituto Superior de Ciências da Saúde do Norte, ISCS-N.

Mitoxantrone (MTX) is used in the treatment of multiple sclerosis and several types of cancers, including in children. One of its major adverse effects is cardiac toxicity that can affect up to 18% of MTX-treated patients [1]. At this point, little is known about the overall risks of the pediatric population to develop cumulative cardiotoxicity after MTX-treatment [2]. The present study aimed to evaluate MTX-toxicity in a pediatric animal model in comparison to adult animals. Two different populations were used: juvenile (3 weeks-old) and adult (8-10 weeks-old) male CD-1 mice. Each group was treated with a total cumulative dose of 9.0 mg/kg of MTX, resulting from multiple administrations (6 intraperitoneal injections, 2 injections *per week*) in order to mimic the human MTX therapy. To the respective controls NaCl 0.9% was administered in equal volumes as the treated groups. The animals were maintained in a drug-free period for three weeks after the last administration, to allow the development of late cumulative toxicity (experiment 1), or sacrificed 24h after the last MTX administration (experiment 2) to assess early toxic events. In experiment 1, the average body weight of juvenile mice did not vary with time, while in adult population it significantly decreased after the last MTX-administration, possibly as a result of lower food and water intake. Also, in experiment 1, no adult animals survived to the end of the protocol, while 33% of juveniles reached the end of the protocol. Elevation of the plasma aspartate aminotransferase/alanine aminotransferase ratio (AST/ALT) in the juvenile survivors suggested heart damage. Moreover, a significant high cardiac reduced/oxidized glutathione ratio (GSH/GSSG) was found in the MTX-treated juvenile mice indicating a possible adaptation mechanism. Regarding the early events of MTX-administration (experiment 2), significant decrease in creatine-kinase MB (CK-MB) levels in juveniles occurred and may indicate altered cardiac protein turnover. Cardiac histology showed that both MTX-treated populations had inflammatory activity, cellular degeneration, with cellular edema and vacuolization, and sporadic zones of necrosis, but in a lower degree in juveniles. Furthermore, in experiment 2 the MTX-treated adults had higher values of cardiac GSSG with no changes observed in cardiac GSSG or GSH in the juveniles. Regarding the liver, the MTX-treated juveniles showed lower GSH/GSSG ratio in experiment 1. In experiment 2, hepatic energetic impairment occurred in all MTX-treated animals (decrease in ATP), while lipid peroxidation decreased in juvenile mice when compared to their control counterparts. Altogether, these results suggest that juvenile mice might be more protected from MTX-induced damage than adult mice. Even so, time elapsed after MTX-administration is a major factor to appraise the mechanisms involved in MTX-cardiotoxicity, since animals showed time-dependent compensatory mechanisms in the same age group.

Acknowledgments: This work was supported by FCT [EXPL/DTP-FTO/0290/2012]-QREN initiative with EU/FEDER financing through COMPETE. VMC acknowledges FCT for her Post-doc grant SFRH/BPD/63746/2009.

References:

- [1] Costa, V.M., Carvalho, F., Duarte, J.A., Bastos, M.L. and Remião, F. (2013), *The Heart As a Target for Xenobiotic Toxicity: The Cardiac Susceptibility to Oxidative Stress*, *Chemical Research in Toxicology*, 26 (9), 1285-1311.
- [2] van Dalen, E.C., van der Pal, H.J.H., Bakker, P.J.M., Caron H.N. and Kremer, L.C.M. (2004), *Cumulative incidence and risk factors of mitoxantrone-induced cardiotoxicity in children: a systematic review*, *European Journal of Cancer*, 40, 642-652.

Expanding our therapeutic options: β -blockers for colon cancer?

M. Coelho¹, M. Moz¹, G. Correia¹, A. Teixeira², R. Medeiros², L. Ribeiro^{1,3}

¹ Department of Biochemistry, Faculty of Medicine of the University of Porto, Porto, Portugal,

²Molecular Oncology Group, Portuguese, Institute of Oncology of Porto, Porto, Portugal

³Center for Medical Education, Faculty of Medicine, University of Porto, Portugal

Colon cancer is one of the most common causes of cancer mortality worldwide. Several lines of evidence suggest that stress increases the incidence and promotes the development of several types of cancers. Adrenaline (AD) and noradrenaline, the main mediators of the sympathoadrenomedullary response, are chronically elevated after chronic exposure to stress, and have been implicated in tumor cell proliferation, adhesion, migration and invasion. Both hormones exert their effects through interaction with alpha- and beta-adrenergic receptors (AR). In colon cancer cells, beta-adrenergic activation has been implicated in carcinogenesis and tumor progression. Thus, beta-AR blockers have been studied as possible additions to cancer treatment [1]. This study aimed to investigate the effect of adrenergic ligands upon proliferation of the human colon adenocarcinoma cell line, HT-29. Cells were incubated during 24h in the absence (control) or presence of the AR-agonists, AD and isoprenaline (ISO) (1, 10 μ M), as well as pretreated with the beta-blockers, propranolol (PRO, 50 μ M), carvedilol (CAR, 5 μ M), atenolol (ATE, 50 μ M) and ICI 118,551 (ICI, 5 μ M), for 45 minutes prior, and simultaneously with, to the incubation with each of the AR agonists. Cell proliferation was assessed by 5' bromodeoxyuridine (BrdU) incorporation assay. AD, respectively at 1 μ M and 10 μ M, significantly increased HT-29 proliferation by 241% ($p < 0.001$; $n = 9$) and 185% ($p = 0.009$; $n = 9$). ISO enhanced by 179% ($p = 0.010$; $n = 9$) and 190% ($p = 0.008$; $n = 9$), respectively at 1 and 10 μ M, comparing to controls. AD-induced cell proliferation was markedly reduced by PRO to 12 ± 3 % ($n = 6$) and 32 ± 10 % ($n = 5$), when AD was respectively used at 1 and 10 μ M. PRO greatly decreased cell proliferation evoked by ISO to 20 ± 2 % ($n = 5$) and 23 ± 5 % ($n = 5$), when ISO was respectively used at 1 and 10 μ M. PRO per se induced a significant proliferation decrease to 44 ± 10 % ($n = 6$), comparing to control. CAR decreased the proliferation induced by AD to 28% ($n = 6$) and 56% ($n = 6$), when AD was respectively applied at 1 and 10 μ M, and to 27% ($n = 6$) and 36% ($n = 6$) when ISO at 1 and 10 μ M was used. Contrary to PRO, CAR per se did not affect the proliferation of HT-29 cells. ATE significantly decreased cell proliferation induced by AD at 1 and 10 μ M to 56 ± 6 % ($n = 6$) and 53 ± 4 % ($n = 6$), respectively and to 46 ± 9 % ($n = 6$) and 32 ± 5 % ($n = 6$) for ISO respectively at 1 and 10 μ M. Furthermore, when applied alone, ATE decreased proliferation to 55 ± 14 % ($n = 6$), comparing to control. ICI did not significantly affect the proliferation induced by AD, whereas diminished to 64 ± 11 % ($n = 7$) the effect evoked by ISO 10 μ M. ICI per se had no effect on HT-29 cell proliferation.

In conclusion, the adrenergic agonists under study significantly increased the proliferation of HT-29 cells, and all the β -blockers were able to revert the proliferative effect of these agonists, being propranolol the most effective in this action.

Supported by U.Porto/Santander Totta (IJUP) (PP-IJUP2011-320)

[1] Cole S.W., Sood A.K. (2012), *Molecular pathways: beta-adrenergic signalling in cancer*, Clin Cancer Res, 18 (5), pp. 1201–1206.

Design of a Web-based Application towards Improving Education in Clinical Anatomy

B. Guimarães^{1,3}, M. Severo^{1,2} and M.A. Ferreira^{1,3}

¹ Center for Medical Education, Faculty of Medicine of the University of Porto, Porto, Portugal

² Department of Clinical Epidemiology, Predictive Medicine and Public Health, University of Porto Medical School, Porto, Portugal

³ Department of Anatomy, Faculty of Medicine of the University of Porto, Porto, Portugal

Clinical medicine has evolved in a tremendous way in the last decades, coping with the revolution in technology that supports medical practice. As medical education complexity increases, education supporting software is becoming more prevalent. Anatomy has been adopted as a core scientific and educational area to implement new software designed to cope with the new demands of medical education.

Following the development of “Virtual Quiz” [1], a software build with the objective of improve identification of anatomical structures, we designed a new web-based application which functions as a study manager towards the improvement of students cognitive competences in clinical anatomy.

This work aims to present this application and discuss its use as a tool for teaching/learning in a core area of medical curriculum at the undergraduate level and of medical education at its different levels.

The study manager application has five main sections. In two of these sections, “Virtual Quiz” and “Identifier”, students can practice their capacity to identify anatomical structures in X-ray, TC and MRI films, as well as images of sectional anatomy. “Clinical Vignettes” section consists in a group of multiple choice questions, presented as clinical cases. “Flash Cards” and “Animated Graphics” enable a quick and directed revision of the course. The progress is indicated by statistical analysis, which incorporates the results of the students.

With this tool different indicators can be collected: the quantitative individual and general performance of students in each section, the amount of time expended in each sections, the percentage of correct/wrong of each question, as well as the answer assigned by the students.

The development of this tool will provide a solid technological support in a new curricular reform, facilitating the approach to reach the anatomical competence towards its clinical application.

References:

[1] Tavares, M.A., Ribeiro, M., Amaral, M., Ribeiro, H., Machado, J., Povo, A., Severo, M. (2009), *Towards the development of skills in medical education: “the virtual quiz identification station”*. Proc. of ICERI 2009 Conference, Madrid, November 16 – November 18, 2009, pp 2315-2319.

Modulation of visceral adipose tissue through exercise in obese animals: analysis of oxidative and apoptotic signalling markers

J. Beleza¹, S. Rocha-Rodrigues¹, I.O. Gonçalves¹, E. Passos^{1,2}, A. Ascensão¹ and J. Magalhães¹

¹ CIAFEL – Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal

² Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Portugal

Introduction: we aimed to analyse the effects of different exercise models (endurance training program and voluntary physical activity) against oxidative damage and apoptotic signalling markers in epididymal adipose tissue from rats fed with hypercaloric diets-induced obesity.

Experimental description: male-Sprague-Dawley rats were randomly assigned into sedentary (SED), voluntary physical activity (VPA) and endurance training (ET) groups fed with two isocaloric diets, a standard and high-fat diet (35% or 70% fat-derived Kcal, respectively) as follows: SED35, VPA35, ET35, SED70, VPA70 and ET70. VPA-animals had free access to voluntary running wheel throughout the entire protocol. After 9-wks of hypercaloric diet regimens, ET-animals were submitted to 8-wks ET maintaining dietary treatments. Total-thiols (T-SH), non-proteic thiols (NP-SH), malondialdehyde (MDA) levels, aconitase, caspases 3, 8 and 9 activities, Bax and Bcl-2 protein levels were determined in epididymal fat pad.

Results: animals fed with high fat-diet (HFD) showed an increase of body weight and visceral adiposity index (VAI). HFD significantly decreased T-SH levels in SED70 vs.SED35 and increased Bax/Bcl-2 ratio by rising Bax (SED70 vs. SED35) protein levels while decreasing Bcl-2 levels (SED70 vs.SED35).

VPA and 8-wk of ET decreased body weight (VPA35 and ET35 vs.SED35 and ET70 vs.SED70 and VPA70), abdominal perimeter (VPA35 and ET35 vs. SED35; ET70 vs.SED70 and VPA70), and VAI (VPA35 and ET35 vs. SED35; VPA70 and ET70 vs.SED70). ET groups exhibited a significantly increased heart-to-body weight ratio. VPA and ET increased T-SH levels (VPA70 and ET70 vs. SED70) as well as NP-SH levels (VPA35 and ET35 vs.SED35; VPA70 and ET70 vs. SED70) and considerably decreased MDA content in epididymal adipose tissue in both diets when compared with SED groups (VPA35 and ET35 vs. SED35; VPA70 and ET70 vs. SED70). No alterations were found in aconitase activity. Epididymal adipose ET70 showed an increase in caspase 9 activity when compared to SED70, although no alterations were observed in caspase 3 and 8 activities. ET increased Bcl-2 protein levels in groups submitted to 35%-fat derived Kcal diet (ET35 vs.SED35 and VPA35).

Conclusions: our results suggest that both exercise models positively modulate visceral adiposity and redox state in epididymal adipose tissue in rats fed with a high fat diet.

Acknowledgments: this work was supported by the FCT: PTDC/DES/113580/2009–FCT/FCOMP-01-0124 FEDER-009573; PEstOE/SAU/UI0617/2011, CIAFEL; SFRH/BPD/4225/2007, AA; SFRH/BD/71149/2010, EP; SFRH/BD/89807/2012, SRR.

A meta-analysis of obesity, and overweight/obesity in 10 year old Portuguese children

S. Pereira¹, F. dos Santos^{1,2}, T. N. Gomes¹, D. Santos¹, M. Souza^{1,2}, R. Chaves¹, A. Borges¹, R. Garganta¹, J. Maia¹

¹CIFI²D, Faculty of Sport, University of Porto, Portugal.

² CAPES Foundation, Ministry of Education of Brazil, Brasília – DF, Brazil

Introduction: Obesity is a major public health epidemic worldwide in children and adults [1]. The prevalence of childhood obesity is dramatically increasing with a corresponding proliferation of cardiometabolic risk [2]. There is no known trend about the prevalences of overweight and obesity in Portuguese children, namely in the last ~10 years.

Purpose: We aim to present information from a meta-analysis concerning obesity and overweight/obesity trends in Portuguese 10 year old children assessed from 2004 to 2012.

Sample and methods: Nine studies were available with reported prevalences; further, 3 were national and 6 regional (different mainland areas, n=4, and 2 from Madeira and Azores islands). IOTF cut-points [3] were used in all studies to define obesity and overweight. Fixed and random-effects models were used to compute effect sizes and the final estimate based on ~10 year data. Heterogeneity was assessed with the Q test, and the Funnel plot was used to investigate possible bias. The Comprehensive-Meta analysis v.2 was used in all computations.

Results: Sample sizes of 10 year old children varied from 106 to 1197, and obesity prevalences ranged from 4.1% (Madeira Islands, year 2009) to 10.6% (mainland, year 2004); overweight/obesity prevalences ranged from 9.5% (Lisbon, year 2005) to 36.3% (Porto, 2012). Obesity fixed and random effects final estimates were 7.6% (95%CI=6.9% to 8.4%), and 7.5% (95%CI=5.9% to 9.4%) respectively; for overweight/obesity the estimates were, respectively, 27.8% (95%CI=26.6% to 29.0%), and 23.7% (95%CI=18.9% to 29.2%). There was a significant heterogeneity in the samples for obesity (Q=39.94, p<0.001), and for overweight/obesity (Q=157.05, p<0.001). No significant trend (p>0.05) was observed in obesity over the ~10 year lag, whereas in overweight/obesity, a positive trend (p<0.001) was suggested. A possible bias was identified in the Funnel plot namely for the overweight/obesity situation.

Conclusions: over a ~10 year lag, the prevalence of obesity in 10 year old children remained constant, 7.5%; in overweight/obesity the value is 27.8%, and a positive trend was observed. In any case, the overweight/obesity value is to be taken cautiously, given the high heterogeneity and bias.

References:

- [1] Karnik S, Kanekar A (2012). *Childhood Obesity: A Global Public Health Crisis*. *Int J Prev Med*. 3(1):1-7.
- [2] Reilly JJ, Kelly J (2011). *Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review*. *Int J Obes* 35(7):891-898.
- [3] Cole TJ, Bellizzi MC, Flegal KM, Dietz WH (2000). *Establishing a standard definition for child overweight and obesity worldwide: international survey*. *BMJ* 320 (7244):1240-1243.

A2

ARCHITECTURE & ARTS II

VIII
PARALLEL
ORAL
SESSIONS

The entrance as element conceptual design

A. Lima¹

¹ Department of Architecture, Faculty of Architecture, University of Porto, Portugal.

The entrance is something that is thought from the beginning of a project and, if it isn't the most important area, it is the one that will undoubtedly influence the whole project. [1] What is the precise delimitation of the entrance? What is our understanding of "entrance"? The door, the hall, the exterior space that precedes the door or the interior that immediately follows? These and other questions were the motto for this investigation that claims to deconstruct and understand the concept of "entrance".

The investigation came from personal concerns – resultant from the education in architecture –, whose in-depth study proved to be of interest and relevance in this context. It occurred during the academic year 2012/2013, within the scope of Architectural Theory 2, of the Integrated Master's Degree in Architecture.

From the medieval cities to the modern ones, the historical analysis allowed us to check that the entrance space always deserved Man's attention. [2] The same thing happens in housing. The attention given to the appearance and the concept of "entrance" itself was altered along History. It is worth remember the rural houses that were showing carelessness and devaluation of this space. [1]

“But, against what man sometimes thinks, the forms that he creates, the spaces that he organizes are not created or organized in regime of total freedom, but deeply conditioned by an infinite sum of factors, some of which the man has full awareness of while others are unconsciously acting upon him.” [3] In any project the entrance has an important role, as an indicator or an advisor of the remaining spaces. Especially for someone with architectural knowledge it is almost instinctive to try to realize firstly where the entrance is located when reading a plan, or even when enumerating it with the number one in a legend of project drawings. [1]

The entrance concept varies from person to person and in order to complement this investigation the project “The entrance of his house” was carried out. It was proposed to a group of persons to photograph the space that they understand by entrance of their residence and send it together with a word that characterizes it. The target group varied between students, professionals and individuals with no architectural expertise, with the intent of realizing the differences between each perspective. The study allowed me to conclude that sometimes the “affection” to the space is more important than its appearance, and it also shows the capacity of distancing and critical eye of the population influenced by knowledge in architecture.

References:

[1] Portas, Nuno, *A Habitação Social: proposta para a metodologia da sua arquitectura*. Porto: FAUP publications, 2004.

[2] Benevolo, Leonardo, *Diseño de la Ciudad*. Barcelona: Gustavo Gili, 1977.

[3] (Author's translation: “Mas, contra o que o homem por vezes pensa, as formas que ele cria, os espaços que ele organiza não são criados ou organizados em regime de liberdade total, mas antes profundamente condicionados por uma soma infinita de fatores, de alguns dos quais o homem tem plena consciência e agindo outros inconscientemente sobre ele.”) Távora, Fernando, *Da Organização do Espaço*. Porto: FAUP publications, 2006, pp. 21.

The viewer as a performer

G. Milhazes

Department of Masters of Painting, Faculty of Fine Artes, University of Porto, Portugal.

The painting, more than the end result, as two dimensional pictorial image that is perceived by the viewer as a static image, is the culmination of a whole creative process in which the artist acts and decides. Allied to science, the painting process can be revealed and redo as a performative act in which the viewer is called to equation.

The research project emerge to hypothesise a painting that only can be revealed with the participation of the viewer. The search for the answer to the problem turns to the field of chemical sciences. And if instead of using conventional paint mediums we choose chemical reagents, which are normally used in the Chemical Sciences?

The junction of two colorless chemicals (phenolphthalein alcohol solution and aqueous solution of sodium hydroxide) results, with the naked eye, in the change of color (magenta) that with the passage of time becomes again colorless. Appropriating these characteristics to the field of arts, the canvas is previously prepared and painted with these solutions. When exposed, the canvas is shown white. The viewer has at its disposal, on a shelf, a paintbrush and one of this solutions and, acting on canvas painting resurfaces, it changes and reacts with the intervention of the spectator, until saturation - due to the chemical reaction, the screen stops being white and shows the painting (magenta coloring) was hidden, and can be manipulated by the viewer.

As a performative act, the viewer experiences and as role in the result that do not want to be final, but rather an ongoing process - being here contained the principles and concepts of painting, as the expressiveness and direction of the stroke.

Visual Technologies applied to Human Rights: Photography on *Programa USP Diversidade* (University of São Paulo Diversity Program)

M. Silva¹,

¹ Communication and Arts School, University of São Paulo, Brazil.

This work aims to promote reflection on prejudice related to the diversities within the São Paulo University campus, through photographic register and elaboration of a multifunctional data bank integrating images and texts, which will be available for academic researchers on digital media.

As Methods and procedures, the bibliographic revision is one of the adopted procedures, aiming at the relation between photographic images and gender, sexual, racial, social, intellectual diversities. In parallel, connecting to groups organized within University of São Paulo with the real objective of implementing and maintaining the rights of the individuals which form these “diversities”. So to interconnect theory and reality, an event/meeting was proposed with the people and groups working with issues related to these diversities, to reflect about identities and the image of diversity, using the ‘Project Unbreakable’ as reference.

As results, we can highlight the event production success and the participants’ photographs with their posters (Fig.1), articulating USP (University of São Paulo) “diversities” groups and fronts to divulgate the proposal through blogs and social networks. The photographs will be available on the web address www.fotoreflexoes.com.br, with image use license to *USP Diversidade* (University of São Paulo Diversity Program) as data bank, allowing exhibitions and to use them on academic events debating the diversity theme.



Fig.1 - Gabi

Talking about Diversity is talking about identity and prejudice. During this process it was possible to realise how much these issues are only talked about but so little in deepened. There is fragility when debating this issue on society at large, and that includes USP. Even though the debate is fragile in general and many times reinforces the prejudice, some groups and individuals know clearly what they are fighting for. With Project USP Diversity, São Paulo State University opens its doors and eyes for dialoguing about it. But, perhaps, there should be more action and participation among the groups and the institution. The results of this work aim at helping straighten these bonds in a reflective and artistic way.

The space of the School, a Silent educator...

A. Moreira¹

¹ Department of Science of Education, Faculty of Fine Arts and Faculty of Psychology and Education Science, University of Porto, Portugal.

The work approaches the importance of the spatial dimension in human activity and, in particular, educational activity, identifying and formalizing it both on its guiding sense and explored thematic.

The research became more intense with the process of observation developed in the field work as part of the intern program done during the academic year of 2012/13 in *Escola Secundária de Rio Tinto* in Oporto, Portugal to complete the Master's Degree in Visual Arts Teaching for Teachers of the 3rd Cycle of Basic and Secondary Education.

The document written to obtain the Master's degree titled "The space of the School, a Silent educator..." reproduces the observation process and the several activities that were developed with a eleventh grade Class, in the subject of A Drawing as part of the Visual Arts Course. After the observation, reflection and analysis made upon the different spatial impositions the class faced during the whole academic year, the work shows how environmental conditions affect cognitive skills, social and emotional behaviour through the analysis people's perception and interpretation of the surrounding space.

The configuration and organization of the space of the school is, by itself, an invisible and silent program [1] that complies cultural and pedagogic functions. These functions can form a central core of spatial experiences (of variable extension and individual or collective) that are crucial in the development of relational, sensory and cognitive skills of their users [2]. Therefore, the purpose of reflecting upon the functions referred and the consideration of its reciprocal implications brings with it the need to make a joint analysis of two aspects: space and education. The space is an important part of an individual's contextualization because every human being is defined and built by his (co)existence and mind maps. Undoubtedly, it is something that is present in the teaching/learning process, something that shapes the way teaching is understood [3]. Following the space of the "home", the space of the school is a fundamental for the creation of the primal spatial structures and in the formation the scheme of the body [4]. The impregnation of spatial and relational experiences of each individual can reflect both on his artistic production and on his gesture (writing and others.)

References:

- [1] Frago, A. V. and Escolano, A. (1998), *Currículo, espaço e subjectividade: a arquitectura como programa*, Editor Paulo, Rio de Janeiro.
- [2] Hall, E. T. (1986), *A dimensão oculta*, Relógio d'Água, Lisbon.
- [3] Guerra, M. Á. S. (2002), *Entre bastidores: o lado oculto da organização escolar*, Editions Asa, Oporto.
- [4] Carneiro, A., Leite, E. and Malpique, M. (1983), *O espaço pedagógico. Corpo/ espaço/ comunicação*. Editions Afrontamento, Oporto.

The Church of Nossa Senhora da Atalaia: an approach to the scientific methods of the Renaissance

N. Silva¹, C. Azevedo¹, F. Pereira¹, A. Coelho¹, P. Silva¹ and R. Santos¹

¹ Department of Architecture, Faculty of Architecture, University of Porto, Portugal.

The object of study of this research is the Church of *Nossa Senhora da Atalaia*, which is located in Fronteira, Portalegre. Therefore, this study begins with the attempt of understanding the connections between the church, the territory, the urban structure of Fronteira and in a closest approach, the public space, within an historical period since the village's formation until the XVI century, when the church was built, never neglecting the study of the church as the object itself.

Drawing will always be our main instrument, which allows us to assess these connections, both in the study of the church and its surroundings and the pre-existences.

Whether the purpose of this investigation, whether our motivation, are intimately related to our interests as architecture students. One of those interests is related to the architectural methodology of the Renaissance: to understand the way that the rules of an artistic movement affect our work as architects. We also consider relevant to study the architects approach to the place: to understand if the work should adapt to the context or if the architect's job is to build that context in order to better serve the work or even if the Church is built as an isolated object.

To achieve our purpose, in a first stage, we started by studying the object in multiple scales and over several centuries, on an attempt to characterize the church's urban and historical context. After this historical analysis of the church we can finally, through the fundamental principles of the Renaissance, draw the structural axes of this space and understand its place inside the renaissance movement in Portugal.

In what concerns the church's authoring, our process will include the analysis and comparison of several churches designed by António Rodrigues and Jerónimo de Ruão, to who is attributed (by some authors), the draft of the Church of *Nossa Senhora da Atalaia*.

References:

- [1] Pina, F. C. (2001) *Fronteira: subsídios para uma monografia*, Câmara Municipal de Fronteira, Fronteira.
- [2] Rodrigues, A. (1579), *Tratado de Arquitectura*.
- [3] Tavares, D., collaboration with Xavier, J. P. (2007), *António Rodrigues – Renascimento em Portugal*, Dafne Editora (sebentas de Historia da arquitectura Moderna, Porto).

Tongobriga's Civic Center.

Designed contributions for its conjectural reconstruction.

C. Rocha¹

¹ Postgraduate Student, Faculty of Architecture, University of Porto, Portugal.

A roman civic center, was built by equipment that corresponded to different functions in the city, such as religion, politics, commercial trading and leisure.

The aim of this research project was the study of the Roman Civic Center, in the ancient city of *Tongobriga*. This civic center was the result of the expansion of urban areas (around the 2nd century AD), and grew to a set of public facilities that imposed *Tongobriga* as a roman center of attraction and decision (*civitas*).

With this study, it was intended to understand how the Civic Center was lively experience and how it related to the landscape, as well as reach it's conjectural reconstruction.

In the first part of the study, it is shown to the reader, the Roman city of *Tongobriga*; a step, that in the research context, is the equivalent to a phase of analysis and familiarization with the surroundings of the studied object. The approach to the Civic Center's area is made, from the general to the particular, as well as gradually, into it.

In the second part of the study, a brief review is made of the city of *Tongobriga*, according to elements that were unfolded in this investigation; then, a deep analyze is made to the buildings from the civic center: *forum*, theater and hypothetical amphitheater.

In the case of the *forum*, we analyze the existing traces in order to see how its space was occupied, as well as see how its elements were distributed (composition, proportion, between others).

In the case of the theater, we propose a draft thought the analysis of the existing remains and use it to compare with other known theaters of the same time; and insert it, finally, in the civic center.

In the hypothetical amphitheater case, we analyze a set of traces, identified by us, that seem to configure a curvature, oriented in the same direction as the theater, and that seems to suggest the position of a *cavea*.

In the end it is presented a hypothetic design for the reconstitution of *Tongobriga's* civic center, in which the elements adopted are directly result from the performed experiments.

This study highlights the interdisciplinary importance between fields of knowledge when it comes to the interpretation of archaeological remains. In the architectural fragment case, an approach, a look, from an architect is essential for the understanding of its past.

The drawing, as a research tool, is a method used by architects in the designing process of a project. In the archeological field, this method allows an approach to the discovery of new data, new ideas, without the need for precipitated excavations, in order to acquire new information.

We built this city: Architecture in Espinho, Portugal (1900-1943)

Hugo Barreira¹

¹Master in History of Portuguese Art, Faculty of Arts of the University of Porto; CITCEM.

This project is based on our Master's Dissertation in History of Portuguese Art, entitled *Improvisos de Progresso – Arquiteturas em Espinho (1900-1943) – Impromptus of Progress – Architectures in Espinho (1900-1943)*. The first half of the title adapts a quote by a Portuguese traveler of the nineteenth century in reference to Espinho's steadfast evolution from a small fishermen's village to an impressive village. As we will see, this expression suits itself as a metaphor for the growth and architectural development of Espinho in the first half of the twentieth century.

The main objective of this investigation is to understand and characterize the architectures of Espinho, between 1900 and 1943, trying to apprehend what conditions configured and supported its development and how this reflected the journey of the Portuguese architecture of the same period, associated to the emergence of modernism in the arts and in architecture. The research was based on the analysis of the documentation from the city's archive, which, confronted with other sources, allowed us to reconstruct the built environment.

The development of Espinho, throughout the nineteenth century, was originally due to the practice of sea bathing, for which the site offered excellent conditions, attracting people from neighboring communities. Throughout the century, stimulated by the arrival of the train, Espinho has become a striving cluster that won its independence as a town in 1899, only ten years after the creation as an autonomous parish. This transformation was supported by an ambitious bourgeoisie, by trading, reinforced with the creation of a market and the industry's development.

The built environment of the period was dominated by the common housing, punctuated with a few noticeable exceptions, alongside some chief buildings, particularly the ones built at the end of the 1930s, which reflected more deeply the ways through which the Portuguese architecture of the period was reaching its modernity. The main transformations were anchored in the action of a set of local builders, responsible for the introduction and dissemination of new solutions and languages. Although in a superficial way, they nevertheless manage to progressively renew the built environment.

Residential buildings can be organized into different types, depending on their occupation, presenting common characteristics. Larger buildings were erected, in result of the combination of several independent houses, preserving its autonomous entrance.

In the early 1940's, Espinho reflected the image of a modern village to visitors' eyes. This image was reinforced and consolidated by the Commission of Aesthetics' action from 1938 onwards. The adoption of the principles of public health and the existence of Espinho's general plan of 1900, contributed greatly to this situation, which circumscribed the buildings to a regular set of streets. The town improvised thus its progress, initially supported by the individual efforts of its builders, strengthened and consolidated, but also somewhat sanitized by the action of the city council at the dawn of a new decade, one century after the first masonry buildings.



A3

**BIOLOGICAL
SCIENCES VI**

VIII
PARALLEL
ORAL
SESSIONS

In vitro tools to explore the communication between injured bone and nervous system: the humoral pathway

J.P. Martins, C.J. Alves, E. Neto, D. Sousa, D. Leite, M. Xavier and M. Lamghari

NewTherapies Group – INEB, Instituto de Engenharia Biomédica

Nervous system is recognized as a key regulator of bone homeostasis. Among the different pathways known to be involved in bone remodeling is the Neuropeptide Y (NPY) neuronal pathway. Recently, we showed that the NPY hypothalamic system also responds to bone injury, but the processes of feedback from injured bone to NPY neuronal pathway remain unknown. We hypothesize that systemic factors released after bone injury may cross the blood brain barrier (BBB) and contribute to the modulation of the NPYergic hypothalamic response. To address this hypothesis, we aimed to establish two crucial tools for an in vitro approach: in vitro BBB cultures and hypothalamic organotypic cultures.

The in vitro model of the BBB was performed using brain microvascular endothelial cell line (bEnd.3) and primary astrocytes obtained from the cortices of 8-days-old C57BL/6 mice, and the two types of cells were co-cultured. The barrier integrity was assessed by transendothelial electrical resistance (TEER) and transendothelial flux evaluation, and by the expression of tight and adherens junctional molecules in the endothelial cells. The hypothalamic organotypic cultures were prepared from 8-days-old C57BL/6 mice brains, based on the air-medium interface culture method. The optimization of the model was assessed through the evaluation of tissue viability and capacity to express NPY.

Our results show that in the BBB cultures the expression of tight and adherens junctional molecules, ZO-1 and CD144 respectively, was observed in the bEnd.3 cell-cell contact, confirming the BBB phenotype of these endothelial cells. Moreover, the values of the transendothelial electrical resistance (TEER) obtained, around $70 \Omega \cdot \text{cm}^2$, indicate a high integrity of the barrier. The integrity was further confirmed by the evaluation of the transendothelial flux, showing a minor permeability to FITC-conjugated dextran 40KDa. Concerning the hypothalamic organotypic cultures we were able to achieve high cell viability, and the establishment of a dense neuronal network. Moreover, the treatment of the organotypic cultures with dexamethasone stimulated the expression of NPY, confirming the responsiveness of the NPY system within the hypothalamic organotypic cultures.

Overall, our data support that a functional in vitro BBB model was achieved, as demonstrated by cell tightness, and TEER and transendothelial flux values. Regarding the hypothalamic organotypic cultures, we have optimized the slice thickness and culture conditions that allowed the NPY system responsiveness. The establishment of the experimental applications presented in this work will provide us the necessary means to further explore the role of the humoral pathway on the feedback communication from injured bone to the NPY hypothalamic system.

Acknowledgements: This work was financed by FCT – Fundação para a Ciência e a Tecnologia in the framework of the project PEst-C/SAU/LA0002/2011, and by the Project NPwhY (PTDC/SAU-OSM/101469/2008). Alves CJ, Alencastre I and Neto E were granted by FCT (SFRH/BPD/63618/2009, SFRH/BPD/75285/2010, SFRH/BD/81152/2011).

OXIDASES AND DEHYDROGENASES METABOLIZING MANNITOL AND OTHER ALCOHOLS IN THE DIGESTIVE GLAND OF GASTROPODS

D. Carvalho¹ and A. Lobo-da-Cunha^{1,2}

¹ Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal.

² Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Porto, Portugal.

Mannitol is a 6 carbon polyalcohol present in algae, fungi and plants, being one of the most abundant sugar alcohols in nature. In many species this compound is a storage substance, but it is also important for osmoregulation and may act as a scavenger of reactive oxygen species. Thus, enzymes capable of converting mannitol present in algae and plants into sugar will be valuable for herbivorous gastropods. Mannitol oxidase, an enzyme that catalyses the oxidation of D-mannitol using molecular oxygen as hydrogen acceptor generating mannose and hydrogen peroxide, was previously reported in the digestive gland and crop of herbivorous land snails and slugs (Heterobranchia, Stylommatophora) [1] and in the digestive gland of the herbivorous sea slug *Aplysia depilans* (Heterobranchia, Euopisthobranchia) [2]. Total homogenates of digestive glands were sonicated in an ice/water bath and centrifuged for 5 min at 1,000 g (4° C) to remove debris. Enzyme activities were assayed in the supernatant, at 25° C. Oxidases were assayed by monitoring the production of H₂O₂, and dehydrogenases were assayed by monitoring the formation of NADH. Six or more animals of each species were used. The current study revealed for the first time mannitol oxidase activity in the digestive gland of herbivorous caenogastropods, namely *Littorina littorea*, *Marisa cornuarietis* and *Pomacea bridgesii*, and in the herbivorous heterobranchs *Siphonaria pectinata*, *Bulla striata* and *Planorbarius corneus*. This enzyme was neither detected in the digestive gland of the carnivores *Calliostoma zizyphinum*, *Nucella lapillus*, *Nassarius reticulatus* and *Ocenebra erinacea*, nor in the herbivores *Patella vulgata* (Patellogastropoda) and *Phorcus lineatus* (Vetigastropoda). Nevertheless, these last two species contained a high mannitol dehydrogenase activity in the digestive gland. Moreover, mannitol dehydrogenase activity was detected in the digestive gland of several other species. Enzymatic activities were also investigated with other alcohols. High oxidase and dehydrogenase activities were recorded with the aromatic cinnamyl alcohol in all species tested so far. Dehydrogenase activity could also be detected with D-sorbitol as substrate. Ethanol oxidase or dehydrogenase activities were low and detectable only in a few species. Available data suggest that mannitol oxidase is present only in the digestive gland of herbivorous caenogastropods and heterobranchs, but mannitol dehydrogenase has a broader distribution in gastropods.

References:

[1] Knigge, T., Mann, N., Parveen, Z., Perry, C., Gernhofer, M., Triebkorn, R., Kohler, H.R., Connock, M. (2002), *Mannosomes: a molluscan intracellular tubular membrane system related to heavy metal stress?* Comparative Biochemistry and Physiology C - Toxicology & Pharmacology, 131(3), 259-269.

[2] Lobo-da-Cunha, A. (1999), *Ultrastructural and cytochemical aspects of the basophilic cells in the hepatopancreas of Aplysia depilans (Mollusca, Opisthobranchia)*, Tissue & Cell, 31, 8-16.

Modulation of angiogenic pathways by 8-prenylnaringenin

AC. Guerra¹, A. Faria^{1,2,3}, C. Calhau^{1,4}, R. Soares¹ and R. Negrão¹

¹ Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Portugal.

² Chemistry Investigation Centre (CIQ), Department of Chemistry, Faculty of Sciences, University of Porto, Portugal.

³ Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

⁴ CINTESIS - Center for Research in Health Technologies and Information Systems, University of Porto, Portugal.

8-Prenylnaringenin (8PN) is a polyphenol and a powerful phytoestrogen with a binding affinity to estrogen receptors alpha (ER) α three times higher than ER β [1]. 17 β -estradiol stimulates angiogenesis [2]. Recent work has attributed angiogenic properties to 8PN [3], but its effect on the modulation of angiogenic process is not consistent.

We aimed to evaluate the effect of 8PN on angiogenic pathways and verify the dependence on the activation of the ER.

Human umbilical vein endothelial cells (HUVEC) were stimulated with vascular endothelial growth factor (VEGF) and treated with 10 μ M 8PN in the absence or presence of an ER α antagonist (MPP) or an ER β antagonist (PHTPP). ER α and ER β gene expression was evaluated by RT-PCR. VEGFR2, pAkt, pERK 1/2 and Tie2 expression was quantified by western blotting. Ang2 was evaluated by ELISA assay. The modulation of angiogenic pathways by 8PN was confirmed in vitro by quantification of tubular structures formed by HUVEC cultivated in matrigel. Quantifications are expressed as mean \pm SEM. Samples were evaluated by ANOVA followed by Bonferroni. A difference between experimental groups was considered significant whenever $p \leq 0.05$

HUVEC only transcribed ER β . 8PN increased the expression of VEGFR2 (151.4 \pm 23.3%), pAkt (116.1 \pm 8.4%), pERK (144.5 \pm 23.9%) and the formation of tubular structures, in a process dependent on ER β . 8PN stimulated the release of Ang2 (122.3 \pm 0.29%) and expression of Tie2 (134.2 \pm 11.2%), independently of ER β . The matrigel assay showed a tendency to increase the formation of tubular structures after treatment with this polyphenol.

The results showed that 8PN seems to have pro-angiogenic properties that may be regulated by binding to ER β , which can be interesting considering pathologies associated to poor angiogenesis, as myocardial ischemia, peripheral arterial disease and neurological diseases.

References:

[1] Schaefer, O. and Schleunig W.D. (2003), *8-Prenylnaringenin is a potent ER α selective phytoestrogen present in hops and beer*, The Journal of Steroid Biochemistry and Molecular Biology, 84, 359-360.

[2] Gencel, V.B. and Khalil R.A. (2012), *Vascular effects of phytoestrogens and alternative menopausal hormone therapy in cardiovascular disease*, Mini Reviews in Medicinal Chemistry, 12, 149-174.

[3] Negrão R. and Soares R. (2010), *Angiogenesis and inflammation signaling are targets of beer polyphenols on vascular cells*, J Cell Biochem, 111, 1270-1279.

Potential hepatotoxicity caused by synthetic cathinones acquired in 'smartshops'

A. M. Araújo¹, **M. J. Valente**¹, **D. Dias da Silva**¹, **M. Carvalho**^{1,2}, **H. Gaspar**³, **F. Carvalho**¹, **M. L. Bastos**¹, **P. Guedes de Pinho**¹

¹REQUIMTE, Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

²CEBIMED, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Porto, Portugal

³Centro de Química e Bioquímica (CQB), Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal

The use of recreational drugs has changed drastically in recent years due to the rapid emergence of new psychoactive substances (NPS) known as 'legal highs', which have been sold over the Internet or in specialized shops, such as 'smartshops'. Although NPS have the biased reputation of being safe for consumption, the vast majority has not been yet tested and several fatal cases have been reported, with pathological patterns comparable to those reported for well-known stimulant drugs, such as amphetamines.

This study reports, for the first time, the *in vitro* hepatotoxic effects of individual synthetic cathinones, namely methylone, pentedrone, 4-methylethcathinone (4-MEC) and 3,4-methylenedioxypyrovalerone (MDPV). Gas Chromatography/Mass Spectrometry (GC/MS) and Nuclear Magnetic Resonance Spectroscopy (NMR) techniques were used to chemically characterize two commercial mixtures used by consumers for recreational purposes. These products sold as 'plant feeders' revealed mainly the presence of cathinone derivatives and were also used in the hepatotoxicity assays. 'Bloom' presented methylone (43%), 4-MEC (29%), pentedrone (26%), isopentedrone and dimethocaine (less than 1% each), while 'Blow' was constituted by 4-MEC (86%), MDPV (14%) and 3-MEC (less than 1%). 3,4-Methylenedioxymethamphetamine (MDMA) was used as the reference drug.

Hepatotoxic effects were evaluated in primary cultured rat hepatocytes and in HepaRG cells through the MTT assay. Concentration-response curves were obtained after 48h incubations of cells with test drugs (0.05 – 5 mM), at 37 °C. Data show that all tested agents induce a concentration-dependent decrease in cell viability. In primary cultured rat hepatocytes, pentedrone and MDPV proved to be the most potent individual agents, with EC₅₀ values of 0.647 and 0.742 mM, respectively, followed by MDMA (EC₅₀ value of 0.754 mM). 4-MEC and methylone were the least toxic substances, with EC₅₀ values significantly higher ($p < 0.05$) than MDMA (1.29 and 1.18 mM, respectively). 'Bloom' and 'Blow' exhibited similar hepatotoxic effects when compared to MDMA (EC₅₀ values of 0.788 and 0.870 mM, respectively). HepaRG cells were significantly less sensitive ($p < 0.001$ vs. primary cultured rat hepatocytes) to the hepatotoxic effects induced by these substances revealing a lower metabolic competence. Nonetheless, the tendency of toxic effects observed in the primary cultured hepatocytes was also observed in the HepaRG model.

Mathematical models of independent action (IA) and concentration addition (CA) were applied to calculate the expected effects of the mixtures, which were then compared to the obtained results. The tested drugs exhibited a higher cytotoxic potential when present in combination as they contribute additively to the overall toxicological effect. These data contribute to increase the awareness on 'plant feeders' toxic potential and unveil the health risks posed by NPS.

Evolutionary population genetics and historical demography: a new method of crossing surnames and Y-chromosome

M. Trigo^{1,2}, M.J. Prata^{1,2}, A. Amorim^{1,2}, L. Alvarez²,

¹ FCUP - Faculty of Sciences, University of Porto, Portugal.

² IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal

Isonimy is defined as the quality of having the same or similar names; Isonimy methods have been used extensively to estimate inbreeding in human populations [1], whose level depends on population size and migration rates, and increases with the number of generations since the population was founded [2]. Complementarily, surname distributions can also be informative about the genetic relationships in populations, especially in some cultures where the paternal name is passed onto children, with the potential of associating surnames to Y profiles [3-5].

Given the irregular surname transmission and difficulties in obtaining data in Portugal, in this study, we focused on the Zamora Province (Spain), previously analysed using isonymic methods [6]. The Zamora Province, which can be subdivided into eleven regions, is located in the North Western portion of the Iberian Peninsula, being one of the current administrative divisions of the autonomous region of Castile-Leon.

The main aim of this study is to apply the statistical methodology of analysis used in population genetics to the paternal (P) and maternal (M) surname distribution of 166,349 individuals of the Zamora Province, in order to investigate its bio-demographical patterns. Obtained results were compared with those obtained in the analysis of STRs Y-Chromosome profiles from 235 unrelated males from the same population (Alvarez, personal communication).

Generally, significant differences were observed in all regions when the P and M surnames were confronted. Moreover, the patterns of diversity reduction in surnames were correlated with the Y-STRs values. The diversity gain in the P surnames was quantified and the probabilities of origin of gained surnames were calculated and distributed among the regions, with a clear negative correlation between geographic distance and origin probability. Finally, we focus in the different municipalities of the Aliste region in order to understand the observed differences in a micro-geographic scale. In this analysis, the departure from the HW equilibrium was confirmed with an excess of homozygosity in almost all considered subdivisions.

References:

- [1] Crow, J.F., and Mange, A.P. (1965). *Measurement of Inbreeding from the Frequency of Marriages between Persons of the Same Surname*. Eugen Q 12(4): 199–203.
- [2] Crow, J.F., and Kimura, M. (1970). *An introduction to population genetics theory*, xiv. Harper & Row, New York. 591 p.p.
- [3] Colantonio, S.E., Lasker, G.W., Kaplan, B.A., and Fuster, V. (2003). *Use of Surname Models in Human Population Biology: A Review of Recent Developments*. Hum Biol 75(6): 785–807.
- [4] Jobling, M.A. (2011). *In the name of the father: surnames and genetics*. Trends in Genetics 17, 353-357.
- [5] Sykes, B. and Irven, C. (2000). *Surnames and the Y chromosome*. American Journal of Human Genetics 66, 1417-1419.
- [6] Alvarez L., Mendoza A. et al (2010), *Biodemographic and Genetic Structure of Zamora Province (Spain): Insights from Surname Analysis*, Human Ecology 38:831–839.

Solid lipid nanoparticles as a vehicle for brain-targeted delivery of resveratrol

J. F. Queiroz, A. R. Neves, J. L. F. C. Lima and S. Reis

REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Portugal

Resveratrol is a polyphenolic compound with interesting functions namely as antioxidant, anticarcinogenic, anti-inflammatory and neuroprotector. The purpose of this work is to take advantage of the beneficial effects of resveratrol as a neuroprotector reducing the risk of neurodegenerative disorders, especially Alzheimer's disease and Parkinson's disease and reducing the risk of brain cancer. However when resveratrol is administered in its free form, little or no drug reach the brain due to its poor bioavailability, low solubility, and its chemical instability. Here the nanotechnology can be an important tool to improve the pharmacokinetic properties and the affinity of some drugs to cross the BBB.

Solid lipid nanoparticles (SLNs) loaded with resveratrol were produced and successfully functionalized with apolipoprotein E (ApoE) in order to mediate the transport of nanoparticles into the brain. ApoE-functionalized SLNs may mimic lipoprotein particles that are endocytosed into the BBB and transcytosed through the BBB endothelium to the brain.

The formulations were completely characterized to evaluate the quality of the developed resveratrol-loaded nanoparticles for brain-targeted drug delivery. TEM images revealed spherical nanoparticles, dynamic light scattering measurements gave a Z-average under 200 nm, with a polydispersity index below 0.2, a reasonable negative zeta potential of around -13 mV, and the entrapment efficiency studies showed over 90% of resveratrol encapsulation. These characteristics remained unchanged for at least 6 months. We also performed release studies simulating the bloodstream which showed that the system provides a controlled and prolonged release of resveratrol with small losses until it reaches the target. Functionalization of SLNs with ApoE was clearly demonstrated through fluorometric assays and evaluating the infrared spectra (using FTIR). The effect of these nanoparticles on enhancing resveratrol permeation through BBB was also evaluated using hCMEC/D3 cell line as a model of the human BBB. The cell viability and the nanosystems toxicity were tested by MTT and LDH assays.

The results suggest that the functionalization of SLNs with ApoE resulted in dynamic stable systems capable of being used as controlled-release models for brain delivery of resveratrol, conferring protection to this drug, targeting to the brain and allowing a controlled release after achieving the therapeutic target. This new approach of SLNs functionalized with ApoE had never been described before and it was quite successfully developed.

Acknowledgments:

ARN acknowledges the FCT (Fundação para a Ciência e a Tecnologia) for financial support through the Ph.D. grant (SFRH/BD/73379/2010).

References:

- [1] Neves, A. R.; et al., *Current Medicinal Chemistry*, 2012, 19(11):1663-1681.
- [2] Neves, A. R.; et al., *International Journal of Nanomedicine*, 2013, 8: 177–187.



A4

**PSYCHOLOGY &
EDUCATION
SCIENCES V**

VIII
PARALLEL
ORAL
SESSIONS

Aging: perspectives and representations and intergenerational solidarity

C., Maria (2013) Department of Sciences of education, the Faculty of Psychology and Sciences of Education, University of Porto, Portugal.

Demographic changes, characterized by the increased longevity and the lower birth- rates, have played a significant role in the aging of the population and in the increase of the elderly population. In addition, the changes in democratic societies recognize the value of the individual in its specificity and difference encouraging the taking of a position. Nevertheless the organization of societies and communities don't seem to favor intergenerational exchange and/or changes in the representations of the elderly. This study focuses on the impact of a meeting of community intervention whose aim was to change the representations and prejudices associated to the different age ranges and promote intergenerational solidarity. This intergenerational education meeting was designed in a way that it would focus on the development/ sharing of knowledge, attitudes and skills that provide generational interaction. Age related prejudices (Ageism) of both the young and the old who participated and who didn't participate were analyzed in the intergenerational meeting. In the theoretical part, we begin by analyzing the family and its evolution relating it to the decreasing of opportunities given to intergenerational solidarity and citizenship. Then, we approach the aging of the population and feature the elder and the young people, the issues of poverty, inequality and exclusion, as well as the prejudice according to age (Ageism). Finally we reflect on the community intervention particularly in the field of intergenerational education. On the empirical component of this work we start by the defining objectives and methodological approach. We describe the Intergenerational meeting organized at the Faculty of Psychology and Educational Sciences of the University of Porto, within the framework of the European Year for Active Ageing and Intergenerational Solidarity that counted with the presence of President Dr. Joaquina Madeira. We have tabled and discussed the results obtained through the administration of the Ageism Range of Fraboni (Grandson, 2004) with 100 young people and elderly people who participated and who did not participate in the meeting, in order to estimate its impact. The results suggest that interventions which aim to promote intergenerational solidarity can be particularly effective, especially in the group of young people: those who participated have significantly less negative representations of the elderly as compared to those who did not participate. In addition it is important to mention that the **Ageism** is higher in the elderly than in the young people. The results also suggest the contribution of this kind of meetings to fight age related prejudice, but also a broader and sustained intervention in time have to be weighted in order to contribute to the deconstruction of young aged related stereotypes in the group of the elderly people. This way, community intervention, for the purposes of intergenerational education, can help fight any prejudice and its crystallizations and strengthen/promote intergenerational solidarity policies.

Encounters: A Psychological Intervention Program with Older Adults

A. Campinho¹ and R. Barbosa²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

² Center of Psychology, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

The average life expectancy has raised substantially in the last decades, making the last stage of life, the elderly age, one to be especially aware of. Even though there are losses associated with this stage, the investigation shows that it can be a period of development and personal growth. This aspect increased the interest and investigation about the way the elders live this period of their lives, and what part does psychology take in it. The psychological interventions in elderly people have shown to be effective whereby this investigation consisted in the development, implementation and evaluation of a group intervention with elderly people. This program is based on Erikson Psychosocial Development, and promotes the resolution of psychosocial crisis. The main goal of this program is to increase the well-being and integrity as well as decrease the depressive and anxiety symptoms.

Therefore, in this investigation-action project, the methodological approach was quantitative and qualitative. In the beginning and in the end of the program, the following instruments were administered: subscale integrity of Inventory of Psychosocial Balance (IPB; Domino & Affonso, 1990), the Geriatric Depression Scale (Yesavage, Brink, Rose, Lum, Huand, Adey & Leirer, 1983; Portuguese version by Barreto, Leuschener, Santos & Sobral, 2007) and the Geriatric Anxiety Inventory (Pachana, Byrne, Siddle, Koloski & Arnold, 2006; Portuguese version by Ribeiro, Paúl, Simões & Firmino, 2007). On the other hand, the subjects were invited to create a meeting diary of every session and to provide a final qualitative evaluation. The program consisted in 9 sessions of two hours each in Faculty of Psychology and Educational Sciences, University of Porto, and the group was composed by five participants.

The results show that this intervention promoted the increase of personal integrity and psychological well-being. There were further observations that had shown a decrease in depressive and anxiety symptoms, even though the magnitude of the decrease didn't have statistical relevance. In conclusion, the study suggests the importance of psychological intervention on elderly people, because, beyond the importance of a deeper knowledge about the aging process and all that it implies, these intervention programs will be important for the promotion of well-being and life quality in the lives of the elderly, and will help to prevent problems related to mental health.

CHARACTERIZATION OF PROCEDURES AND FORENSIC PSYCHOLOGICAL APPRAISAL OF CHILDREN AND YOUNG VICTIMS OF MALTREATMENT: 10 YEARS OF EVALUATION IN GEAV

A. Lopes¹, C. Manita¹

¹ Department of Psychology, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Maltreatment towards children in Portugal shows a worrying incidence rate and that is why there is such a high number of crime procedures as well as promotion and protection procedures in the national Courts. As a consequence, the number of requests for forensic psychological evaluations to the victims is increasing more and more every day. Due to this reality, our case study tried to further the knowledge about the forensic psychological evaluations done in cases of maltreatment towards children and young people, characterizing the requests, the protagonists and the maltreatment situations, as well as the used methodologies and the contents most emphasized by the experts in the elaboration of the reports of psychological appraisal. We used as an analyzer of this reality the forensic psychological evaluation performed in the Office of Studies and Attendance of Batterers and Victims of FPCEUP in the last ten years. Forty seven records of physical and psychological maltreatment and neglect, existing in the archives of this office, were analyzed. It was used a mixed investigation methodology, in which, in addition to the examination of the documents included in the contents of the reports, it was made a quantification and statistic treatment of the data obtained through the analysis grid of those contents. It was observed that the sex distribution of the victims in our sample is equitable being that most of the perpetrators are the paternal figure. The physical maltreatment is the type of maltreatment most represented in these records and it wasn't found a direct relation between the maltreatment and the socioeconomic level of the family. There are no significant differences between the requests that are made by the Courts in crime procedures and the promotion and protection procedures, not being identified significant statistically differences in the methodology and the appraisal results made in both cases. There were observed, however, some significant statistically associations between evaluated dimensions and different conclusions of the experts, which are displayed and analyzed in this dissertation.

“Your past counts”: Leaders’ (il)legitimacy as a guide to reaction to deviance

A. Marques, I. R. Pinto, A. Leite, and J. M. Marques

Laboratory of Social Psychology, Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

According to Subjective Group Dynamics (SGD, e.g., Marques & Páez, 1994; Pinto, Marques, Levine & Abrams, 2010), deviance within groups jeopardizes group normative standards, contributes negatively to the positive value of the group and consequently, to its members’ positive social identity. Only through an extreme negative reaction directed to the deviant members, groups are able to restore the value of the violated norms and their members’ positive social identity.

However, according to Transgression Credit (TC, Randsley de Moura & Abrams, 2013), such negative reaction does not apply to deviant leaders. Facing a deviant leader, other members may turn a “blind eye” to deviance and give the deviant leader, license to deviate.

We present an experiment aiming to test the idea that the TC should occur only with leaders that gather legitimacy among other group members, and especially facing an absence of formal group reaction toward the deviant leader. Indeed, only when facing a legitimate deviant leader, information about the social control measures implemented by the group toward deviance should guide participants’ willingness to engage in informal negative reactions. Thus, when they are informed that the group does not react, participants should engage less with informal reaction and tolerate the deviant conduct. In contrast, reaction to an illegitimate deviant leader should always trigger negative extreme reactions, irrespectively of the presence or absence of group social control.

A 2 (Leader’s Legitimacy: Legitimate vs. Illegitimate) x 2 (Presence of Justice: Present vs. Absent) between-participants experimental design was followed. Participants ($N = 112$) were invited to give their opinion about “Political behavior in Portugal”. Participants were presented with a piece of a regional newspaper about a deviant leader. According to the “Leader’s Legitimacy” factor, the deviant leader was described either with a good background that confirmed his competency, credibility, and other legitimate-related aspects among the other members (Legitimate condition), whereas in the Illegitimate condition, the opposite was described. Participants evaluated the deviant target and gave their agreement on punitive reactions. A second piece of the same newspaper informed participants about how the Portuguese judicial system dealt with this deviant case (prosecution of the deviant Present vs Absent). Finally, participants expressed their emotions and agreement with collective punitive actions.

As predicted, results show that participants are more lenient toward legitimate deviant than illegitimate deviant leaders. Indeed, legitimate deviant leaders were more positively evaluated and less punished than illegitimate leaders. Additional results revealed that negative emotional climate and participants’ willingness to engage in informal collective justice considering the deviant leader was lower when the leader was legitimate and the judicial system was absent. Our results suggest that TC is especially directed towards legitimate deviant leaders in situation of perceived absence of group reaction. Otherwise, and consistent with SGD, groups react negatively toward deviance.

References:

- [1] J. M. Marques, and D. Páez. (1994), *The black sheep effect: social categorization, rejection of ingroup deviates, and perception of group variability*, in Stroebe, W., and Hewstone, M. (Eds) *European Review of Social Psychology*, 5, 37-68.
- [2] I. R. Pinto, J. M. Marques, J. M. Levine, and D Abrams. (2010), *Membership status and subjective group dynamics: Who triggers the black sheep effect?*, *Journal of Personality and Social Psychology*, 99, 107-119.
- [3] G. Randsley de Moura, and D. Abrams. (2013), *Bribery, blackmail, and the double standard for leader transgression*, *Group Dynamics: Theory, Research, and Practice*, 17, 43-52.

The verb: a study on creativity and individuation.

Ana Luísa Abreu, Joaquim Luís Coimbra

Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

In having creativity as its primary object, the current study is dedicated, based on the urgency of theorization and interdisciplinarity, in a first moment, to the exploration of the contributions from Psychology to understand the creative process. Thus, as this analysis constitutes a platform to question the methodological options and the social-political implications that underlie the scientific discourse, this investigation aimed to conduct a comparative, integrated analysis of the contributions from Psychology, alongside those of the works by Margaret Boden (regarding creativity in Artificial Intelligence) and of Gilbert Simondon (with his proposed theorization of individuation in Philosophy).

In this sense, the current work – based on a theoretical methodology and on Critical Psychology – claims the urgency to include the developmental history of the psychological subject as a central dimension to conceptualize creativity in Psychology, additionally alerting to the importance of reversing the question by presenting the idea for future exploration of the impact of creativity in the process of individuation (Fig.1).

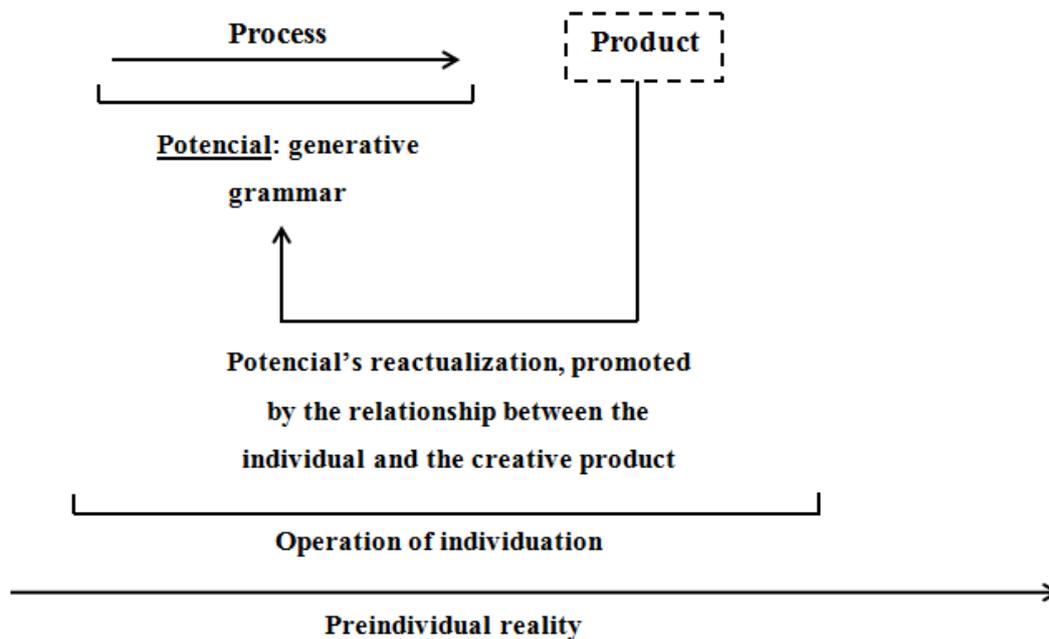


Fig.1: Representation of the conceptual progress on the definition of creativity.

Challenges of the “almost Psychologist”: the effects of personal and social skills in Psychology entails in how well psychology students tend to respond to requests for support or counseling or speeches that reflect on unadaptive beliefs regarding the psychologist and exercise psychology.

Nascimento, I. Faculty of Science Education and Psychology, University of Porto and Pombo, L. Faculty of Science Education and Psychology, University of Porto

As found through a literature review, the lack of research in this area, had aims to analyze the effects of personal and social skills in Psychology entails in how well psychology students tend to respond to requests for support / opinion and / or counseling or speeches that reflect on unadaptive beliefs regarding the psychologist and exercise psychology, from elements of their informal network of relationships. In order to reach this goal, we developed a qualitative-quantitative methodological study which would be tested amongst the MSc Psychology from the University of Porto (N = 234). In order to accumulate the necessary figures, we decided to hold a focus-group and then administered a questionnaire to students who attended within the five years of training. In order to explore the differences within these years we had to ensure that we took into account areas of specialization. Having completed this study and accumulated the results it seems that (1) psychology students were able to be more personal within a social surrounding. It could be suggested that this has resulted from the knowledge and experience which comes from studying psychology. It seems that those who were in the first year are those who have a mean significantly lower in terms of effects and also highlights their perception of personal effects. On the other hand, those who attended or planned to attend a specialized area of Clinical Psychology and Health are those who produced the highest average values; (2) students were apprehensive about the consequences of the social stereotypes surrounding the beliefs about psychology, the psychologist and the psychological practices which take place; (3) nevertheless, as for informal support requests, opinions and counselling, our outcome showed us that psychology students were more likely to resist the temptation to respond to them. The analysis and discussion of these results are used as a base set of reflections on how the training in psychology can contribute to increase the sense of competence of students regarding learning mobilization, when it comes to responding to challenges resulting from their condition "almost psychologists."



A5

ENGINEERING IV

VIII
PARALLEL
ORAL
SESSIONS

Instrumentation for Surface Electromyography

Nuno Nebeker¹ and José Araújo²

^{1,2} Department of Electrical Engineering, Faculty of Engineering, University of Porto, Portugal.

The object of the project is to design an instrument to capture, amplify and filter electrical signals associated with muscle control in the human body. This proved to be very challenging, particularly because of low voltages found (under 1 mV in amplitude) and high susceptibility to interference from the power grid, as demonstrated by noise at 50 Hz. The circuit includes a notch filter for this frequency, as an optional block. The authors conclude that the instrument designed is adequate for first-stage signal acquisition, filtering and amplification.

The proposed characteristics for this circuit are lowest possible cost, without compromising accuracy and reliability, option for battery operation and versatility, offering different approaches to signal filtering.

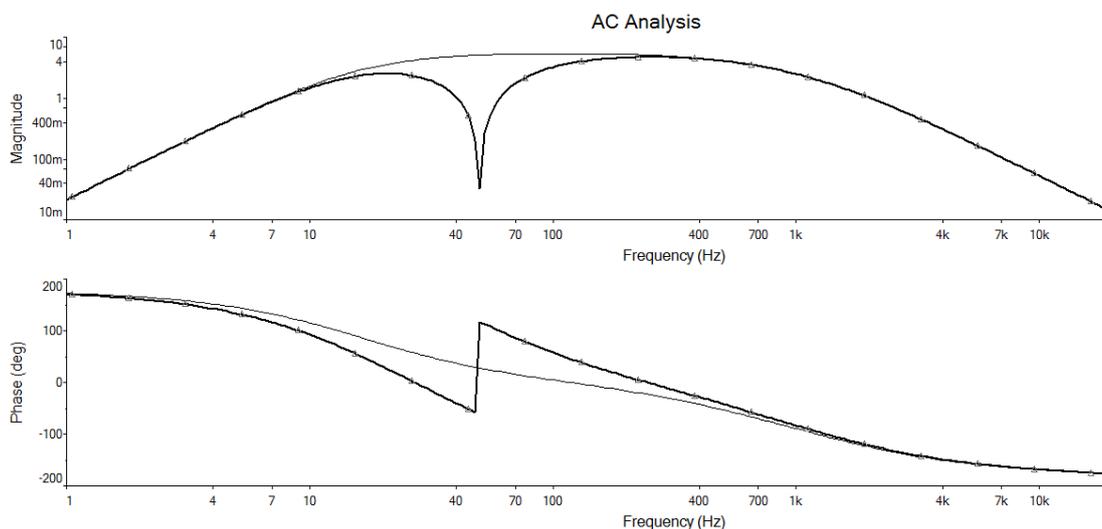
The first phase of development was researching about sEMG, focusing on the characteristics of the expected signals – amplitude, frequency range. We also studied the equivalent model of electrodes, concluding that actual values for the impedance of the electrodes in use were not available.

The development process included researching the state of the art, fundamental to finding an adequate front-end with an instrumentation amplifier (IA) to use as a starting point.

The next challenge was designing filters for signal conditioning. Considering the frequency range of interest, we used a high-pass and a low-pass filter.

After filtering, the signal is amplified by an operational amplifier (Op Amp) in a simple non-inverting configuration.

We also designed a notch filter, to filter 50 Hz noise, that is optional, because this is a frequency of interest. The end-user can select to bypass this filter and retains the option of using software filter.



Complete circuit AC analysis. The thin graph is the output before the notch filter and the thick graph is the output after the notch filter. Note the high attenuation and phase shift at 50 Hz and neighboring frequencies.

The project was supervised by Assistant Professor Manuel Cândido Duarte dos Santos.

Hydrogen generation from catalytic hydrolysis of sodium borohydride on a novel mini-reactor for portable applications

H. X. Nunes, M. J. F. Ferreira, A. M. F. R. Pinto

Department of Chemical Engineering, CEFT/Transport Phenomena Research Center, Faculty of Engineering, University of Porto, Portugal.

The need to reduce greenhouse gas concentrations to sustainable levels has resulted in a demand for clean and abundant energy to substitute fossil fuels. Hydrogen (H₂) is recognized as the environmentally desirable clean fuel of the future, and it can be used directly in a proton exchange membrane (PEM) fuel cell to provide electricity to small portable applications (laptops, cell phones, iPods, iPads, etc.) with high autonomy.

Chemical hydrides are viewed as having great potential to store and generate hydrogen, by a simple hydrolysis reaction in the presence of a suitable catalyst, mainly because they exhibit greater gravimetric energy densities and are stable during long periods of storage without usage. Among the chemical hydrides, sodium borohydride (NaBH₄) stands out thanks to their high hydrogen content (10.8 wt%) [1] and high energy density combined with a good storage stability and nontoxicity. NaBH₄ reacts with water to generate molecular hydrogen according to the hydrolysis reaction shown in Eq. (1):



Catalytic NaBH₄ hydrolysis for H₂ generation has currently problems to solve such as high induction times, low H₂ yields and rates and small H₂ storage capacities.

In this work, a novel portable batch mini-reactor (~ 10 cm³) was designed with an ovoid optimized geometry, to generate and storage H₂ according to Eq. (1). The reactor material was chosen considering the following factors: hydrogen diffusion effect; hydrogen embrittlement effect; material density and material costs. Most of the experiments were done with solid NaBH₄ and stoichiometric liquid water (alkali free) in the presence of a reused nano-particulate Ni-Ru based catalyst. The impact of the following parameters on the hydrolysis reaction was studied: H₂O/NaBH₄ ratio; catalyst/NaBH₄ ratio; temperature and successive feeds effect. Once most of the studies in this area report the H₂ production from aqueous alkaline solution of NaBH₄, the effect of the presence of the inhibitor sodium hydroxide was also studied.

The results reveal not only the importance of considering the role of the reactor inside geometry [2] but also the catalytic capability of the used Ni-Ru based catalyst (~ 47 times reused) to generate hydrogen with ~ 6 wt% and ~ 60 kg H₂/m³ (materials-only basis), with exclusion of any excess of water ($x = 0$), with a H₂ yield of almost 80 %, at room temperature. Also, the novel mini-reactor, with dimensions likely that of a small “egg”, is suitable for carrying in bags and supply on-demand low-power H₂-PEMFCs.

References:

- [1] Demirci, U. B., Akdim, O., Andrieux, J., Hannauer, J., Chamoun, R. and Miele P. (2010), *Sodium Borohydride Hydrolysis as Hydrogen Generator: Issues, State of the Art and Applicability Upstream from a Fuel Cell*, in *Fuel Cells*, 10 (3), 335-350.
- [2] Ferreira, M.J.F., Rangel, C.M., Pinto, A.M.F.R. (2012), *Water Handling Challenge on Hydrolysis of Sodium Borohydride in Batch Reactors*, in *Int. Journal of Hydrogen Energy*, 37, 6985-6994

TelePalestra – A better way to share knowledge

E. Soares, P. Brandão, R. Prior

Department of Computer Science, Faculty of Science, University of Porto, Portugal.

Sharing knowledge is one of the important things on the modern society. One of the universities' objectives is to achieve this, thus creating or educating professionals on many areas. However, some knowledge is difficult to properly be shared and learned, much by the complexity of the receiver to obtain or assimilate it. One example is the medical area, where teachers need to share and discuss with students videos or images with huge quality and sometimes even live captures of biomedical information.

Our work addresses some of the problems inherent to this issue. It enables an easy and secure setup for wireless sharing with a huge range of compatible devices where the content sharing is done in an efficient and reliable way, supporting a large number of persons providing and/or receiving the content. The result is a software library that works on most of the Android devices and can be integrated on an existing application, thus extending their capabilities or improving them. It allows the user to easily create a session for sharing content and to personalize it (name and description), making easy for interested users to find the session.

For the intended use case, authentication is mandatory for the users to access the session and the transmitted content. The users authenticate to the session owner and receive a key that protects the shared content, such that the content can only be seen by correctly authenticated users.

One of the biggest problems this work addresses is scalability regarding the sharing of information. Usually information is sent in a one-to-one connection, which would imply that for sharing from one to N persons it would be necessary to send N copies of information in N connections. This wastes bandwidth and overcharges the network, increasing the delivery time of the content. This work tries to improve this taking advantage of multicast to only send one copy for N recipients at the same time [1].

Multicast communication however does not provide reliability, that is, the correct and ordered delivery of data that mechanisms for one-to-one communications provide. Our library ensures reliability in a way that does not compromise the scalability and performs better than the traditional one-to-one solution [2].

All the software is built to work over Wi-Fi 802.11 that is commonly found, with the only requirement being multicast support from the network equipment. This enables having a large number of users on a session across a few tens of meters (depending on the access point and configuration). This software library also opens up the opportunity for more interactivity between members of a session, giving the session owner the possibility to authorize users to emit content for all other users.

We are currently working to expand the current implementation to be used on a simple web browser taking advantage of the new draft standard for real time communication in the web (WebRTC).

References:

- [1] Diot, Christophe, et al. "Deployment issues for the IP multicast service and architecture." Network, IEEE 14.1 (2000)
- [2] Gemmell, Jim, et al. "The PGM reliable multicast protocol." Network, IEEE 17.1 (2003)

Analysis of the Morphology and Dynamic Behaviour of populations of particles of FeSi in Heavy Media Separation.

I. Pinto¹, J. Rocha e Silva^{1,2}(jarochoa@fe.up.pt)

¹ Department of Mine Engineering, Faculty of Engineering, University of Porto, Portugal.

² ENEAS – Faculty of Engineering, University of Porto, Portugal

Heavy media separation is a well-established density separation process, ideally suited for the separation of minerals, ores and scrap metals, with specific gravities ranging from 2 – 4.5. Modern dense media plants use a suspension of dense powder in water to act as an artificial ‘heavy liquid’ in separating mineral particles in a sink-float process. Ferrosilicon powders with a relative density of 6.7-7.1 containing 13 to 16% silicon are commonly used as the dense powder.

The rheology of the suspension, thus its capability as a separator, depends on the morphology of the particles and their granulometric distribution and can be fine-tuned at different dilutions and sizes. The usage of ENEAS image analysis equipment and the freshly acquired rehometer were able to define the optimal configuration of the Heavy Media and to discriminate between several FeSi providers.

We started the work by comparing two samples of FeSi coming from different providers in the image analysis equipment of ENEAS. We were able to ascertain the different provenience of both powders and to provide a forecast of their behaviour. A thorough statistical description of the 3D morphology of the particles done in a different equipment followed; it enabled us to understand the internal hydrodynamic stress of the heavy media. Lastly the provisional behaviour of the mix at different dilutions was determined in a state-of-the art rheometer. The client was given the information needed for the optimization of the FeSi powders to be used in the separation plant.

Acknowledgements

We are grateful to Dr. Eduardo Crespo, former Chief Metallurgist at Sojitz Beralit Tin for the samples of FeSi provided (with the consent of the Board of Directors), and for all the fruitful discussion about the technical subject.

References

- [1] Ronald, Rousseau (1987). *Handbook of Separation Process Technology*, JohnWiley and Sons
- [2] Ungarish, Marius (1993). *Hydrodynamics of Suspensions: Fundamentals of Centrifugal and Gravity Separation*, Springer Verlag
- [3] King, Judson (2013). *Separation Processes*, Dover Publications.
- [4] Collins, B., Napier-Munn, TJ, Sciarone M. (1974). *The production, properties, and selection of ferrosilicon powders for heavy-medium separation*, JS Afri. Inst. Min. Metall,
- [5] Hea, Mingzhao; Wanga, Yanmin; Forssberg, Eric (2007). *Slurry rheology in wet ultrafine grinding of industrial minerals: a review*, Powder Technology 147 (2004) 94–112

Introduction of artificial magnetic flux pinning centers in MgB₂ multifilamentary superconducting wire

L. H. M. Antunes¹, L. B. S. da Silva¹ and D. Rodrigues Jr.¹

¹ Department of Materials Engineering, Engineering School of Lorena, University of São Paulo, Brazil.

The objective of the present work is to develop a multifilamentary superconducting wire of MgB₂ with artificial pinning centers (APC) of VB₂ and co-doping with carbon nanotubes. The doping with VB₂ will allow the formation of new pinning centers and will substitute vanadium atoms into magnesium sites [1]. Doping with carbon nanotubes will allow the formation of pinning centers with some alignment to the magnetic flux lines, besides the substitution of boron by carbon atoms, allowing the increase of the upper magnetic critical field (H_{C2}), hence increasing the critical current densities (J_C).

The MgB₂ powder was mixed with the VB₂ powder accordingly to the stoichiometric composition Mg_{0.95}V_{0.05}B₂ [2]. In addition, co-doping was made with 3wt.% of carbon nanotubes. This mixture was milled for 300 minutes in a high energy ball mill in an argon atmosphere glove-box, to avoid the formation of oxide contaminants during the milling process. The monofilament was prepared by inserting the MgB₂ APC powder into a niobium tube, which was inserted in an OFHC (Oxygen Free High Conductivity) copper tube. The set was deformed in a swage down to the diameter of 4.70 mm. For the bundling, 7 monofilaments were organized in an OFHC Cu tube and the set was swaged and wire-drawn to the final diameter of 2.3 mm. The wire was analyzed with a scanning electron microscope and heat-treated for superconducting characterization.

During the manufacturing process of the superconducting wire, samples were taken for metallographic analysis. It was noted that the niobium layer does not keep an uniform thickness around the powder during deformation, reducing the mechanical strength of the set. This was proved during the wire-drawing process, when the wire broke after more severe diameter reductions. The solution for the problem was to put the set inside another OFHC Cu tube in order to increase the mechanical strength.

Samples of the wire were heat-treated at 600°C/2h, 700°C/1h and 800°C/30 minutes and characterized in a PPMS (Physical Properties Measurement System). All samples presented superconductive transition at approximately 38.5K, as expected for MgB₂, and another around 9.2K, in respect to the niobium diffusion barrier. The transition related to the MgB₂ was always very wide, indicating that ruptures of the niobium layers could lead to the formation of unexpected phases. The results will be discussed concerning to the deformation process and to the formation of phases during heat treatments. The obtained results were satisfactory, even with some difficulties during the fabrication process.

Acknowledgements: The authors wish to thank CNPq and FAPESP, Brazil. DRJ is a CNPq researcher.

[1] Nagamatsu, J., Nakagawa, N., Muranaka, T, Zenitani, Y and Akimaitu, J (2001), *Nature*, v.410, p.63.

[2] Rodrigues Jr., D., Senkowicz, B. J., Hanson, J. M., Larbalestier, d. C. and Hellstrom, E. E. (2008), *Advances in Cryogenic Engineering*, v.54, p.359-366.

Geostatistics applied to electrical resistivity data FEUP Geophysical Experimental Field – A case study

R. Sousa¹, J. Carvalho¹

¹ Department of Mining Engineering, Faculty of Engineering, University of Porto, Portugal.

The main objective of this work is mapping the spatial variability of the electrical apparent resistivity at the FEUP geophysical experimental field, CEG, using data obtained during a practical survey developed by 2013 Projeto FEUP students, using a resistivity meter and the Schlumberger electrode array. Different interpolation methods were used and compared, particularly geostatistical kriging.

The electrical resistivity method uses four linearly spaced electrodes, two for injecting current into the ground and the other two for measuring the in-between potential difference, subsequently converted in the so-called apparent resistivity value.

The acquired data treatment comprises the following five steps: exploratory statistical data analysis; non-geostatistical spatial description. These two first steps including the statistical univariate characterization of the data and its initial spatial description help, namely, to evaluate the mean and variance stationarity assumptions. The following steps: variogram model inference: aiming at quantitatively characterizing the spatial data continuity based on a continuous variogram function model derived from directional discrete experimental variograms; cross-validation method: used in order to choose the possibly best variogram model among the previously selected ones; geostatistical and non-geostatistical interpolation: using geometric and geostatistical kriging interpolation procedures aiming at mapping the apparent resistivity spatial variability at the CEG. To carry on the referred procedures and obtaining the respective graphical outputs the softwares MatLab and Surfer were used.

Geostatistical inference methods allow incorporating the calculated spatial continuity of the process, quantitatively captured by the variogram model, which is often a significant advantage in modeling geo-systems when compared with other non-geostatistical interpolation procedures. In addition, it is possible to obtain, for each interpolated point, a measure of the kriging estimation error variance, which may be understood as a measure of the uncertainty related to each particular point.

Being part of a more comprehensive CEG subsurface characterization program, this work allows namely comparing the outputs of different interpolation methods. Although so far in a preliminary way it is perceptible the more realistic kriging outputs when compared with the non-geostatistical ones. At this point, it is possible to distinguish generically to subareas with lower and higher mean apparent resistivity values whose interpretation needs additional information. Nevertheless, and although not confirmed at the moment, a possible cause for the lower values may be previously buried metallic waste.

Acknowledgments: gratitude to Professor Jorge M. C. M de Carvalho for all support and contribution and to the 2013 Projeto FEUP students who obtained the data.

A6

ASTRONOMY

VIII
PARALLEL
ORAL
SESSIONS

Magnetic characterization of meteorites using a mobile facility

B. Alves^{1,2}, M. Côtó^{1,2}, J. Teixeira^{1,2}, T. Seixas^{1,2}, M. Salgueiro da Silva^{1,2}, D. Flores^{3,4}, H. Sant'Ovaia^{3,4} and F. Noronha^{3,4}

¹ Department of Physics and Astronomy, Faculty of Science, University of Porto, Portugal.

² Research Centre of the Earth and Space, University of Coimbra, Portugal.

³ Department of Geosciences, Environment and Spatial Planning, Faculty of Science, University of Porto, Portugal.

⁴ Centre of Geology, University of Porto, Portugal.

In this project, we have performed a basic structural and magnetic characterization of collections of meteorites with non-destructive and non-invasive techniques. We have used a mobile facility (KT-10 PLUS Magnetic Susceptibility Meter) to measure the magnetic susceptibility of meteorites. This technique is particularly useful not only for a rapid classification of new meteorites, but also as a check against curation errors in large collections (i.e., unweathered meteorites, whose measured susceptibility is likely outside the expected range, may well be misclassified or misidentified samples) [1].

The project website features a database of magnetic susceptibility measurements [2,3]. The results of this study will soon be added to existing planetology research databases, in order to allow a reliable interpretation of magnetic information carried in extraterrestrial materials. Such information provides constraints on ancient magnetic field intensities and on the evolution of minor bodies in our Solar System. We will continue to publish the results using multimedia content for attracting new students, young scientists and the general public.

References:

[1] Rochette et al., Magnetic Classification of Stony Meteorites: Ordinary Chondrites. *Meteoritics & Planetary Science*, 38, Nr 2, (2003) 251-268.

[2] <http://osmeteoritos.wordpress.com/>

[3] <http://www.facebook.com/meteoritos>

Fundamental Cosmology with ESPRESSO and ELT-HIRES

A. C. O. Leite^{1,2}, P. O. J. Pedrosa^{1,2}, C. J. A. P. Martins²

¹ Departamento de Física e Astronomia, Faculdade de Ciências, Universidade do Porto, Portugal.

² Centro de Astrofísica da Universidade do Porto, Portugal.

The observational evidence for the acceleration of the universe demonstrates that canonical theories of cosmology and particle physics are incomplete, if not incorrect, and that new physics is out there, waiting to be discovered. Forthcoming high-resolution ultra-stable spectrographs will play an important role in this quest, by enabling a new generation of precision consistency tests and complementing other facilities in terms of redshift coverage.

In previous work [1], we have studied how astrophysical measurements of varying constants can constrain dark energy (specifically, its equation of state), either on their own or in combination with Type Ia supernova measurements.

In this talk we will present the results in [2] where we quantify the improvements that can be expected with the ESPRESSO and ELT-HIRES spectrographs, and their impact on fundamental cosmology. We find that the current E-ELT configuration has the potential to constrain dark energy more strongly than standard surveys with thousands of supernovae. We will also discuss improvements in dark energy constraints (quantified through various figures of merit) obtained by combining measurements of fundamental couplings with Type Ia supernovae from Euclid and the ELTs.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal), with additional support from grant PP-IJUP2011-212 (funded by U. Porto and Santander-Totta). C.J.M. is supported by an FCT Research Professorship, contract reference IF/00064/2012, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

References:

- [1] Amendola, L. Leite, A.C.O. Martins, C.J.A.P. Nunes, N.J. Pedrosa, P.O.J. Seganti, A. Variation of fundamental parameters and dark energy. A principal component approach Phys.Rev. 063515 D86 (2012)
- [2] Pedrosa, P.O.J., Leite, A.C.O., Martins, C.J.A.P., Observational strategies for varying constants with ESPRESSO and ELT-HIRES, Mem. S.A.It. Vol. 85, in press (2014).

Different Views of Cosmic Defect Evolution

J. R. C. C. Correia ¹, I. S. C. R. Leite¹ and C. J. A. P. Martins¹

¹ Centro de Astrofísica da Universidade do Porto, Rua das Estrelas, 4150-762 Porto, Portugal.

The breaking of spontaneous symmetries at phase transitions that are thought to have happened in the early Universe led to the formation of topological defects [1]. Most of the work that has been done on the subject has focused on cosmic strings, and lately on superstrings, because if proven they exist, they may help explain some of the large-scale structures seen in the Universe today. On the other hand, the study of domain walls has been fairly neglected since it was first remarked by Zel'dovich, Kobzarev and Okun [2] that the restoration of spontaneously broken discrete symmetries at high temperatures in the early universe stands serious problems for its subsequent evolution.

However, domain walls can be of interest to. Being the simplest of topological defects – they can be described by a single scalar field – domain walls are very useful in providing simple toy models where one can study how different physical mechanisms shape the network evolution and this knowledge can be applied to more complex defects.

Our work has studied the extent to which the well known linear scaling as the attractor solution for the evolution of domain walls networks [3] would still abide in some relevant standard and non-standard scenarios. For the various studied networks we based our high-resolution simulations on the PRS [4] algorithm and drew our conclusions from the results obtained for the evolution of the velocity and density of the walls in conformal units.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT, Portugal. C.J.M. is supported by an FCT Research Professorship, contract reference IF/00064/2012, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

References:

- [1] T.W. B. Kibble, *J. Phys. A* 9, 1387 (1976).
- [2] Y. B. Zeldovich, I. Y. Kobzarev, and L. B. Okun, *Zh.Eksp. Teor. Fiz.* 67, 3 (1974).
- [3] A. Vilenkin and E. P. S. Shellard, *Cosmic Strings and other Topological Defects* (Cambridge University Press, Cambridge, U.K., 1994).
- [4] W. H. Press, B. S. Ryden, D. N. Spergel (1989), *The evolution of domain walls and cosmic strings*, *Astrophys. J.* 347, 590.

Consistency tests of the stability of fundamental couplings and unification scenarios

M. C. Ferreira^{1,2}, O. Frigola³, C. J. A. P. Martins¹, A. M. R. V. L. Monteiro^{1,2} and J. Solà⁴

¹ Centro de Astrofísica da Universidade do Porto, Portugal;

² Faculdade de Ciências, Universidade do Porto, Portugal;

³ Institut S'Agullà, Carretera de Malgrat 13, 17300 Blanes, Spain

⁴Institut Manuel Blancafort, Avinguda 11 de Setembre 29, 08530 La Garriga, Spain

Fundamental couplings are dimensionless quantities that are used to compare magnitudes of interactions. Current experimental data provides evidence for the existence of four fundamental interactions in Nature. Fundamental means that all phenomena are ultimately described in terms of these interactions. So, if it is assumed that these couplings are constants in time and space, it is expected that physical laws are the same everywhere at every time. But, what if these couplings vary? What if one interaction has a greater magnitude in one place than in the other? Being true, variation of couplings would be a seminal finding.

There are many theories of unification that encompass variation of fundamental couplings. The best way to probe them is to compare their predictions with experimental data. In a wide class of unification theories it's possible to squeeze into two coefficients, R and S, their predictions on variation of couplings. Different theories produce different coefficients. Translating experimental data into these coefficients allows one to sort out theories for which the coefficients don't agree with the ones found with the data.

Following our previous work[1-2], we use the most recent atomic clocks and quasars absorption spectra data to obtain constraints to the variations of several dimensionless couplings and to test their self-consistency. Our work also includes a study of the consequences of our results on the theoretical freedom of a specific class of unification theories.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal), with additional support from grant PP-IJUP2011-212 (funded by U. Porto and Santander-Totta). The work of CJM is supported by a Ciência2007 Research Contract, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

References:

[1] M. C. Ferreira, M. D. Julião, C. J. A. P. Martins and A.M. R. V. L. Monteiro, *Phys. Rev. D* **86**, 125025 (2012).

[2] M. C. Ferreira, M. D. Julio, C. J. A. P. Martins, and A. M. R. V. L. Monteiro, *Phys.Lett. B* **724**, 1 (2013).

The effects of biased initial conditions on domain wall evolution

J. R. C. C. Correia¹, I. S. C. R. Leite¹ and C. J. A. P. Martins¹

¹ Centro de Astrofísica da Universidade do Porto, Rua das Estrelas, 4150-762 Porto, Portugal.

It is known that topological defects necessarily form at phase transitions in the early Universe [1]. Depending on the details of the phase transitions, the resulting networks can have a range of astrophysical and cosmological consequences, ranging from highly desirable ones (such as contributing to the origin of large-scale structures) to the undesirable ones that can be used to rule out some classes of models.

A specific type of topological defect (known as ‘domain wall’) normally evolves according to an attractor linear scaling solution. If this persists for a sufficiently long time, the wall network will end up dominating the energy density of the universe, a scenario that is in disagreement with current observational data.

In order to further understand the range of conditions leading to such catastrophic behavior, we delve into the consequences of evolving domain walls, specifically considering scenarios involving biased initial conditions. We evolve our defects using numerical simulations as derived from the PRS algorithm [2] and then review the results by observing the velocity, the density, and their dependency on conformal time. We also test an analytic fitting formula provided by [3]. Our results also provide some relevant insights on the longevity of walls.

References:

- [1] T. W. B. Kibble (1976), *Topology of cosmic domains and strings*, J. Phys. A9 1387.
- [2] W. H. Press, B. S. Ryden, D. N. Spergel (1989), *The evolution of domain walls and cosmic strings*, Astrophys. J. 347, 590.
- [3] M. Hindmarsh (1996), *Analytic scaling solutions for cosmic domain walls*, Phys. Rev. Lett. 77, 4495, hep-ph/9605332.

Analysis of the yearly sunspot number with the Empirical Mode Decomposition

R. Albuquerque¹, A. C. O. Leite^{1,2}, R. Almeida^{3,4,5} and A. P. Rocha^{3,4}

¹ Department of Physics and Astronomy, Faculty of Sciences, University of Porto, Portugal.

² Centre of Astrophysics of the University of Porto, Portugal.

³ Department of Mathematics, Faculty of Sciences, University of Porto, Portugal.

⁴ Centre of Mathematics of the University of Porto, CMUP, Portugal.

⁵ GTC-I3A, University of Zaragoza and CIBER-BBN, Spain.

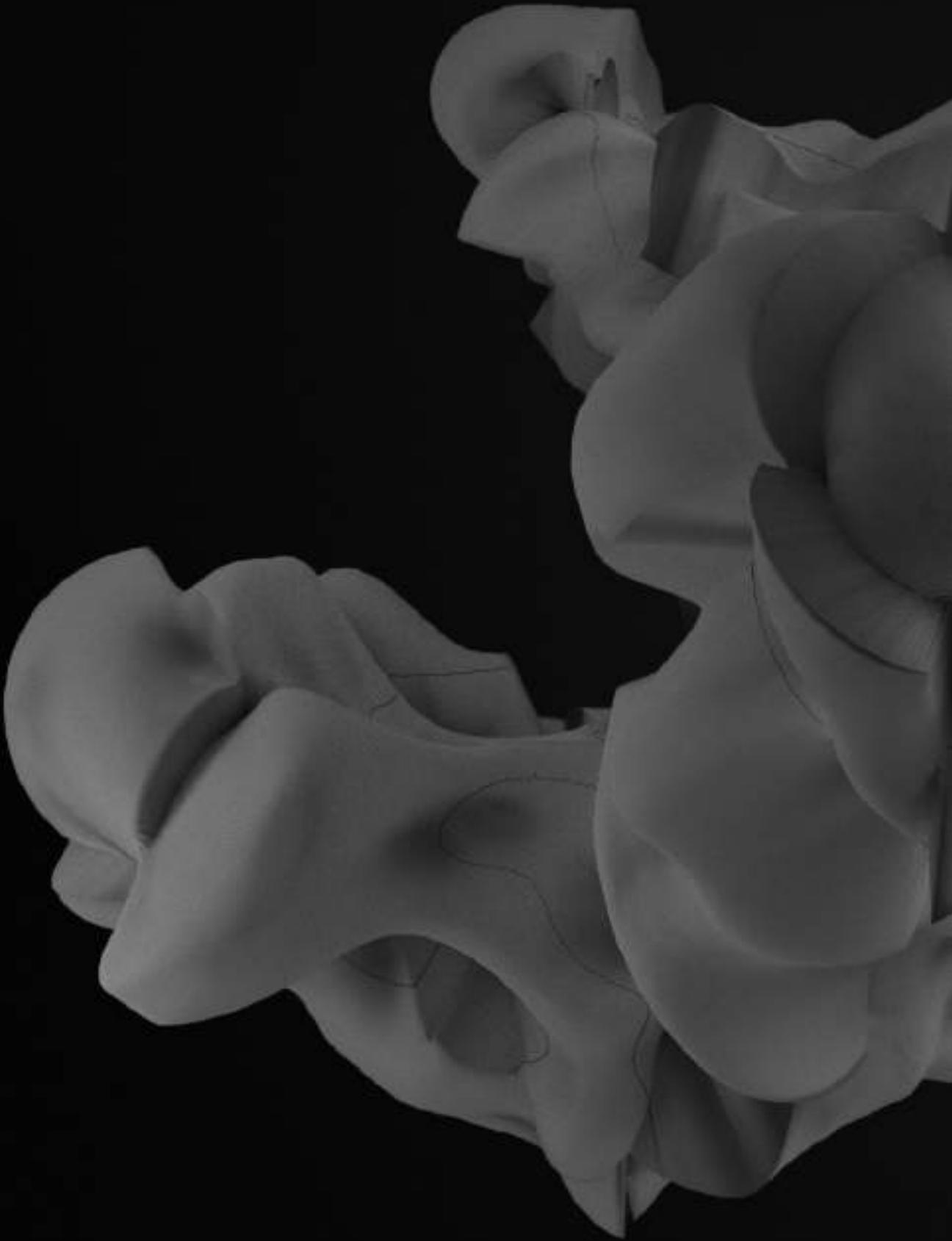
During the XVII century, the astronomers Galileo Galilei and Christoph Scheiner observed that the Sun presented groups of dark spots on the surface. The designated sunspots are cooler regions of the photosphere and are manifestations of magnetic activity of the Sun [1]. In 1843, Samuel Schawbe discovered a cyclic behavior in the number of sunspots, which periodicity is nowadays estimated in around 11 years [2].

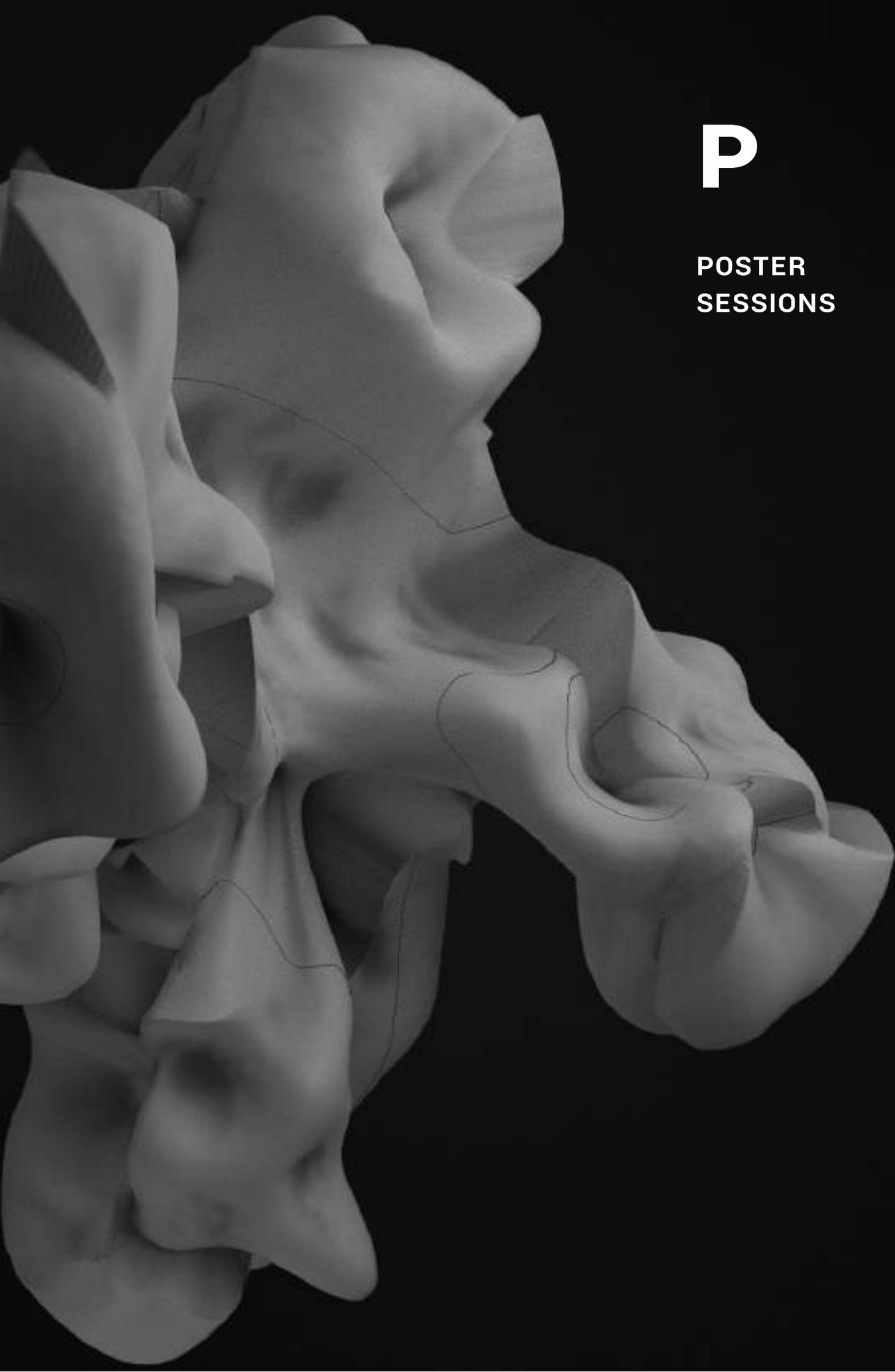
The sunspot number time series are probably the data set most studied in astronomy [3]. Nevertheless, besides the 11-year cycle, there are other hidden cycles with larger periodicities. In order to find them, it will be applied the Empirical Mode Decomposition (EMD) [4] to yearly sunspot number data, between 1700 and 2012, obtained by the Solar Influences Data Analysis Center (SIDC) from the Royal Observatory of Belgium [5]. Afterwards, Fourier Transform is applied over the intrinsic mode functions. Also, some methods regarding the prediction of the number of sunspots will be presented.

By analyzing the results, it was possible to identify the Schwabe cycle (~11 years), the Hale cycle (~22 years), the quasi-Hale cycle (~50 years) and the Gleissberg cycle (~100 years) [6,7]. Thus, the application of the EMD together with Fourier Transform was able to correctly identify the expected periodicities, without a more sophisticated transform as the Hilbert Transform, commonly used in the literature.

References:

- [1] Santos, N. C., Tirapicos, L. and Crato, N. (2012), *Outras Terras no Universo*, Gradiva Publicações, S. A..
- [2] Priest, E. R. (2000), *Solar Magneto-hydrodynamics*, Reidel Publishing Company.
- [3] Rao, T. S., Priestley, M. B. & Lessi, O. (1997), *Applications of Time Series Analysis in Astronomy and Meteorology*, Chapman & Hall.
- [4] Huang, N. E., Shen, Z. et al. (1998), *The empirical mode decomposition and the Hilbert spectrum for non-linear and non-stationary time series analysis*, Proc. Royal Soc. London A, Vol. 454, pp. 903-995.
- [5] SIDC-team, World Data Center for the Sunspot Index, Royal Observatory of Belgium, Monthly Report on the International Sunspot Number, online catalogue of the sunspot index: <http://www.sidc.be/sunspot-data>, '1700-2012-of-data'.
- [6] Barnhart, B. L., Eichinger, W. E. (2011), *Analysis of sunspots variability using the Hilbert-Huang Transform*, Solar Physics, 269:439-449.
- [7] Wang, Y., Ding, Y., Luo, Q., Miao, Q. (2011), *Application of EMD and Wavelet Method to Sunspot Data*, International Conference on Information Science and Technology, March 26-28, Nanjing, Jiangsu, China.





P

**POSTER
SESSIONS**



P

POSTERS

**WEDNESDAY
12TH**

**POSTER
SESSIONS**

Urban floods, flash floods and wave-overtopping in the city of Santa Cruz, Madeira: affected areas and frequency

Peixoto, Ana Isabel¹

¹ Department of Geography, Faculty of Letters, University of Porto, Portugal

Historically, the city of Santa Cruz, in Madeira Island, is affected by flash flood processes – alluviums [1], urban floods and wave-overtopping, highlighting the interest in the definition of susceptible areas for each process, inventory and definition of their frequency.

This work is dedicated to the analysis of the urban area of Santa Cruz susceptible to extreme hydric events, which began with the preparation of the events chronology, based on the dates and information given by the municipality. After the identification of these events, it was made an historic survey to obtain the frequency of each event/process, analysing the news of the regional newspapers, *Diário de Notícias da Madeira* (1956-2010) and *Jornal de Notícias da Madeira* (1956-2010) for the occurrences dates. The information collected was gathered in a geodatabase on the ArcMap 10.1. software, associating the elements affected by the runoff and the height of the water column for each flood event. This data allowed the definition of the flood area for each event and the identification of the exposed elements, like: building, street, port number, property type, functionality on the time of the event and the height of the water column. Finally, it was identified the affected streets featuring the behavior of streets in the network drainage and respective flow direction [2].

The obtained data allowed the definition of the flood perimeters for each occurrence, according to the type of event: flash floods, coastal flood and urban flooding. The superimposition of the perimeter for each event was used for the calculation of the occurrences frequency in the city of Santa Cruz. Another result derives from the water column height measured for the flash flood of 3-11-1956 and for the urban flood of 20-02-2010, considering the positions of the door sills in relation to the road [2].

Concluding, this analysis reveals that the most frequent events (20th and 21st centuries) are urban floods due to: i) incapacity of the sewage drainage network, ii) increase of sealed areas, iii) the increase of runoff from the higher altitude areas that drain to the urban center. The analyzed data show that the occurrence of floods in Santa Cruz mainly occurred in the area defined by four streets: Rua 17 de Julho de 1876, Rua da Praça, Rua Conselheiro Luís Freitas Branco and Rua da Praia.

References:

[1] Abreu, U., Tavares, A., & Rodrigues, D. (2008). *Processos de perigosidade natural no município de Câmara de Lobos–Madeira. Contributo para a gestão do risco e da emergência*. *Territorium*, 15, 53-71

[2] Peixoto, Ana (2013). *Inundações urbanas, cheias rápidas e galgamentos costeiros na cidade de Santa Cruz, Ilha da Madeira: áreas afetadas, frequência e avaliação da vulnerabilidade funcional*. (Dissertação de Mestrado Riscos, Cidades e Ordenamento do Território), FLUP

Regression Methods for Multiple Outcomes in Health Research

Oliveira, R. *

Faculdade de Medicina do Porto, Porto, Portugal rcoliveira@med.up.pt
Teixeira-Pinto, A.

University of Sydney, Sydney, Australia armando.teixeira-pinto@sydney.edu.au

Abstract: In research problems, particularly in biomedical research studies, it is common that the outcome of interest is characterized by multiple variables rather than a single measure per individual in order, for example, to examine therapeutic efficacy, treatment effectiveness or associations with various covariates of interest. A typical approach when multiple outcomes are present in a study is to analyze each outcome independently, in a univariate framework, ignoring the most likely correlation between the outcomes and the multivariate structure of the data. At first glance, this approach may seem less efficient than applying multivariate methods, because it ignores the additional information contained on the correlation between the outcomes. Surprisingly, this is not always the case. In Seemingly Unrelated Regression (SUR) model, Zellner (1962), allowed each outcome of interest to be associated with its own set of covariates, i.e., he showed that SUR is an extension to the classical multivariate linear regression (MvLR) in the sense that if all the outcomes are modeled using the same covariates, the SUR model reduces to the classic MLR. The SUR estimator proposed by Zellner (1963) was shown to be more efficient than the ordinary least squares (OLS) estimator. Later, Srivastava (1970) studied SUR model with second-order moments and also found that as the correlation increases, the relative efficiency decreases which agrees with Zellner (1962) study. Breiman (1997) also investigated different procedures in order for SUR to be more efficient than OLS. All authors concluded that if there is no correlation between the outcomes, or if the setting is the same that in the classical multivariate linear regression, the coefficients estimators in the multivariate setting is the OLS Estimator. We show the analytical estimates for the parameters associated with share and unshared covariates and present a Monte Carlo simulation for different settings. In this paper we study the relationship of the gain / loss of efficiency of coefficients estimates in sets of equations of multiple multivariate regression when one or more covariates are correlated. We study a mix setting where the outcomes share some covariates but are also associated with specific covariates. We demonstrate that for the coefficients associated with shared covariates there are efficiency gains, while for the outcome-specific covariates the efficiency gains depend on the correlation between the outcomes.

Key Words: Seemingly Unrelated Regression, Ordinary Least Squares, Correlated errors, Generalized Least Squares.

The concept of cultural district: reflection about the form and the function

F. Rodrigues¹

¹ Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

The identification of the limits and conditions of the cultural markers furthers our understanding of the spatial and functional stability of a cultural district. The description of a language, religion, economic and political aspects, for example, when studied, situated in an environment, indicate significant differences to determine the cultural boundaries of a geographical area.

The theoretical reflection that presents, proposes: to clarify the definition of the cultural district, based on their appearance and on their functionality.

Considering how culture, the acquisition, through learning, behavioral characteristics, identified a group of people who meet the standards of similar behavior. This condition warrants the complexity and dynamism in the conceptualization of a cultural area, mainly due to the ability of initiative and creation of man. Also translates into limits of folk base, common recognition of the region and represented by the subjectivity that exists in people's minds.

This thought classify the cultural district with a little scattered and little manageable.

Otherwise, the functions of daily life classified cultural district through the most typical social roles. This sets clear boundaries and set in an exclusive area.

Thus, the strong influence of social actors as cultural mediators of a political subdivision with a variable function: be religious, be military, be economic.

However, clarification of a cultural district that is possible to describe the highest cultural stability in an area and the common brands in shared areas.

The cultural characteristics shall mean that speech, but not so stable, unlike be understood that such features are not present. There is some cultural boundaries and thin lines and transitional.

The resulting representation of the understanding of this concept is a cultural landscape of a non-stationary area that is being remodeled for the valuation assigned by the meanings and points of view that they'll be intensified by what was experienced by each person.

References:

[1] Claval, P.C.C. (2011), Geografia cultural: um balanço, *Revista Geografia*, Londrina, 20 (3), 5-24.

[2] Hui, D. (2013), *Cultural Geography of the World*, in: "EDX Courses", PekingX's University.

The Corporate Social Responsibility - A lever for sustainability? A case study: The Nestlé Group and cocoa plantations in Ivory Coast

J. Fernandes¹

¹ Department of History, Political and International Studies, Faculty of Arts, University of Porto, Portugal.

It was mostly in turn of the 20th century that Corporate Social Responsibility (CSR) became widely noticed by the media, the business world, the academy and society in general. Considered as one of the landmarks for the sustainable management of macroeconomic world, CSR promotes policies concerning working condition's quality, environmental aggressions, and the eradication of human-rights transgressions. In a society where the global overthrows the particular, enterprises look to establish rules and orienting principles, to share responsibilities that promote sustainability (social, environmental, cultural and political) in the communities where they invest [1].

This study compares CSR's international social responsibility certification norms with Nestlé's social responsibility plan (Cocoa Plan) in Ivory Coast's cocoa plantations. The research evolved from a descriptive and exploratory qualitative approach, with no intention of measuring results and impacts quantitatively.

For this case study was important the creation of an analysis model, where we apply the external dimensions, (local communities, business partners, suppliers and consumers, human rights and global environmental concerns) their indicators are contemplated in the *Livro Verde - Promover um quadro europeu para a responsabilidade social das empresas*[2], in confrontation with the specific performance of Nestlé in developing its Cocoa Plan in Ivory Coast, verify compliance with the guidelines of SA8000 (child labor, forced labor, health and safety, freedom of association and collective bargaining, discrimination, disciplinary practices; remuneration, management system).

The results obtained at this research, show's that Nestlé with its Cocoa Plan, follow the guidelines for social responsibility enshrined in the "*Livro Verde - Promover um quadro europeu para a responsabilidade social das empresas.*" as well the certification of specific codes of conduct for the area of cocoa production, as they are implemented the UTZ Certified Cocoa Programme and Fair Trade Foundation. However, is important refer the Nestlé don't have the international certification for Social Responsibility, the SA8000 norm.

References:

[1] REGO, Arménio [et al.] - *Gestão Ética e Socialmente Responsável*. Lisboa: Teoria e Prática, 2006, p. 130-136.

[2] *Livro Verde: Promover um quadro europeu para a responsabilidade social das empresas*, Bruxelas, Comissão Europeia, 2001.

Toxicity evaluation at different trophic levels of pharmaceutical drugs

D. Silvério¹, S. C. Antunes^{1,2}, O. M. Lage^{1,3}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² Centre of Environmental And Marine Studies (CESAM), Campus of Santiago, University of Aveiro, Portugal.

³ Centre of Marine and Environmental Research (CIIMAR), Porto, Portugal

In recent years and, due to anthropogenic activities, water pollution has increased significantly, and has drawn the worlds' attention to this matter. Pharmaceutical drugs are one of the many pollutants increasing in concentration in water bodies [1], due mostly to the residential or agricultural origin, or direct disposal from industry. Despite contaminated water treatment, many of these products aren't eliminated from the water course, and may then be responsible for significant alterations in aquatic ecosystems. In this study, the effect of pharmacological water pollutants was tested in non-target species with ecological relevance, namely *Daphnia magna* (an aquatic planktonic microcrustacea member of the order Cladocera) and *Rhodopirellula rubra* (a bacteria belonging to the phylum *Plantomycetes*). Both species were submitted to acute tests, in order to determine the toxicity of several pharmaceutical compounds. *R. rubra* showed resistance to the nonsteroidal anti-inflammatory sodium salicylate at least up to 4g/L, but was affected significantly by the quinolone antibiotic ciprofloxacin (almost no growth obtained at 50 mg/L after 30 minutes exposure), the disinfectant benzalkonium chloride (no growth at 10 mg/L) and the antiseptic boric acid (significant growth reduction at 16 g/L). *Daphnia magna* showed resistance to almost all the substances within environmental concentrations [2], not presenting mortality up to 1g/L of sodium salicylate and boric acid (which are further higher than the environmental values, 1.0 µg/L and 0.1-4.5 mg/L, respectively). These results demonstrated that both species tested, *D. magna* and *R. rubra* have high resistance to the tested compounds, and the concentrations that cause significant mortality are far higher than the environmental concentrations, not posing threat to this organisms, with the exception of benzalkonium chloride, which shows environmental concentrations (6 mg/L) similar to those who caused *R. rubra* death (10 mg/L).

[1] Han, G. H., Hur, H. G., Kim, S. D. (2005). Ecotoxicological risk of pharmaceuticals from wastewater treatment plants in Korea: occurrence and toxicity to *Daphnia magna*, *Environmental Toxicology and Chemistry*, 25(1), 265-271.

[2] Darcan, C., Kahyaoglu, M. (2012). The effect of Some Boron Derivatives on Kanamycin Resistance and Survival of *E. coli* and *P. aeruginosa* in Lake Water. *Biomed Environ Sci*, 25(4), 476-482.

Acknowledgment: This research was supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology, under the projects PEst-C/MAR/LA0015/2013 and PEst-C/MAR/LA0017/2013.

Toxic effects of single and combined nickel and microplastics on juveniles of *Pomatoschistus microps*

R. Norberto^{1,2}, L. Guilhermino^{1,2}

¹ Department of Populations Studies, Laboratory of Ecotoxicology of Institute of Biomedical Sciences of Abel Salazar of University of Porto, Portugal.

² CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Laboratory of Ecotoxicology and Ecology, University of Porto, Portugal.

Microplastics are synthetic organic polymers (< 5mm) made from nonrenewable resources, which are widely used in several types of industries. They are considered emerging pollutants of high concern mainly because: (i) due to their low degradation rate, they tend to accumulate in both terrestrial and aquatic ecosystems; (ii) they are able to cause adverse effects on the biota because some organisms confound them with food leading to starvation; and (iii) they might act as carriers for very toxic chemicals allowing their entrance in food webs. Thus, the objective of the present study was to investigate the acute toxic effects of microplastics alone and in mixture with nickel that is a common environmental contaminant, especially in estuaries and other coastal areas, on early juveniles of the common goby, *Pomatoschistus microps*, a species with a relevant role in trophic webs lagoons and estuaries of Europe.

Juveniles from wild populations were acclimatized to laboratory conditions. After the acclimatization period, two bioassays were carried out: one with nickel alone and the second where fish were exposed to nickel in the presence of microplastics. Effect criteria were mortality, predatory behaviour and several biomarkers were determined (glutathione S-transferases - GST, acetylcholinesterase - AChE, lipid peroxidation - LPO and 7-ethoxyresorufin-O-deethylase - EROD). The results are discussed in relation to the effects of nickel and microplastics on fish health.

Acknowledgements: This study was done in the scope of the project “*SIGNAL - Effects of pollution on estuarine ecological interactions zooplankton-zooplanktivorous fish in relation to climate changes*” funded by The Portuguese Foundation for the Science and Technology (PTDC/AAC-AMB/110331/2009) with FEDER funds of the COMPETE programme (FCOMP-01-24-FEDER-01387601).

Extended-spectrum beta-lactamase producing *Escherichia coli* in river water of a rural area in the North of Portugal

M. Monteiro¹ and H. Ferreira^{1,2}

¹ Microbiology, Biological Sciences Department, Faculty of Pharmacy, University of Porto, Portugal

² REQUIMTE University of Porto, Portugal

Rivers are used for many purposes including water collection for drinking water production, recreation and agriculture field irrigation. Microbiological contamination of natural waters from different origins should be taken in consideration namely in terms of recreation use of river water. Microbiological quality of recreational waters is addressed in terms of faecal contamination indicators as are coliforms and enterococci. *Escherichia coli* is a relevant representative of coliforms, in terms of river water microbiological quality. *Escherichia coli* is an opportunistic pathogen able to carry antimicrobial resistance mechanisms of clinical relevance, as are extended-spectrum beta-lactamases (ESBLs).

The aim of our study was the detection of antimicrobial resistant bacteria in water from river samples, with special attention to ESBL producing coliforms.

For that purpose, river water was collected in two different points, a recreation area and water from a free running zone. Water samples were analysed by the membrane filtration method. A defined volume of water was submitted to a ramp vacuum filtration, followed by placement of the filters in Mac Conkey and in Mac Conkey with aztreonam, ceftazidime, cefotaxime and meropenem. Colonies of lactose fermenters were randomly selected for antimicrobial susceptibility study which was determined by agar diffusion method, according to the Clinical Laboratory Standards Institute (CLSI) and screened for ESBL production, by the double disc synergy test and clavulanic acid addition, according to the CLSI guidelines. Identification of the selected strains was achieved by ID 32 GN.

Non repetitive ESBL producing *Escherichia coli* isolates were detected in the collected samples, showing three different beta-lactam resistance phenotypes.

This finding is of relevance because the unawareness of the presence of this kind of bacteria may lead to a health risk, increased resistance to treatment in the community and respective dissemination of these bacteria in soil, waters and other different environments. Unexpected contamination by this kind of bacteria might reflect direct or indirect anthropogenic impact in natural waters, namely by animal production in the surrounding area or clandestine wastewater runoffs.

Acknowledgements: Tânia Vilaça, Susana Rocha

Benzene sorption isotherms of soils contaminated with biofuels

F. Rohden¹, **M. Carvalho**², **M. Rosas**³, **M. Vila**⁴, **A. Danko**⁵, **T. Oliva-Teles**⁶, **J. Dias**⁷ and **A. Fiúza**⁸

^{1,2,3,4,5,7,8} Faculty of Engineering, University of Porto, Portugal.

^{2,6} REQUIMTE, Institute of Engineering of Porto, Polytechnic Institute of Porto

The remediation of soils contaminated by aromatic hydrocarbons are largely studied, but it is not yet known how the presence of biofuels impacts the efficiency of remediation of these volatile contaminants [1],[2].

The present work focus on the adsorption of benzene on a real limestone soil previously contaminated with two different biofuels: butanol and biodiesel, with the goal of studying the partition of benzene between the three phases (solid, liquid and gas) of the soil when it is has been previously contaminated by different biofuels with different concentrations.

Materials and methods: Four Erlenmeyer flasks of 1L were used that contained 630 g of soil which was wetted by the addition of 70 mL of demineralized water in order to make up a moisture content of 11.1%. Two of the samples were contaminated with different contents of n-butanol (200 μ L and 1000 μ L, corresponding to de concentration of 0.23 mg/kg and 1.16 mg/kg), and the other two samples were contaminated with a soybean biodiesel in the same amount as used with n-butanol. The flasks were kept at constant temperature of 25°C. The addition of benzene was made by successive increments from 0.4 μ L to 220 μ L, corresponding to the concentrations from 0.5 μ g/L to a final concentration of 275.6 μ g/L. The time of each addition of benzene was determined by the equilibrium conditions.

Results and discussion: As can be seen in Figure 1, when the soil was contaminated with butanol, it showed lower capacity to sorb benzene than when it has contaminated with biodiesel. It should also be emphasized that the sorption of benzene increase when the concentration of biodiesel in the soil increases. Mathematical models (Freundlich, Langmuir and polynomial) were adjusted to the experimental data. For soil contaminated with butanol, the best fits were obtained with the Langmuir model ($R^2 = 0.998$ and $R^2 = 0.994$ for the contamination levels of 0.23 mg/kg and 1.16 mg/kg respectively). For soil contaminated with biodiesel the model that best fitted the experimental data was a 3rd order polinomial ($R^2 = 1.000$ for the two levels of contamination).

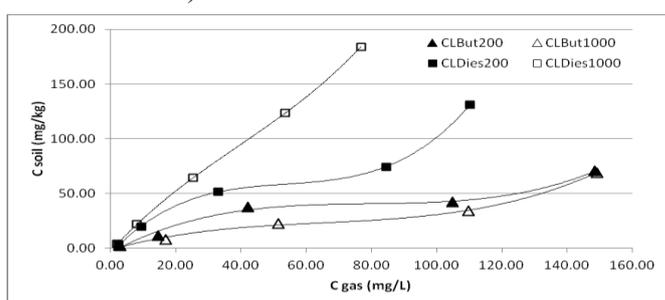


Figure 1- Benzene isotherms in limestone soil contaminated with biofuels.

References: [1] Albergaria, J. T., Alvim-Ferraz, M. and Delerue-Matos, C. (2010), *Estimation of pollutant partition in sandy soils with different water contents*, Environ Monit Assess, vol. 171, pp. 171-180.

[2] Carvalho, M., Vila, M., Soeiro de Carvalho, J., Domingues, V., Delerue-Matos, C., Oliva-Teles, M.T. and Fiúza, A. (2012) "Xylene sorption in three different Portuguese subsoils". 1st Symposium on subsoil characterization and remediation, FEUP, Porto.

Bioaccumulation of cyanotoxins in microalgae and plants – A laboratorial approach towards risk assessment of toxic cyanobacteria

C. Santos^{1,2}, A. L. Pereira¹, J. Azevedo¹, V. Vasconcelos^{1,2} and A. Campos¹

¹ Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Rua dos Bragas 289, P 450-123 Porto, Portugal

² Department of Biology, Faculty of Sciences of the University of Porto, Rua do Campo Alegre, 4069-007 Porto, Portugal

Toxic cyanobacteria are known as an emergent environmental threat since water contaminated with cyanotoxins represents a potential risk in humans due to the ingestion of contaminated food or water. Research demonstrated toxic effects of those cyanotoxins in microalgae and plants, with changes in seed germination, growth rates and oxidative stress. In addition, phytoplankton and aquatic macrophytes exposed to those cyanotoxins may accumulate and transfer them to higher trophic levels.

The aim of this work is to observe 1) if there is uptake of cylindrospermopsin (CYN) by *Chlorella vulgaris* and *Azolla filiculoides* and 2) if CYN affects the growth rate of the microalgae and fern.

A bioassay using the crude extract of a cylindrospermopsin-producing cyanobacterium *Aphanizomenon ovalisporum* (strain X001) was made with *C. vulgaris* and *A. filiculoides*. For *C. vulgaris* a cellular density of 1×10^5 cells/mL and two concentrations of crude extract (0.055 and 0.15 $\mu\text{g/mL}$) during 14 days was chosen. With *A. filiculoides* the concentration of crude extract was 0.05, 0.5 and 5 $\mu\text{g/mL}$ during 7 days. High performance liquid chromatography was used to analyze the culture media Z8 and H-40 where *C. vulgaris* and *A. filiculoides* grown.

In *C. vulgaris* cell density increased for both control and exposure assays (0.055 and 0.15 $\mu\text{g/mL}$ CYN) during the 14 days of culture. But no statistical significant differences were observed in the cell density between control and exposure assays. These results may indicate that CYN, at this interval of concentration, has no toxic effects on *C. vulgaris* growth.

In case of *A. filiculoides* it was observed an increase on the relative growth rate (RGR) in 0.05 and 0.5 $\mu\text{g/mL}$ crude extract and a marked decrease in RGR in 5 $\mu\text{g/mL}$ crude extract. This could be due to the root detach from the pteridophyte especially on the highest crude extract concentration. Perhaps this could indicate that the CYN induced stress on the fern. The culture medium showed no significant changes in the CYN concentrations and the pteridophyte show low CYN contents.

Acknowledgments: Porto University under the project IJUP2011_3, the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and FCT – Foundation for Science and Technology, under the project PEst-C/MAR/LA0015/2013

Stress alleviation capability of exogenous brassinosteroids applied to *Solanum nigrum* L. exposed to high levels of Zn

F. Oliveira, J. Teixeira and F. Fidalgo

Biosystems & Integrative Sciences Institute (BioISI), Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

Contamination of soil with heavy metals, such as zinc (Zn), by anthropogenic activities is becoming more common than ever and is a serious environmental problem. At higher levels Zn can indirectly enhance the production of reactive oxygen species (ROS) resulting in oxidative damage. To protect themselves from oxidative stress induced by excessive ROS, plants developed an antioxidant defense system composed by enzymatic and non-enzymatic constituents. Superoxide dismutase (SOD) is the most effective intracellular enzymatic antioxidant and it is present in all aerobic organisms. SOD catalyses the dismutation of superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen and it is in the first line of defense against the toxic effects of elevated levels of ROS [1]. Brassinosteroids (BRs) are steroidal phytohormones that play crucial roles in plant development and also promote tolerance to a range of biotic and abiotic stresses [2].

Solanum nigrum L. plants were grown in three situations: G1-control plants watered with Hoagland solution (HS), G2-plants watered with HS with 500 μ M Zn and G3-pretreated plants with 24-epibrassinolide (EBR) and subsequently watered with HS with 500 μ M Zn. To study the influence of BRs on *S. nigrum* plants tolerance to this high level of Zn, biochemical parameters associated with the antioxidant metabolism were analyzed. Zn quantification, both in shoots and roots, showed a significant increase in all the plants treated with Zn (G2 and G3) compared to control (G1). H_2O_2 levels decreased 69% in roots of Zn treated plants (G2) relatively to G1. In contrast, the pretreatment with EBR (G3) did not cause significant differences compared to G1. In shoots, H_2O_2 concentration was not significantly affected. There was a significant increase of about 31% and 19% of the soluble protein content in the shoots of the treated plants (G2 and G3), respectively, in comparison to the control (G1). In roots, a decrease of 32% in the G2 situation relatively to G1 was found. The analysis by SDS-PAGE of soluble proteins revealed only the occurrence of some quantitative differences between the treated and control plants. The evaluation of SOD activity by native PAGE in shoots of the three groups of plants, revealed five major bands corresponding to SOD isozymes: one MnSOD, one FeSOD, two Fe+Cu/Zn SOD and one Cu/ZnSOD. The analysis of zymograms showed there was an increase in SOD activity when the plants were pretreated with EBR and a decrease in Zn treated plants. The results suggest that EBR pre-treatment can provide some protection to plants during Zn exposure and that its protective role may be through induction/activation of the antioxidant system.

References:

- [1] Gill S. S. and Tuteja N. (2010), *Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants*, Plant Physiology and Biochemistry, 48, 909-930.
- [2] Bajguz, A. and Hayat, S. (2009), *Effects of brassinosteroids on the plant responses to environmental stresses*, Plant Physiology and Biochemistry, 47, 1-8.

Impact of Microcystin contaminated irrigation water on the photosynthesis and growth of carrot (*Daucus carota*) – implications in water management and crop production

J. Machado¹, J. Azevedo¹, M. Freitas¹, E. Pinto², V. Vasconcelos^{1,3} and A. Campos¹

¹ Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Porto, Portugal

² REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Porto, Portugal

³ Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

Cyanobacteria blooms are serious sources of pollution in aquatic environments because many of them produce a large diversity of potent toxins [1]. Contaminated waters are commonly used in agriculture for irrigation. Introduction of these toxins into the human food chain is therefore a possibility due to the contamination of vegetable food products. However, the impact of cyanotoxins on plants and its ability to enter the food chain via this pathway is not fully understood thereby extensive research is needed [2]. Microcystin (MC) is the cyanotoxin most frequently present in eutrophic freshwaters causing serious problems to human health [1]. Carrots (*Daucus carota*) are root-vegetables with great importance for human nourishment and economy [3]. Therefore, it is important to evaluate the possible negative effects of the use of water contaminated with MC in the growth and production of this horticultural crop. For this purpose we grew young carrots (~ 1 month old) in soil during 1 month in greenhouse conditions. Three groups were performed with plants being irrigated with non-contaminated water (control group) or with a crude *Microcystis aeruginosa* extract containing respectively with 10 and 50 µg/L MC twice a week. Pulse-amplitude modulated (PAM) fluorometry was applied to investigate photosynthetic performance of plants in vivo (noninvasive method). Additionally fresh and dry weight of carrots and leaves were measured to assess plant growth. The results show that both short and prolonged exposure to MC increases photosynthetic efficiency of plants. However no significant variations in fresh and dry weight of leaves and carrots were observed at any concentration of toxin. The future work will focus on the evaluation of the toxin concentrations and the nutritional value in carrots

Acknowledgments: Porto University under the project IJUP2011_3, the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and FCT – Foundation for Science and Technology, under the project PEst-C/MAR/LA0015/2013.

References:

- [1] Chorus, I.; Falconer, I.R.; Slas, H.J.; Bartam, J. (2000). *Health risks caused by freshwater cyanobacteria in recreational waters*. Journal of Toxicology and Environmental Health, Part B 3, 323-347.
- [2] Kittler, K., Schreiner, M., Krumbein, A., Manzei, S., Koch, M., Rohn, S., Maul, R. (2012). *Uptake of the cyanobacterial toxin cylindrospermopsin in Brassica vegetables*. Food Chemistry, 133(3), 875–879.
- [3] Singh, D. P., Beloy, J., McNerney, J.K., Day, L. 2012. *Impact of boron, calcium and genetic factors on vitamin C, carotenoids, phenolic acids, anthocyanins and antioxidant capacity of carrots (Daucus carota)*. Food Chemistry, 132, 1161–1170.

Effects of Ozone in *Salix atrocinerea* pollen

Bárbara Ventura¹, Helena Ribeiro², Susana Pereira^{1,3} & Ilda Abreu^{1,2}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² Geology Center of the University of Porto, Portugal.

³ BioFIG- Center for Biodiversity, Functional and Integrative Genomics, Portugal.

Pollen is the microgametophyte of seed plants, producing the male gametes. It can be spread by the wind, and influenced by atmospheric pollutants such as ozone (O₃). The tropospheric ozone is generated by photochemical reactions in the presence of NO_x emitted by vehicles and industries. It is one of the most important air pollutants causing severe health effects on humans.

In this study was intended to assess the ozone effects on the viability, germination rate, protein content and allergenicity of *Salix atrocinerea* exposed pollen samples in comparison to non-exposed control ones.

Salix atrocinerea pollen collected directly from the anthers was exposed to O₃ in an environmental chamber during 6 and 24 hours with concentrations equal to the limit value for the protection of human health established by the Directive 2008/50/EC.

After exposure pollen, pollen fertility was determined by means of pollen viability and in vitro germination. Pollen total soluble proteins were quantified and the polypeptide profiles were determined by SDS-gel electrophoresis and the immunoreactivity was assessed by Western blot using patient sera sensitized to pollen.

O₃ exposure decreased significantly the viability, germination rate and total soluble protein content of *Salix atrocinerea* pollen. No changes were observed in the SDS-PAGE protein profiles; however the sera tested revealed increased IgE reactivity to proteins of *Salix atrocinerea* pollen exposed to the pollutant compared with the non-exposed one.

Our study demonstrated that the pollen exposed to O₃, at limit levels for human health protection induced pollen changes that can decrease its fertility and may induce higher IgE reactivity in allergic patients to the pollen of this plant species.

This work was supported by FEDER funds through the COMPETE and National funds through FCT (Ref^o PTDC/AAC-AMB/102796/2008).

Aerobiology of *Populus* and effects of different O₃ levels on its pollen fertility, proteins and allergenicity

Ângela Pires¹, Ilda Abreu^{1,2}, Helena Ribeiro² & Susana Pereira^{1,3}

¹Department of Biology, Faculty of Sciences of the University of Porto, Portugal.

²Geology Centre of the University of Porto, Portugal.

³BioFIG – Center for Biodiversity, Functional and Integrative Genomics, Portugal.

Over the past years, an increase in prevalence and severity of respiratory allergic diseases related with atmospheric pollen has been observed, particularly in urbanized environments. *Populus* spp. is a plant used for ornamental purposes in gardens and parks of the cities in spite of being considered an allergenic pollen producer. The aim of this research was to study the aerobiology and the effects of in vitro exposure of *Populus* pollen to Ozone (O₃).

Airborne pollen monitoring was continuously performed during 2012, using a 7-day Hirst-type volumetric trap set on the roof of the Faculty of Sciences in Porto. Pollen grains were trapped on a Melinex tape coated with silicone oil, which was then cut into daily segments and mounted on slides with glycerol jelly. The daily mean concentration of *Populus* pollen was estimated using an optical microscope (x400) along 4 full lengthwise traverses. Pollen counts were expressed as the sum of the number of pollen per cubic meter of air for a 24-hour period. *Populus* pollen samples, directly collected from the anthers, were exposed to O₃ in an environmental chamber, in which the temperature and relative humidity were controlled and sunlight was simulated. The pollen was fumigated with O₃ for 24h to three different levels: three times, two times and to the hour-limit value acceptable for human health protection in Europe (Directive 2008/50/EC). Its effects on pollen viability, total soluble protein content polypeptide profiles and human sera IgE reactivity were investigated. Pollen viability was determined by FDA, trypan blue and lugol tests and the results compared. Pollen total soluble proteins were quantified colourimetrically and the polypeptide profiles were determined by SDS-gel electrophoresis and the immunoreactivity was assessed by Western blot using patient sera sensitized to pollen.

Populus pollen is mainly present in the atmosphere during March until early April, with the maximum airborne concentration found in the end of March. A decrease in the viability of exposed in contrast to non-exposed pollen was observed. The total soluble proteins of all the pollen samples exposed to O₃ decreased significantly when compared with the non-exposed, however no significant differences were observed among the exposed samples. The polypeptide profiles did not show differences but an increase in IgE reactivity by sera used was observed in exposed samples.

Our study showed an influence of different O₃ concentrations on fertility, total soluble protein content and IgE reactivity of *Populus* pollen.

This work was supported by FEDER funds through the COMPETE and National funds through FCT (Ref^a PTDC/AAC-AMB/102796/2008).

Impact of urbanization level on *Acer negundo* pollen: fertility, protein content and allergenicity

Renata Fernandes¹, Helena Ribeiro², Susana Pereira^{1,3} and Ilda Abreu^{1,2}

¹Department of Biology, Faculty of Sciences of the University of Porto, Portugal.

²Geology Centre of the University of Porto, Portugal.

³BioFIG – Center for Biodiversity, Functional and Integrative Genomics, Portugal.

The aim of this work was to study the fertility, protein profile and allergenicity of *Acer negundo* pollen collected from polluted and non-polluted areas in order to analyze the effects due to the urbanization level.

Pollen grains of *Acer negundo* were collected during its flowering season from a more-polluted urban area, with heavy traffic, and a less-polluted one during 2012 in the city of Oporto.

After separation of extraneous materials, the anthers were dried at 27 °C, gently crushed and the pollen thus released was passed through different grades of sieves to obtain pure pollen. Pollen samples were then stored at –20 °C.

Pollen viability was compared using FDA and Tripan blue tests and germination rate was determined using an optimized medium [1]. The proteins extracted from the *Acer negundo* more-polluted and less-polluted pollen were separated by SDS -PAGE, and the allergenic profiles were analyzed by immunoblotting using sera tree polysensitized patients. The oxidative stress was evaluated through reactive oxygen species (ROS) fluorescent detection.

Acer negundo polluted pollen viability, germination rates and total soluble protein content presented significantly lower values than the pollen from less-polluted area. However a similar SDS-PAGE protein extract profile was observed. Immunoblotting tests showed labelling of various IgE binding components with different densitometric values, some of them corresponding to yet non-characterized allergens. The IgE binding proteins presented greater optical density values in the more-polluted pollen protein extracts. It was also observed an almost 24% increase on ROS in the more-polluted pollen.

Our results indicate that environments with higher atmospheric pollution levels can influence airborne pollen fertility and IgE reactivity. Also, can lead to an increase in the concentration of reactive oxygen species (ROS) and hence oxidative stress, that can be correlated with an increased allergenicity.

These results constitute preliminary data that suggest an interaction between pollution and pollen proteins that somehow may interfere with normal pollen metabolism.

Acknowledgments: This work was supported by FEDER funds through the COMPETE and National funds through FCT (Ref^a PTDC/AAC-AMB/102796/2008).

Reference:

[1] Sousa, R., Duque, L., Duarte, A.J., Gomes, C.R., Ribeiro, H., Cruz, A., Silva, J.C.G.E. and Abreu, I. (2012), *In vitro exposure of Acer negundo pollen to atmospheric levels of SO₂ and NO₂: Effects on allergenicity and germination*. Environmental Science and Technology, 46 (4), 2406–2412.

Interaction between metallic NPs and the salt marsh plant *Halimione portulacoides*

F. Andreotti^{1,2,3}, Ana P. Mucha², P. Rodrigues², C. Marisa R. Almeida²

¹ Department of Agriculture and Environment Sciences, Faculty of Agriculture University of Milan, Italy.

² CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal.

³ Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal.

The nanomaterials discharged into the environment find their way through waste disposal routes, with estuaries and the near costal shore eventually being their final repository. The impact of nanomaterials on the health and on the environment must be understood in detailed. Health and environmental effects of common metals are well-known, however, when metals take the form of nanoparticles (NPs) consequential hazards based on shape and size are yet to be explored. Although a lot of information exists regarding plants interaction with metals there is a lack of information among metallic NPs interaction with plants [1], including with salt marsh plants.

This work aims to study the interactions among a Cu metallic NPs (CuO) and the salt marsh plant *Halimione portulacoides* and compare those interactions with those observed when the selected metals are in their ionic form. In addition, it aims to evaluate the potential of these plants for the phytoremediation of these nanomaterials. The chosen plant has shown to uptake considerable amount of Cu when in its ionic form [2].

Experiments were conducted with sediment elutriate, a simplified natural medium, being the plant expose for 1 week to medium contaminated either with CuO or with Cu (II). To evaluate metal uptake, total metal concentrations were determined by atomic absorption spectroscopy in plant tissues at the beginning and at the end of the experiments.

Results indicate that *H. portulacoides* had the ability to accumulate Cu in its roots, being the accumulation 10 times lower when the metal was in the nanoparticle form, a fact that need to be taken in consideration when applying this plant for the phytoremediation of contaminated sediments.

References:

[1] Ma X, Geiser-Lee J, Deng Y, Kolmakov A. (2010) Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. *Sci. Total Environ.* 408: 3053-3061

[2] CMR Almeida, A.C. Dias, A.P. Mucha, A.A. Bordalo, M.T.S.D. Vasconcelos (2009), Study of the influence of different organic pollutants on Cu accumulation by *H. portulacoides*. *Estuarine Coastal and Shelf Science*, 85: 627-632

ACKNOWLEDGMENTS: Research partially supported by the European Regional Development Fund (ERDF) through COMPETE - Operational Competitiveness Program and national funds through FCT, under PEst-C/MAR/LA0015/2013 and REEQ/304/QUI/2005 and ECORISK (reference NORTE-07-0124-FEDER-000055) within the SR&TD Integrated Program MARVALOR funded by Programa Operacional Regional do Norte (ON.2 – O Novo Norte) and by ERDF.

Reactivity of green zero-valent iron nanoparticles produced using citrus wastes

Y. Pereira, A.F. Rodrigues, I.M. Gonçalves, S. Machado, J.P. Grosso, H.P.A. Nouws, J.T. Albergaria, C. Delerue-Matos, A. Fiúza.

REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal. Email jtva@isep.ipp.pt.

Nanotechnologies applied to the environmental remediation are growing interest on the scientific and technical community because of their low costs and high overall efficiencies. These technologies involve the utilization of reactive nanoscale materials for the transformation and degradation of pollutants through chemical reduction or catalytic processes [1]. Recently, a new and green method for the production of zero-valent iron nanoparticles (nZVIs) has been proposed by Hoag et al. [2] and Machado et al. [3]. This production method consists of the utilization of natural products that have high polyphenol contents as well as high antioxidant capacity, which react with iron (III) solutions to produce nZVI. This green production method avoids the use of traditional methods which are expensive and include toxic compounds, such as sodium borohydride. Parameters such as size, agglomeration tendency and reactivity should be evaluated. Among these characteristics, reactivity is one of the most important properties because it determines the capacity of these particles to act as a remediation agent and their efficiency to remove/degrade pollutants.

In this work, the study of the reactivity of the green nZVI produced using citrus wastes such as orange, mandarin, limon and lime is presented. Through this, it was expected to produce a remediation agent (added value product) valorizing a common waste of the juice industry. Hexavalent chromium, a common contaminant in soil and water and often treated with zero valent iron, was selected as the target compound. The results showed that the mandarine wastes showed to be the most reactive namely the peel and the albedo parts.

Acknowledgements

This work has been supported by Universidade do Porto through the project PP_IJUP2011_264 and also by the Fundação para a Ciência e a Tecnologia through the projects PEst-C/EQB/LA0006/2013 and PTDC/AAG-TEC/2692/2012. J.G. Pacheco also thanks the Fundação para a Ciência e a Tecnologia for financial support through his pos-doc grant SFRH/BPD/73943/2010.

References:

- [1] Karn B., Kuiken T., Otto M. (2009), *Nanotechnology and situ Remediation: A review of the benefits and potential risks*, Environmental Health Perspectives, 117, 1823-1831.
- [2] Hoag G.E., Collins J.B., Holcomb J.L., Hoag J.R., Nadagouda M.N., Varma R.S. (2009), *Degradation of bromothymol blue by 'greener' nano-scale zero-valent iron synthesized using tea polyphenols*, Journal of Materials Chemistry, 19, 8671-8677.
- [3] Machado S., Pinto S.L., Grosso J.P., Nouws H.P.A., Albergaria J.T., Delerue-Matos C. (2013), *Green production of zero-valent iron nanoparticles using tree leaf extracts*, Science of the Total Environment, 445, 1-8.

N-doped TiO₂ photocatalytic activity towards diphenhydramine oxidation and *E. coli* inactivation

R. Monteiro¹, S. Miranda^{1,2,4}, M. Silva⁴, V. Vilar¹, L. Pastrana-Martínez², P. Tavares³, R. Boaventura¹, J. Faria², E. Pinto⁴ and A. Silva²

¹LSRE - Laboratory of Separation and Reaction Engineering

²LCM - Laboratory of Catalysis and Materials

Associate Laboratory LSRE/LCM, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

³CQVR Centro de Química – Vila Real, Departamento de Química, Universidade de Trás-os-Montes e Alto Douro, 5001-911 Vila Real, Portugal

⁴CEQUIMED-UP, Microbiology Service, Biological Sciences Department, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, N° 228, 4050-313 Porto, Portugal

TiO₂ is a semiconductor with unique optoelectronic and physiochemical properties [1]. Nowadays, lot of attention has been paid towards the shifting of the TiO₂ absorption spectrum into the visible light range [2]. TiO₂ modified with nitrogen is one of the most investigated photocatalysts in the field of visible light photocatalysis [3].

Within this work, N-TiO₂ samples with different N:Ti ratios were prepared by manual grinding the TiO₂ photocatalyst (P25, Evonik Degussa Corporation) with urea and calcined at 380 °C. Multiple techniques were carried out to obtain information about the crystallite structure and chemical binding of N-TiO₂. The photoactivity of the prepared materials was evaluated using diphenhydramine hydrochloride pharmaceutical (DP) under visible light and *E. coli* under UVA light.

The prepared materials showed visible-light absorption and no changes on the crystallite structure of TiO₂ were detected. The analysis made on the chemical states of N_{0.50}P25-380 revealed that the nitrogen is located at an interstitial site of the TiO₂ structure. N_{0.50}P25-380 presented the highest photoactivity towards DP degradation under VIS light and *E. coli* inactivation under UVA light.

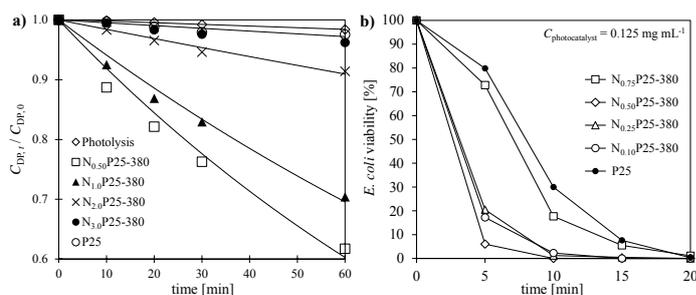


Fig. 1 a) Photodegradation of DP under VIS light; b) *E. coli* viability under UVA light.

[1] Fujishima, A., Hashimoto, K. and Watanabe, T. (1999), *TiO₂ Photocatalysis: Fundamentals and Applications*, Bkc, Incorporated, Tokyo.

[2] Takeuchi, K., Nakamura, I., Matsumoto, O., Sugihara, S., Ando, M. and Ihara, T. (2000), *Preparation of Visible-Light-Responsive Titanium Oxide Photocatalysts by Plasma Treatment*, Chemistry Letters, 29, 1354.

[3] Asahi, R., Morikawa, T., Ohwaki, T., Aoki, K., and Taga, Y. (2001), *Visible-light photocatalysis in nitrogen-doped titanium oxides*, Science, 293, 269.

Photocatalytic disinfection of bioaerosols generated from wastewater treatment plants (WWTPs) (Projeto 183)

M. Silva^{1,2}, A. Almeida^{1,2}, S. Miranda^{2,4}, J. Teixeira^{1,2}, V. Vilar⁴, A. Silva⁴, J. Faria⁴, D. Gonçalves^{2,5}, P. Cecílio^{2,5}, H. Ferreira^{2,5}, E. Pinto^{1,2,3}

¹CEQUIMED/ ²laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia/ ³Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR)/

⁴Laboratório Associado LSRE-LCM, Departamento de Engenharia Química, Faculdade de Engenharia. Universidade do Porto, Portugal/ ⁵REQUIMTE

Indoor air pollution by microbial contaminants, particularly antimicrobial resistant microorganisms, is increasingly receiving attention as a public health problem. WWTPs are important sources of bioaerosols that can constitute a serious health risk to plant workers and to the population of the surrounding area. In this context, will be urgent to develop cost-effective means to eliminate pathogenic microorganisms. One of the most promising antimicrobial technologies is the photocatalytic oxidation, being “clean”, low-cost and effective in the inactivation of bacteria, fungi and virus. The main goal of this work was to develop a photocatalytic system for the decontamination of indoor air containing microorganisms, which included the following steps:

- 1) Characterization of indoor airborne microorganisms from a WWTP: Air samples were collected by impaction (MAS 100-*Merck*) in different media to evaluate total bacteria and fungi, Gram-positive and Gram-negative bacteria, and *Aspergillus fumigatus*. The total cultivable bacteria was found to vary from 60 to >52560 CFU/m³ and the total cultivable fungi concentration ranged from 369 to 14068 CFU/m³.
- 2) Identification of the representative microorganisms isolated: Gram-positive (*Bacillus*, *Corynebacterium*, *Arcanobacterium*, and *Staphylococcus* species) were observed in higher number than Gram-negative (*Enterobacter*, *Klebsiella*, *Serratia*, *Raoultella*, *Escherichia*, *Citrobacter*, *Proteus*, *Acinetobacter*, *Morganella*, *Ochrobactrum*, and *Pseudomonas* species). For fungi, the most representative genera were *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria*. *A. fumigatus* (19 CFU/m³), *A. niger*, *A. lentulus*, *A. terreus*, *A. flavus*, *A. versicolor* were also identified.
- 3) Evaluation of antimicrobial susceptibility: *Enterobacteriaceae* that exhibited multidrug-resistance (GMDR), from 3 to 10 antimicrobial categories, were isolated. The presence of genes encoding different beta-lactamase types, in ESBL producing and carbapenem-resistant *Enterobacteriaceae*, was investigated by PCR. ESBL-positive *E. coli* isolates showed *bla*_{CTX-M} and *bla*_{OXA} genes.
- 4) Evaluate the sensitivity of microorganisms to the photocatalytic treatment in liquid phase: For *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *S. saprophyticus**, *A. niger*, *A. fumigatus*, *Penicillium* spp.*, *Cladosporium* spp.*^{isolated from the WWTP}, it was demonstrated the possibility of liquid phase decontamination using the systems UV/P25 and UV/ZnO. P25 was the most efficient catalyst in the microorganisms inactivation.
- 5) A photoreactor prototype using UV lamps was developed and used for the gas-phase inactivation studies using P25 as photocatalyst and *P. aeruginosa* and *S. aureus* were chosen as model microorganisms.

Acknowledgments: U.Porto /Santander Totta “Projetos Pluridisciplinares 2011”.

Photodegradation of veterinary antibiotics in culture media when exposed to artificial light

S. Andrade¹, M.C.P. Basto^{2,3} and M.S. Baptista²

¹ Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

² CIIMAR/CIMAR- Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal.

³ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Antibiotics are one of the most important groups of pharmaceuticals due to their broad applications, including human and veterinary medicine. A significant amount of antibiotics reaches the aquatic environment, mainly due to direct discharge of wastewater after incomplete metabolism in humans and animals.

Once present in the aquatic environment, they may interact with existing organisms. Therefore, the aim of this study was to determine whether veterinary antibiotics suffer photodegradation when exposed to light, in aquatic ecosystems.

Assays were performed in erlenmeyer flasks containing 150 mL Z8 medium doped individually with minocycline, oxytetracycline, tetracycline, enrofloxacin and ceftiofur. Collections were performed at 0 h, 1 h, 3 h, 7 h, 28 h, 56 h and 240 h of exposure to light. Three concentrations of each antibiotic were tested in triplicate: 0.1; 1 and 10 mg/L. Controls, *i.e.* antibiotics not exposed to the light, were run in parallel.

The analytical procedure [1] was performed using a HPLC Beckman Coulter equipment provided with a DAD detector (module 168) and an automatic sampler (module 508). The analytes were separated on a 100 mm x 4.6 mm 2.6 μ m C18 Kinetex™ column and the detector signal was monitored at $\lambda=280$ nm. The flow gradient was set at 1mL/min and the sample injection volume was 50 μ L. When the concentration of antibiotics was too low a pre-concentration step of solid phase extraction (SPE) was carried out with Oasis HLB cartridges (60 mg, 3cc).

The results indicate that the veterinary antibiotics, in general, suffer photodegradation, this being more evident for tetracyclines, at all concentrations tested. However, for the 10 mg/L concentration of ceftiofur and enrofloxacin there was no photodegradation.

This work supports the evidence that in toxicity bioassays the photodegradation of antibiotics should be considered.

References:

[1] Cavenati S., Carvalho P.N., Almeida C.M.R., Basto M.C.P. and Vasconcelos M.T.S.D. (2012), *Simultaneous determination of several veterinary pharmaceuticals in effluents from urban, livestock and slaughterhouse wastewater treatment plants using a simple chromatographic method*, Water Science & Technology, 66 (3), 603-611.

Characterization, treatment proposal and metal recovery in *Active Implantable Medical Devices*

R. Guimarães¹, J. Carvalho¹, V. Leal¹ and A. Guerner Dias¹

¹Department of Geosciences, Environment and Land Management, Faculty of Sciences, University of Porto, Portugal.

Population growth, more pronounced every day, leads to the exponential rise in consumerism, generating many problems in relation to treatment and final destination for the waste produced. With the improvement of life conditions, more waste is generated, particularly hospital waste, turning to several legal and social issues, in relation to environmental effective treatment solutions.

The hospital waste is divided in four groups^[1], in which groups I and II obey in an efficient way the current waste management hierarchy, while groups III and IV, after a previously treatment, are sent for incineration or landfilled deposition.

The active implantable medical devices (AIMD), after explantation, belonging to group III and classified in chapter 18 of the European Waste Catalogue^[2] (EWC), and according to EWC, it doesn't has a specific waste flow. Therefore arises the necessity to identify the components presents in AIMD, in particular, the metals components, with the aim of development an efficient process for treatment of AIMD's waste.

Were collected AIMD's, previously autoclaved, in hospitals in the north of Portugal, subsequently sent to the laboratories on the Faculty of Science where they were identified and weighed. The devices were dismantled into three principal components: i) Exterior case and electrodes; ii) Printed Circuit Boards (PCB); iii) Batteries. These components were identified and weighed individually, to be analyzed by Scanning Electron Microscopy (SEM).

The results, even considering that the analytic methods had focused in particular components, showed that AIMD's are constituted by a relevant quantity of metals with high economic value, such as, titanium, gold and silver, among other metals with lowest economic value.

The preliminary results allow us to consider that these devices have conditions to be recovered, with particularly incidence in the recovery of the precious metals presents. The investigation, realized in pilot scale until now, continues with the purpose to determinate the advantages of the development a methodology than can be applicable on a larger scale.

References:

[1] PERH – Plano Estratégico dos Resíduos Hospitalares (2011-2016). Agência Portuguesa do Ambiente, Direção Geral da Saúde e Direção Geral de Veterinária. URL: www.apambiente.pt/_cms/view/page_doc.php?id=47

[2] Ministério da Economia, da Agricultura, desenvolvimento Rural e Pescas, da Saúde e das Cidades, Ordenamento do Território e Ambiente, 2004. Portaria n.º 209/2004, de 3 de março. In: Diário da República – I Série – A. 19:1188-1206.

Magnetic susceptibility in contaminated soils by mineral extraction of São Pedro da Cova

Paula Cristina Santos¹

¹ Department of Geosciences, Environment and Spatial Territórios da the Faculty of Sciences of University of Porto, Portugal.

Environmental Magnetism has been recognized as a distinct scientific discipline with applications in several disciplines, for providing important data for studies of global environmental change, climate processes data as well as the anthropic impact on the environment, (Evans & Heller, 2003). The magnetic measurements of the high sensitivity allows the detection of small amounts of magnetic material (Dekkers, 1997). This scientific study is located in north of mainland in Portugal, specifically in São Pedro da Cova, parish belonging to the municipality of Gondomar, of Porto District. Is based on the application of environmental magnetism techniques for magnetic characterization of the samples in order to find possible relationships between extinct mine coal activity (anthracite), the effluents originated inside of the mine and implications on environmental liability, specifically at Ribeiro de Murta and the surrounding land. In the analysis there are the three galleries surrounding soil drainage defunct mining operation, all in an abandonment state.

It were collected a total of 21 samples upstream and downstream of the three galleries of the extinct mine into two different periods: a first group collected at 6 of July, 2012, classified as A1 to A8, and a second group collected at 30 of January 2013, classified as C1 to C6, M1, T1, T2, T3, S2 and S6. The samples were previously prepared in the pedology laboratory of the Department of Geosciences, of Porto University. After being dried, weighted, and divided into three size fractions: <0,5 mm, 0.5 mm to 2 mm, > 2 mm. Each fraction was divided into two samples A and B. Subsequently, the samples were subjected to the measurement of the magnetic properties, in three trials, on the device KAPPABRIGE KLY - 4S brand AGICO with software support SUMEAN of Lab Center of Geology, Porto University. Parameters pH and electrical conductivity were also measured. The MS was expressed in units of mass ($\times 10^{-8} \text{m}^3/\text{kg}$). From the results obtained it was found that: higher concentrations are located in the near of the third gallery; the C samples collected in agricultural fields irrigated directly from Ribeiro de Murta, recorded higher magnetic susceptibility values than the samples in non-agricultural soils; the analysis of the magnetic susceptibility by size fraction generally indicated that the thinner fraction has the highest values; there was not recorded no negative magnetic susceptibility which indicates the absence of diamagnetic particles; the data suggest that the studied soils have ferromagnetic particles from the effluent of the coal mine of São Pedro da Cova and this is affecting the environment of the surrounding areas.

References:

- [1] Dekkers, M.J. (1997). Environmental magnetism. *Geologie en Mijnbouw* 76, 163-182.
- [2] Evans, M.E. & Heller, F. (2003). *Environmental Magnetism. Principles and Applications of Enviromagnetics*. Academic Press, Elsevier. 299 pp.

Economic Growth in Cape Verde and its Impact on Environmental Sustainability

Isa Dias Gomes ¹

¹ Faculty of Economics, University of Porto, Portugal.

For economic growth to be effective, one must likewise register real growth in the remaining aspects that sustain growth itself. Therefore, it is increasingly important to introduce environmental and social variables when it comes to effective analysis of economic growth. One can only speak about sustainability on an economic growth level if resources are properly and effectively rationalized, but it is also very important to consider the reuse of resources, particularly those which are scarce or when they pose serious risks to the environment but also for the economy, among others.

Cape Verde (CV) is one of the few Africa's countries, and unique in its sub-region, which has met with project objectives proposed by Kyoto Protocol, the Framework Convention on Climate Change and the Millennium Development Goals (MDG), and other programs whose goals aim at essentially regulating the emission of carbon dioxide (CO₂). These achievements have allowed this country to conduct its development policies, buoyed in contexts of globally accepted best practices. Infrastructure investments with high added value in capturing and exploring green energies have been channeled.

This dissertation aims to: identify possible environmental impacts in Cape Verde which could follow economic growth and development; suggest improvements at a sustainability level.

The method used was the contingent evaluation through technical willingness to pay for environmental valuation, whose aim is to evaluate the behaviour of Cape-verdean residents and tourists in preserving environmental systems in Cape Verde.

It is concluded, according to our survey, that the Cape-verdeans are willing to contribute financially to environmental protection in Cape Verde.

References:

- [1] Bürgenmeier, B. (2005), *Economia do Desenvolvimento Sustentável*, Lisboa: Instituto Piaget.
- [2] Garrod, G., & Willis, K. G. (1999), *Economic Valuation of the Environment: Methods and Case Studies*, Massachusetts, USA: Edward Elgar Publishing, Inc.
- [3] Ministério do Ambiente, Agricultura e Pescas (2004), *Segundo Plano de Acção Nacional para o Ambiente*, <http://www.governo.cv/documents/PANAII-sintese-final.pdf>, acedido em 04 de Março de 2013.
- [4] Ramos, M. C. P. (2012), Ambiente, Educação e Interculturalidade, *Revista Tempos e Espaços em Educação*, n. 8 Janeiro/Julho, p.27-39, Universidade Federal de Sergipe.

Sustainability assessment in agrosystems, combining terrestrial fauna simple information.

J. Neto¹, P. Santos^{1,2}, and J. Guedes²

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² Ecoinside® - soluções em ecoeficiência e sustentabilidade, Lda.

The agro systems' sustainability concept approaches three strands: economic, social and environmental [1]. In the environmental context, the rural space is considered an important habitat of an important diversity of fauna, so it's essential to maintain the sustainability of this ecosystem [2]. To evaluate this sustainability it becomes indispensable an assessment of the farms through an appropriate methodology. The literature suggests the use of two integrated methods: *sustainability indicators* and *assessment models* [1]. The main goal of this study is to find a simple methodology that allows identifying positive or negative aspects of the productive systems, and in the other hand, allows understanding the level of sustainability of the different farms.

For that three Portuguese regions were chosen with different types of crops and productive systems: strawberry, apple, lettuce and courgette. The richness of the following groups was measured: mammals, birds, amphibian and reptiles. The species data was collected using transects and listening spots on the field and using bibliographic information. The indicators were chosen based on the literature and taking into account the characteristics of the farms [3]. Finally all the data was analysed and the indicators for the species richness were calculated. In this early phase of the study we used species richness indicators, which were the percentage of occurrence for each one of the taxa above indicated and the *Margalef Index* (DMg) modified, which uses the farm area. To obtain a new indicator characterising the farms all values of the species richness indicators were standardised into a 1-5 scale and then the unweighed mean was obtained for each farm.

Our preliminary results show that regardless of the production type all of the farms present very close values, varying from 1,8 to 2,6. The results point that the productive system isn't the most influential factor and our best guess is that the influence of the surrounding habitat diversity and land cover should be incorporated in deeper analysis which is in accordance to other studies [4].

References:

- [1] Costa A. (2012), *Agricultura sustentável II: Avaliação*, Revista de Ciências Agrárias, Universidade de Trás-os-Montes e Alto Douro.
- [2] Comissão Europeia (2012), *Agricultura e Desenvolvimento Rural*. Política Agrícola comum: uma parceria entre a Europa e os Agricultores.
- [3] Bélanger V., Vanasse A., Parent D., Allard G., Pellerin D. (2012), *Development of agri-environmental indicators, to assess dairy farm sustainability in Quebec, Eastern Canada*. Ecological Indicator 23, 421- 430.
- [4] Atauri J. A., Lucio J. V. (2001), *The role of landscape structure in species richness distribution of birds, amphibians, reptiles and lepidopterans in Mediterranean landscapes*, Landscape ecology 16, Kluwer Academic Publishers, Netherlands, pp.147-159.

Dispersion of Carbon Nanotubes by Ionic Surfactants

Bárbara Abreu¹, Bárbara Claro¹, Ricardo M.F. Fernandes^{1,2}, Eduardo F. Marques¹, István Furó²
and Oren Regev³

¹Centro de Investigação em Química, Dept. Chemistry and Biochemistry, University of Porto, Portugal.

²Div. of Applied Physical Chemistry, Dept. Chem., KTH Royal Inst. of Technology, Stockholm, Sweden.

³Dept. Chem. Eng. and Ilse Katz Inst. for Nanotech., Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel.

Carbon nanotubes (CNTs) have several mechanical, electrical and thermal properties that in combination make them unique and very promising materials in many technical applications, such as in molecular sensing, electronic circuitry and drug delivery. CNTs have strong tendency to aggregate due to inter-tube van der Waals attractions. For the vast majority of the applications, however, individual dispersion and alignment of CNTs is needed.

This work aimed at a comparative study of the effect of different ionic surfactants in the dispersion efficiency of carbon nanotubes in aqueous solution, using ultrasonication as the dispersion method. The ultrasonic acoustic vibration causes exfoliation and breakdown of CNTs, favoring the adsorption of the surfactant hydrophobic chains onto the surface of the nanotube [1, 2]. In addition, the steric and/or electrostatic repulsions promoted by the polar groups of the adsorbed surfactant facilitate CNT solubility and stability in the colloidal aqueous carbon dispersion [3, 4].

Herein, the mass concentration of dispersed CNTs was determined by UV-Vis absorption spectroscopy. The critical micelle concentration (*cmc*) of the surfactants in neat water was previously determined by conductometry. Single and multi-walled CNTs were used, whereas the surfactants comprised anionic (SDBS and SDS) and cationic ones (DTAB, TTAB, CTAB and CPyCl). The CNT dispersions were prepared as a function of surfactant concentration under experimental conditions strictly controlled and reproducible. It was found that for a given surfactant the concentration has a significant effect on dispersing ability, and that the *cmc* (and hence the presence of micelles in solution) has a key role in the process [3]. Moreover, it was observed that the degree of dispersion is strongly dependent on the molecular structure of the surfactant (for instance, the presence of aromatic groups, the length and volume of the polar group the hydrocarbon chain). This study thus highlights the importance of a careful selection of the surfactant as a dispersing agent for CNTs.

References:

- [1] Britz, D.A. and Khlobystov, A.N. (2006), *Noncovalent interactions of molecules with single walled carbon nanotubes*, Chem. Soc. Rev.,35, 637–659.
- [2] Vaisman, L., Wagner, H.D. and Marom, G. (2008), *The role of surfactants in dispersion of carbon nanotubes*, Adv. Colloid Interface Sci.,128, 37– 46.
- [3] Marques, E.F. and Silva, B.F.B. (2013), *Surfactant Self-Assembly*, in Tadros, T. (ed.) " Encyclopedia of Colloid and Interface Science", Springer Berlin Heidelberg, pp. 1202–1241.
- [4] Fernandes, R.M.F., Buzalo, M., Shtein, M., Pri-Bar, I., Regev, O., Marques, E.F., and Furó, I. (2014), *Lateral Diffusion of Dispersing Molecules on Nanotubes As Probed by NMR*, J. Phys. Chem. C, 118, 582–589.

Development of a method for simultaneous determination of drugs in natural waters by SPE-UPLC-MS

L. C. Ribeiro¹, A. D. Guimarães², P. Almeida¹ and M. F. Alpendurada²

¹ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

² IAREN- Instituto da Água da Região Norte.

Emerging pollutants may be defined as compounds not included in European monitoring programs, but that may be candidates for future regulation, depending on the toxicity testing results, potential effects on human health and occurrence in environmental. The drugs, like anti-inflammatories, antibiotics, beta-blockers, analgesics, antidepressants and diuretics, are examples of emerging pollutants.

The emerging pollutants reach the environment through direct discharges of waste, or as part of the wastewater, sewage hospitals or breeding. The first stage of processing can begin in living organisms, yielding metabolites. The starting compounds and their metabolites will suffer environmental changes through biotic and abiotic processes.

These compounds are not mineralized in conventional WWTPs and so the effluent discharged daily into waterways has a higher concentration of pollutants. At this level, it is not known the harmful effects on ecosystems and in man.

In this work, it is developed a method for simultaneous determination of drugs (17 α -etinilestradiol, 17 β -estradiol, estrone, progesterone, desogestrel, paracetamol, ciprofloxacin, azithromycin, bisoprolol, furosemide, indapamide, paroxetin, fluoxetin, nimesulide, ketoprofen, naproxen, diclofenac, ibuprofen, gemfibrozil, simvastatin), using solid-phase extraction and ultra performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (SPE-UPLC-MS).

First, the experimental work was based on the optimization of separation of the compounds studied, using UPLC-MS. Standard solutions of the compounds, individually or in a mixture, were used and acetonitrile/water and methanol/water were tested as mobile phases, in order to improve the separation.

The next step will be testing different cartridges and choose the one that has the best recovery rates, to apply in solid-phase extraction (SPE) of natural waters. After that, this method will be applied for determination of those compounds in natural waters, like Leça or Douro river waters.

Comparison of different methods of extraction to quantify vitamin E in rainbow trout (*Oncorhynchus mykiss*) muscle

M. Araújo^{1,2}, R. C. Alves³, F. Pimentel¹, T. Fernandes¹, L. M. P. Valente², M. B. P. P. Oliveira¹

^{1,2} REQUIMTE, Dep. of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² CIIMAR/CIMAR L.A.- Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal.

³ REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal

Vitamin E is a lipid soluble vitamin and is divided in two groups: tocopherols and tocotrienols. Among the four classes of vitamers (α , β , γ and δ -tocopherol and tocotrienols), α -tocopherol has the highest antioxidant activity [1]. In fish, α -tocopherol is the main vitamer found [2] and its deposition in fillet depends on sources rich in tocopherols present in the environment. In aquaculture, synthetic tocopherols or vegetable ingredients are commonly used in aquafeeds as source of vitamin E [3]. Due to its antioxidant properties, α -tocopherol assumes great importance in finfish products, minimizing lipid oxidation during storage [4].

In this work, we tested five different methods of extraction to quantify vitamin E in the muscle of rainbow trout (*Oncorhynchus mykiss*), raised in aquaculture: Soxhlet, a modified Folch procedure, solid-liquid extraction with methanol or *n*-hexane, and saponification (followed by an organic solvent extraction). The final extracts were analyzed by normal phase-HPLC with fluorescence detection.

The results obtained from the different methodologies tested were compared in order to evaluate which one was more effective to extract tocopherols and tocotrienols from rainbow trout muscle.

Acknowledgments: R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

References:

- [1] Schneider, C. (2005), *Chemistry and biology of vitamin E*, Molecular Nutrition and Food Research, 49 (1), 7-30.
- [2] Syvaolja, E. L. and Salminen, K. (1985), *Tocopherols and tocotrienols in Finnish foods: Fish and fish products*, Journal of American Oil Chemist Society, 62 (8), 1245-1248.
- [3] Chen, Yi-Chen, Nguyen, J., Semmens, K., Beamer, S., Jaczynski, J. (2008), *Effects of dietary alpha-tocopheryl acetate on lipid oxidation and alpha-tocopherol content of novel omega-3-enhanced farmed rainbow trout (Oncorhynchus mykiss) fillets*, LWT - Food Science and Technology, 41 (2), 244-253.
- [4] Kamireddy, N., Jittinandana, S., Kenney, P. B., Slider, S. D., Kiser, R. A., Mazik, P. M. and Hankins, J. A. (2011), *Effect of dietary vitamin E supplementation and refrigerated storage on quality of rainbow trout fillets*, Journal of Food Science, 76 (4), 1750-3841.

Performance of monounsaturated oils during potatoes deep-frying

J. F. Martins¹, C.S.P. Santos¹, L. Molina García², R. Cruz¹, S. Cunha¹, S. Casal¹

¹ REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Spain.

Deep-frying comprises one of the oldest and most commonly used techniques of domestic cooking, playing an important role in industrial processing of potato products (chips and crisps). It enhances the sensory quality of potatoes by the formation of flavor compounds, as by the improvement of texture and appearance, being all these factors highly appreciated by consumers. However, the use of high processing temperatures, associated to prolonged time, causes chemical degradation of frying oils, in particular by oxidation, hydrolysis and polymerization.

The purpose of the present work was to assess the behavior of monounsaturated oils, namely extra virgin olive oil (VOO), peanut oil, and canola oil during potatoes deep-frying, evaluating its degradation through the increment of total polar compounds, polar compounds fractions, and anisidine value. Therefore, potato samples were deep-fried at 175°C, for 8h/day during three consecutive days. Total polar compounds were estimated by Food Oil Sensor and latter quantified by high-performance size-exclusion chromatography, distinguishing the main degradation fraction: free fatty acids, diacylglycerols, oxidized triacylglycerols and polymerized triacylglycerol [1]. The anisidine value, an indirect method to evaluate lipids oxidation, was performed according to ISO 6885:2006 [2].

Despite being all recommended for frying, VOO revealed a better performance under real frying conditions. Strong positive and statistically significant *Pearson's* correlations with frying time were observed for all monounsaturated oils but, except for free fatty acids, their increment was always less marked for VOO than other oils. Except for polymerized triacylglycerol, characterized by an exponential increase, all components analyzed described a linear increase with frying time. The anisidine values had a strong positive *Pearson's* correlation with oxidized triacylglycerols, as expected. The presence of higher amounts of several natural substances in VOO, namely phenolic compounds, due to the absence of refining, might be related to the increased resistance to oxidation as observed in the current work. Other chemical and sensorial studies are needed to support these observations.

References:

[1] Márquez-Ruiz, G., Jorge, N., Martín-Polvillo, M. and Dobarganes, M.C. (1996), *Journal of Chromatography A*, 749, 55-60.

[2] International Organization for Standardization, *Animal and vegetables fats and oils – determination of anisidine value*, 3th ed, ISO, Switzerland, 2006, ISO 6885:2006(E).

Antioxidant potential of *Asteraceae* species with botanical relevance in Portugal

Sílvia Bessada¹, João C.M. Barreira^{1,2}, M. Beatriz P.P. Oliveira¹

¹ REQUIMTE/Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal.

²Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, *Campus* de Santa Apolónia, Apartado 1172, 5301-854 Bragança, Portugal.

Oxidative stress is involved in the etiology of several diseases and metabolic disorders, being reasonable to expect that antioxidant compounds might have beneficial effects in health maintenance or disease prevention [1]. Antioxidant compounds might be isolated and characterized from different plant parts like roots, stem, bark, leaves, flowers, fruits and seeds [2].

The *Asteraceae* family has global geographical dimension, with special relevance in the Mediterranean, Eastern Europe and Asia Minor, being acknowledge about 25,000 species integrated in approximately 1,000 genera. In addition to the anti-inflammatory, analgesic and antipyretic potential of some of these species, its high antioxidant power, as proven in research works with extracts of roots, stems, bark, leaves, flowers, fruits and seeds [3], should be highlighted. Herein, a bibliographic screening was performed to identify the *Asteraceae* species with highest potential as sources of natural antioxidants with application in medicine and in pharmaceutical, cosmetic and food industries. The species were selected based on their botanical representativeness in the Portuguese territory, being identified the 9 most relevant species: *Achillea millefolium* L. (*milefólio* or *erva-carpinteira*), *Acmella oleraceae* Murr. (*pimenta d'água*), *Artemisia absinthium* L. (*absinto*), *Bidens pilosa* L. (*agulha espanhola* or *picão preto*), *Carthamus tinctorius* L. (*açafrão-bastardo*), *Inula crithmoides* L., *Otanthus maritimus* L. (*atanásia marítima*), *Matricaria recutita* L. (*camomila*) e *Parthenium hysterophorus* L. (*coentro do mato*).

With the obtained results, it could be concluded that the antioxidant activity of most Portuguese species of *Asteraceae* family still continues to be characterized, constituting a research scope with great potential. However, among species with scientific evidence of antioxidant activity, *A. millefolium* and *M. recutita* may be highlighted as those with the highest potential to be exploited in the development of dietary supplements, bioactive food or pharmaceutical base products with dermocosmetic or medicinal application.

References:

- [1] Niki, E. (2010), *Assessment of antioxidant capacity in vitro and in vivo*. Free Radical Biology & Medicine, 49, 503-515.
- [2] Krishnaiah, D., Rosalam, S., Rajesh, N. (2011). *A review of the antioxidant potential of medicinal plant species*. Food and Bioproducts Processing, 89, 217-233.
- [3] Chetan, J., Kumara, S., Sekhar, S., Prakash H.S. (2012). *Antioxidant, antibacterial and DNA protecting activity of selected medicinally importante Asteraceae plants*. International Journal of Pharmacy and Pharmaceutical Sciences, 4, 257-261.

Cyclodextrin-encapsulated herbicides: A promising technology

A. Cerqueira^{1,2}, **S. Benfeito**^{1,2}, **J. Garrido**^{1,2}, **F. Borges**² and **E.M. Garrido**^{1,2}

¹ Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic of Porto, Portugal.

² Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Some of the major challenges faced nowadays by world agriculture include changing climate, sustainable use of natural resources and environmental issues like runoff and accumulation of pesticides. These problems are further intensified by an alarming increase in food demand that will be needed to feed an estimated population of 9 billion by 2050 [1]. Thus, scientific research has been intense in pesticides area either with the goal of increasing the efficacy and reducing the non-target effects, or for reducing their environmental risks [2,3]. Many technologies have been developed and proposed to increase farm productivity and also reduce the environmental and resource costs related with agricultural production. Among them, controlled release technology promises to improve the effectiveness and to solve environmental problems associated with existing herbicides. Microencapsulation is considered one of the most appealing industrial process for the production of controlled release agricultural formulations.

Cyclodextrins (CDs) are a group of naturally occurring cyclic oligosaccharides derived from starch, with six, seven or eight glucose residues linked by α (1–4) glycosidic bonds in a cylinder-shaped structure. The CDs structural characteristics, a hydrophobic interior cavity and hydrophilic shell, and their ability to alter the physical, chemical, and biological properties of guest molecules have been regarded as a solution to improve herbicide formulations.

This work was aimed to study the inclusion effect of cyclodextrins on the chemical and photochemical properties of several herbicides. Ultraviolet spectroscopy and nuclear magnetic resonance (NMR) studies have been carried out to elucidate the strength and binding mode of association of the complex. The influence of microencapsulation of herbicides by cyclodextrins on its rate of photodegradation was also assessed. The results obtained will be presented and discussed in this communication.

Acknowledgements:

Financial support from Fundação para a Ciência e Tecnologia FCT/MCTES project PTDC/AGRAAM/105044/2008, National Funds PIDDAC also cofinanced by the European Community Fund FEDER through COMPETE–Programa Operacional Factores de Competitividade (POFC), and CIQ-UP (PEst-C/QUI/UI0081/2013) is gratefully acknowledged.

References:

- [1] FAO (2009), *Global agriculture towards 2050*, FAO, Rome.
- [2] Ditta, A. (2012), *How helpful is nanotechnology in agriculture?*, *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 3, 10pp.
- [3] Pérez-de-Luque, A., Hermosín, M.C. (2013), *Nanotechnology and its use in agriculture*, in Bagchi, D., Bagchi, M., Moriyama, H., Shahidi, F., “Bio-nanotechnology: A revolution in food, biomedical and health sciences”, Wiley-Blackwell, West Sussex, pp. 383–398.

Synthesis and application of novel molecularly imprinted material for determination of an aromatase inhibitor.

E. A. Lopes¹, P. Kadhivel¹, M. V. Osório², M. A. Segundo² and M. Azenha¹

¹ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

² Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Aminoglutethimide (AGT) is an aromatase inhibitor which is used therapeutically for the treatment of advanced breast cancer and Cushing syndrome. It is also consumed illegally by bodybuilders and other sportsmen to diminish the cortisol levels in the circulatory system, decreasing muscle loss. Hence, it is controlled in anti-doping testing carried out on athletes contesting in official competitions.

Current methods for determination rely on expensive, time-consuming techniques (capillary electrophoresis and liquid chromatography coupled to mass spectrometry based detectors), not suitable for screening or high throughput analysis. In this research, a novel methodology for determination of AGT is developed, aiming at the screening of illegal consumption by athletes and also as a tool for bioresearch in cancer treatment. For this, state-of-the-art synthesis of molecularly imprinted polymers (MIPs) using sol-gel technique was applied to obtain selective sorptive material to separate and to concentrate AGT. Selective binding sites were achieved by the insertion of a newly synthesized monomer (bis(propyl-trimethoxysilylurelene) pyridine, DPS) into the silica network, an organic tri-alkoxysilane possessing the pyridine 2,6-diamine functionality allowing at least triple hydrogen bond arrangement with the piperidine 2,6-dione sub-structure of AGT (figure 1).

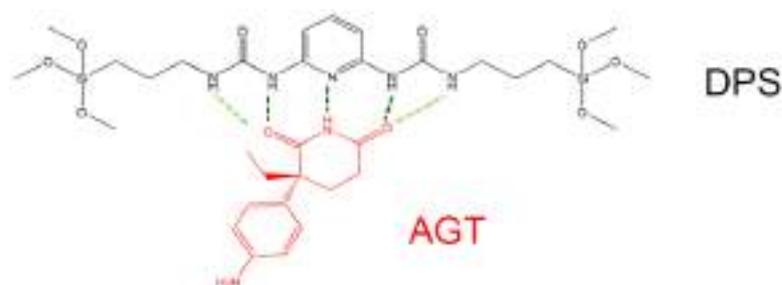


Fig. 1. Schematic hydrogen bonding arrangement between DPS and AGT

The solid phase extraction fostered by the novel material was automated using the bead injection technique performed in a lab-on-valve platform coupled to current HPLC equipment for detection and quantification of AGT. The automated, computer-controlled cycle comprised the main steps present on SPE protocols, including the on-line formation of MIPs sorbent column inside one of the channels of LOV device. The retention of AGT on this sorbent was evaluated by using 1.0 mL of 0.03-0.30 mg L⁻¹ solutions. pH was controlled using a phosphate saline buffer (pH 7.0) as carrier and elution conditions were studied. Application to surrogate samples is currently under development.

Acknowledgments:

Authors are grateful for funding from Reitoria U. Porto (Ref. PP_IJUP2011 87). M. V. Osório (SFRH/BD/91251/2012) thanks Fundação para a Ciência e a Tecnologia for his PhD grant.

Synthesis of novel serine-based surfactants as potential bioactive systems

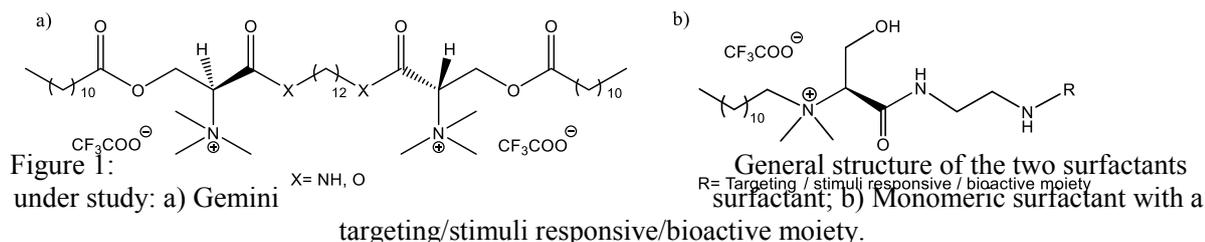
A. R. Dias¹, C. M. Alves¹, E. F. Marques¹, M. J. Araújo¹, M. L. C. do Vale¹

¹ CIQ(UP), Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal

Gemini surfactants represent a new generation of compounds with two surfactant monomers linked chemically at or near the head groups by a rigid or flexible spacer. Owing to their excellent performance and enhanced physicochemical and biological properties, when compared to the conventional monomeric surfactants, they may be suitable for drug or DNA delivery in biological systems [1,2].

Also, there has been an increasing attempt to functionalize such compounds with molecules that can improve their biocompatibility, by adding, for example, anticarcinogenic, antioxidant or targeting properties [3,4].

Following the work developed by our research group during the last years, which included the synthesis and assessment of the physicochemical and toxicological properties of serine-base monomeric and gemini surfactants [2,5], we present herein the synthesis of two new “families” of compounds under development and study in our laboratories (Figure 1).



Acknowledgements: This work was financed with FEDER funds through the Programa Operacional Fatores de Competitividade – COMPETE as well as by national funding provided by FCT- Fundação para a Ciência e Tecnologia through projects Pest/C-QUI/UI0081/2011, PTDC/QUI-QUI/115212/2009 and Re-equipment program REDE/1517/RMN/2005.

References:

- [1] Menger, F. M.; Keiper, J. S. (2000), *Angew. Chem. Int. Ed.* (39), 1906-1920.
- [2] Silva, S. G.; Fernandes, R. F.; Marques, E. F.; Vale, M. L. C. (2012), *Eur. J. Org. Chem.* (2), 345-352.
- [3] Zhang, S.-J.; Ge, Q.-F.; Guo, D.-W.; Hu, W.-X.; Liu, H.-Z. (2010), *Bioorg. Med. Chem. Lett.* (20), 3078–3083
- [4] Low, P. S.; Henne, W. A.; Doorneweerd, D. D. (2008), *Acc. Chem. Res.* (41) 120-129
- [5] Silva, S. G.; Alves, C.; Cardoso, A. M. S.; Jurado, A. S.; Pedroso de Lima, M. C.; Vale, M. L. C.; Marques, E. F. (2013), *Eur. J. Org. Chem.* (2), 1758-1769.

Synthesis of new *N*-acyl homoserine lactones

Sílvia Vinhas¹, Olga Lage^{2,3}, Cidália Pereira¹, José Enrique Rodriguez-Borges¹

¹Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Portugal.

² Department of Biology, Faculty of Science, University of Porto, Portugal.

³Centre of Marine and Environmental Research (CIIMAR), Porto, Portugal

Research on bacterial communication started in the late 1960s. Until then, bacteria were believed to behave as individual cells. However, it was discovered that bacteria are able to sense their cell density by monitoring the accumulation of an activator molecule or "autoinducer" and are capable of coordinated activity that was once believed to be restricted to multicellular organisms. The autoinducers, like *N*-acyl homoserine lactone (acyl-HSL), are small, self-generated signal molecules.[1] This mechanism of cell density sensing was termed quorum sensing and allows bacteria to synchronize the expression of specific genes, encoding for phenotype expressions, like the emission of bioluminescence. Most of the bacteria utilizing quorum-sensing systems are associated in some way with plants or animals through beneficial (symbiotic bacteria) or adversarial (pathogenic bacteria).[1]

Acyl-HSL signaling represents a dedicated communication system that is used by bacteria to control specific genes in response to population density [2]. Acyl-HSLs are small molecule signals with no other known function. These chemical signals are produced by specific enzymes, and they are detected by specific receptors.

In this project, we will explore the inducing molecules mimetic *N*-acyl homoserine lactones. Various ***N*-carbonylaminoalkyl homoserine lactones** and ***N*-carbonyloxyalkyl homoserine lactones** that had never been studied are being synthesized and their influence on cellular communication will be tested.

[1] Kievit T. R., Iglewski B. H., Bacterial Quorum Sensing in Pathogenic Relationships, *Infect. Immun.* 2000, 68(9):4839-4849.

[2] Parsek M. R., Greenberg E. P., Acyl-homoserine lactone quorum sensing in Gram-negative bacteria: A signaling mechanism involved in associations with higher organisms, *PNAS* 2000, 97(16):8789-8793.

Acknowledgment: This research was supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology, under the projects PEst-C/MAR/LA0015/2013.

Ochratoxin A residues in soluble coffee and coffee substitutes

T. Vieira, R. Cruz, S. Cunha and S. Casal

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Contamination of food and feed with mycotoxins is a global health problem. Mycotoxins are fungal secondary metabolites that have adverse effects on humans and animals, resulting in illnesses and economic losses. Although not exclusive to coffee, mycotoxins are highly prevalent in this product, particularly Ochratoxin A (OTA) [1]. Alternative products such as coffee substitutes can contain coffee in various percentages plus roasted cereals, being both important sources of OTA contamination [1].

The aim of this work was to determine the levels of OTA in samples of coffee substitutes (mixtures with and without coffee, barley and chicory), commercialized in Portugal. The analytical method selected uses a solvent extraction with polyethylene glycol followed by OTA isolation and sample clean-up through immunoaffinity columns. OTA amounts were determined by reversed phase high performance liquid chromatography with fluorescence detection (HPLC-FLD). The method was validated in terms of linearity, precision and accuracy. Confirmation of OTA positive samples was achieved by gas chromatography with mass spectrometry.

In forty samples analyzed, including ten of soluble coffee, thirty-seven samples (83%) had OTA levels ranging from 0.05 to 5.76 µg/kg. Coffee samples had significantly higher amounts of OTA ($p < 0.05$), followed by mixtures with and without coffee and later by plain cereal mixtures. A significant linear correlation between OTA amounts and coffee percentage in the mixtures was verified ($p < 0.01$). The daily intake of OTA, estimated by the regular consumption of coffee and substitutes, indicates that these beverages are not an important source of OTA in the diet of the Portuguese population. Based on the maximum consumption of 4 beverages, prepared with 2 g of soluble powder each, soluble coffee contributes with an estimated maximum of 4.5 % (1.0% on average) of the provisional tolerable daily intake (PTDI), while the contribution from substitute with coffee, is about half compared to the previous one. Cereals contribution is extremely low, achieving an estimated maximum of 0.5% of PTDI.

OTA amounts in coffee substitutes are generally low and within the regulated and safety limits but the high incidence of OTA contamination in these products should not be disregarded.

References:

[1] Vieira, T., Cunha, S. and Casal, S. *Mycotoxins in coffee*, in Preedy, V.R., "Coffee in Health and Disease Prevention" Chapter 38, Elsevier, Amsterdam, In Press.

Chemical characterization of fennel (*Foeniculum vulgare*) extracts obtained by salting-out assisted liquid-liquid extraction

P. Neves^{1,2}, M. M. Moreira¹, I. M. Valente¹, L. M. Gonçalves¹ and J. A. Rodrigues¹

¹Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

²Faculty of Animal Science and Food Engineering, University of São Paulo, Brazil.

The salting-out effect has been widely used in analytical chemistry with many different purposes, e.g. to increase the volatility of the analytes in headspace extractions, to precipitate proteins in biological samples or to improve the recoveries in liquid-liquid extractions. An interesting application of this effect is the separation of water-miscible organic solvents (such as propanol, acetone, and acetonitrile) from water. The addition of electrolyte(s) to the solution allows the weakening or the disruption of the solvation forces between the water-miscible organic solvent and water. As a result, two distinguishable liquid phases are formed in which the upper phase is mainly composed by the organic solvent. Simultaneously, it is possible to extract solutes to the organic layer, consisting in a salting-out assisted liquid-liquid extraction (SALLE). The interest in this type of extraction procedure has been increasing and it is nowadays the basis of a widespread sample preparation technique, the QuEChERS methodology [1,2].

The application of SALLE in plants is well documented but mainly aims the analysis of contaminant compounds (e.g. pesticides, toxins). However, the potentialities of SALLE are vast and should be further explored. In this work, the use of SALLE for the chemical profiling of fennel (*Foeniculum vulgare*) is presented. The extracts were analyzed by liquid chromatography with UV and mass spectrometry detections (HPLC-UV-MS/MS) and by gas chromatography with mass spectrometry detection (GC-MS). The samples were firstly extracted by decoction with water. Then, the decoction extracts were subjected to SALLE using acetonitrile and ammonium sulphate. Finally, the organic layer was directly analyzed by HPLC-UV-MS/MS. For GC-MS analysis, a dispersive solid-phase extraction clean-up of the extracts was needed to remove residual water and other interferences. The preliminary results showed that the more polar compounds extracted by water were not detected in SALLE extracts, and phenolic compounds such as quercetin and caffeic acid derivatives were identified. It was also verified that the recovery of some compounds was enhanced, when SALLE was applied. This verification is an important aspect to be considered and is currently under study.

Acknowledgements: This work was supported by Fundos FEDER through “Programa Operacional Factores de Competitividade – COMPETE” and by Fundos Nacionais through FCT - Fundação para a Ciência e a Tecnologia within the project PEst-C/EQB/LA0006/2013, and by QREN (NORTE-07-0124-FEDER-000069). IMV (SFRH/BD/69719/2010) and LMG (SFRH/BPD/76544/2011) wish to acknowledge FCT for PhD studentship and postdoctoral grant, respectively.

[1] Anastassiades, M., Lehotay, S.J., Štajnbaher, D., Schenck, F.J. (2003), *Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce*, Journal of AOAC International, 86 (2), 412-431.

[2] Valente, I.M., Santos, C.M., Moreira, M.M., Rodrigues, J.A. (2013), *New application of the QuEChERS methodology for the determination of volatile phenols in beverages by liquid chromatography*, Journal of Chromatography A, 1271 (1), 27–32.

Synthesis of new chiral diamines as potential bidentated ligands

R. J. Nunes¹, A. G. Fortes¹, M. J. Alves¹, J. E. Borges²

¹ Department of Chemistry, Science School of Minho University, Portugal

² Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Portugal

In the past few years, the field of asymmetric catalysis in organic chemistry have experience an enormous development, due to the enormous demand of enanteomeric pure products and the research to find proper chiral ligands for their obtainment.

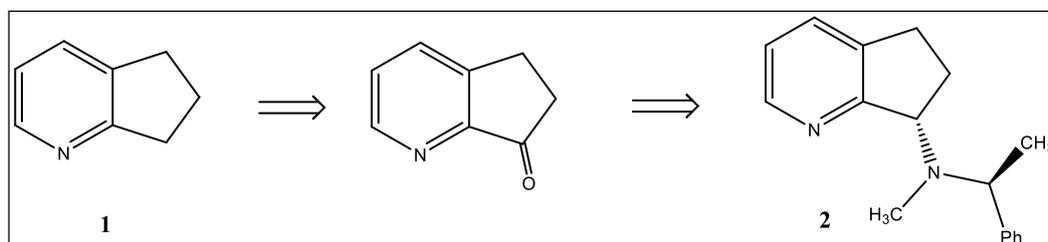
Chiral diamine ligands have been used in different researches and they have been applied to ketone reduction with high enanteomeric yields.

Despite of high activities and selectivity shown by homogeneous catalysts, they demonstrate some fragility typical from this kind of catalysts. They aren't easily reutilized and they aren't also appropriated for continuous oxidation processes.

Conceive a heterogeneous catalyst with similar characteristics to their homogeneous congeners is an attractive strategy.

The principal objective of this work focused on the development of innovative approaches for the preparation of oxidative catalysts which can be easily recovered.

In this project we intended to synthesize a chiral diamine **2** starting from pyrindane **1** (**Scheme 1**), for posterior analysis with the bidentated ligand as a catalytic system in chiral processes.^(1,2) The synthetic strategy used for the preparation of chiral diamine **2** will be presented and discussed.



Scheme 1.

References:

1. Pereira, C.S.; Salgado, S.; Rizzo-Aguiar, F.; Garcia-Mera, X.; Rodriguez Borges, J.E., (2013), *Synthesis of New Propargylated 1-Pyrindane Derivatives as Rasagiline Analogues*, *Synlett*, 24, 837-838.
2. Goretí Silva, J.; Rodriguez-Borges, J.E.; Marques, E.F.; Vale, M.L.C., (2009), *Towards novel efficient monomeric surfactants based on serine, tyrosine and 4-hydroxyproline, synthesis and micellization properties*, *Tetrahedron*, 65, 4156-4164.

Distribution of Resveratrol and its Antioxidant Efficiency in Stripped Corn Oil Emulsions

S. Losada-Barreiro², M. Costa¹, J. Almeida¹, C. Bravo-Díaz², L. S. Romsted³, F. Paiva-Martins¹

¹ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal

² Faculty of Chemistry, Department of Physical-Chemistry, University of Vigo, Spain

³ Department of Chemistry and Chemical Biology, State University of New Jersey, USA

Resveratrol (RES) has shown remarkable health-promoting effects, such as inhibition of platelet aggregation, anti-inflammatory and cardiovascular protective effects and protection against cancer proliferation [1] which explain the great interest shown in consuming grapes, wines and dietary products containing RES. However, this compound has low water solubility, which reduces the dissolution-rate limited cell absorption leading to reduced oral bioavailability. Lipid emulsions are, however, candidates for improving drug solubility. The use of food emulsions containing resveratrol would, therefore, be very attractive for the functional food industry but might be complex from a technological point of view due to the high oxidation susceptibility of unsaturated edible oils usually used as cost effective and non-toxic ingredients.

The antioxidant activity of phenolic compounds depends on multitude factors including the fraction of antioxidant in the interfacial region of emulsion and the ability of for scavenge free radicals. We estimate RES radical scavenging activity, its antioxidant activity in emulsions and its distribution in stripped corn oil emulsions using a well-established kinetic method [2]. The distribution of RES is described by two partition constants, the one between the oil-interfacial region, P_{O}^I , and the one between the aqueous and interfacial region, P_{W}^I , of the emulsion. The high values obtained for partition constants in emulsions indicated that RES is primarily located in the interfacial region, $\%RES_I > 90\%$ at the lowest emulsifier volume fraction, and the percentage of RES in that region is essentially independent of the emulsifier volume fraction (Fig. 1). In spite of its high percentage in the interfacial region, RES exhibit lower antioxidative efficiency in emulsions than caffeic acid (CA) probably due to its radical scavenging activity, 3 times lower than that of CA at $t = 5$ min.

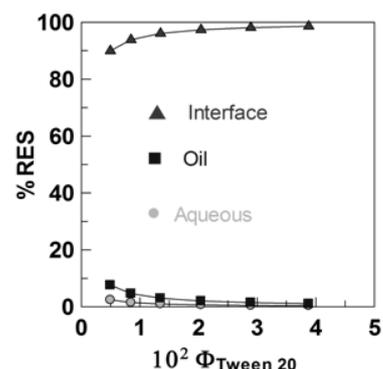


Figure 1 -Distribution of resveratrol in a 4:6 (O:W) corn oil/Tween 20/acidic water emulsion.

Our results indicate that emulsions are, in did, useful for improving RES dispersability but it did not significantly improve emulsions oxidative stability. Since all RES is mainly at the interface, chemical modification of RES that change its lipophilicity will probably not improve its antioxidant capacity.

References:

[1] Vang, O., Ahmad, N., Baile, C., Baur, J. A., et. al. (2011) what is new for an old molecule? Systematic review and recommendations on the use of resveratrol. *PLOS One*, 6, e19881.

[2] Losada-Barreiro, S., Sánchez Paz, V., Bravo Díaz, C., Paiva Martins, F., Romsted, L. S. (2012). Temperature and emulsifier concentration effects on gallic acid distribution in a model food emulsion. *J. Colloid. Interface Sci.*, 370, 73-79.

Application of gas-diffusion microextraction for high-performance liquid chromatographic analysis of volatile compounds in bread

R. Ferreira¹, R. Ramos¹, P. Almeida¹, J. Rodrigues¹

¹ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

In this work a novel extraction approach for volatile and semi-volatile compounds in solid samples is presented. The gas-diffusion microextraction device (GDME) consists in a Teflon module with a microporous hydrophobic membrane (PTFE) at its bottom that, due to its hydrophobicity avoids the passage of the aqueous solvent but allows the diffusion of volatile compounds. This extraction device was recently patented [1]. So far, GDME has only been used for the extraction of compounds from liquid samples, especially in beverages [2,3]. This work intends to enlarge the application field of GDME to solid samples. To highlight this possibility, the GDME approach was applied to the chromatographic analysis of α -dicarbonyl compounds in different types of fermented foods, particularly bread, since these compounds are important fermentation markers and their determination is useful to assess the quality of the product. In a single step, a derivatizing reaction, an extraction and a concentration of the analyte is achieved allowing a very simple instrumental detection.

The influence of several parameters of the methodology was studied, such as temperature and time of extraction, acceptor derivatizing solution's volume and concentration, among others. We were able to determine the content of vicinal diketones (especially diacetyl) on several types of bread. Using vicinal diketones data from different bread origins we are trying to identify correlations between vicinal diketones profile and bread type.

Acknowledgments:

This work was supported by Fundos FEDER through program "Programa Operacional Factores de Competitividade – COMPETE" and by national funds through Fundação para a Ciência e a Tecnologia (FCT) under the project (Pest-C/EQB/LA0006/2013). RMR (SFRH/BD/88166/2012) wishes to acknowledge FCT for his PhD studentship.

References:

- [1] Rodrigues, J.A.; Gonçalves, L.M.; Pacheco, J.G.; Barros, A.A (2011), *Módulo extractor e processo de extração de espécies voláteis e semi-voláteis baseado em difusão gasosa*, PT Patent 104789.
- [2] Ramos, R.M.; Pacheco, J.G.; Gonçalves L.M.; Valente I.M.; Rodrigues J.A.; Barros A.A. (2012), *Determination of free and total diacetyl in wine by HPLC-UV using gas-diffusion microextraction and pre-column derivatization*, Food Control, 24 (1-2), 220-224.
- [3] Valente I.M.; Santos C.M.; Gonçalves L.M.; Rodrigues J.A.; Barros A.A. (2012), *Application of gas-diffusion microextraction for high-performance liquid chromatographic analysis of aliphatic amines in fermented beverages*, Analytical Methods, 4 (8), 2569-2573.

Validation of BPA and phthalates metabolites in urine samples by SPE-GC-MS

L. Correia-Sá^{1,2}, S. Sousa¹, D. Teixeira², D. Pestana², C. Correia de Sá², S. Norberto², R. Monteiro², C. Calhau², V. F. Domingues¹, C. Delerue-Matos¹

¹REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal

²Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, P-4200-319 Porto, Portugal.

Endocrine disruptor chemicals (EDCs) are synthetic compounds that even though being initially designed for a specific function are now being linked with a wide range of side effects [1]. The list of possible EDCs is growing and includes phthalates and bisphenol A (BPA). Phthalates are one of the most widely used plasticizers to improve the extensibility, elasticity and workability of polyvinyl chloride (PVC), polyvinyl acetates, etc. Considered non-toxic and harmless additives for polymers, they were used unrestrainedly all over the world for several decades [2]. However, recent studies have indicated that some phthalates and their metabolic products are reproductive and developmental toxicants in animals and suspected endocrine disruptors in humans [2]. BPA (2,2-bis(4-hydroxyphenyl)propane) is a high production volume chemical mainly used in the production of polycarbonate plastics and epoxy resins. Although BPA was initially considered to be a weak environmental estrogen, nowadays it is known that this compound can stimulate several cellular responses at very low levels of concentrations [3].

The aim of this study was to develop a method to evaluate the presence of phthalates, metabolites and BPA in human urine samples. The analyzed compounds included: dibutyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP), BPA, mono-isobutyl phthalate (MiBP), monobutyl phthalate (MBP), mono-(2-ethyl-5-oxohexyl) (MEOHP) and mono-(2-ethyl-5-carboxypentyl) (MECPP).

Chromatographic analyses were carried out in a Thermo GC ULTRA GC-MS/MS. Two different SPE cartridges were tested (Strata C18-E and Strata-X 33µm Polymeric Reversed Phase). The cartridge with higher recovery was selected. Linear calibration curves were obtained. After validation, the methodology will be applied to human urine samples for phthalates, metabolites and BPA evaluation.

Acknowledgements:

Research relating to this abstract was funded by FCT (SFRH/BPD/40110/2007, SFRH/BD/46640/2008, SFRH/BD/64691/2009, SFRH/BD/87019/2012 and PESt-C/EQB/LA0006/2013) and supported by “Projectos de Investigação na Pré-graduação 2012, Univ.Porto- PP_IJUP2011-243.

References:

- [1]. Schug TT, Janesick A, Blumberg B, Heindel JJ (2011) Endocrine disrupting chemicals and disease susceptibility. *The Journal of Steroid Biochemistry and Molecular Biology* 127 (3–5):204-215. doi:<http://dx.doi.org/10.1016/j.jsbmb.2011.08.007>
- [2]. Guo Z-Y, Gai P-P, Duan J, Zhai J-X, Zhao S-S, Wang S, Wei D-Y (2010) Simultaneous determination of phthalates and adipates in human serum using gas chromatography–mass spectrometry with solid-phase extraction. *Biomed Chromatogr* 24 (10):1094-1099. doi:10.1002/bmc.1410
- [3]. Rubin BS (2011) Bisphenol A: An endocrine disruptor with widespread exposure and multiple effects. *The Journal of Steroid Biochemistry and Molecular Biology* 127 (1–2):27-34. doi:<http://dx.doi.org/10.1016/j.jsbmb.2011.05.002>

Lead in lipstick

Results from a study of products manufactured in Brazil

A. Carvalhido¹, K. Paiva^{1,2}, P. Ramos¹ and A. Almeida¹

¹ REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

² Federal University of Espírito Santo, Brazil.

Lead (Pb) is ubiquitous in the environment and can be present in varying amounts in all raw materials. Due to its well-known toxicity, human exposure to products containing Pb poses safety concerns.

At the end of the 2000s, several reports on the presence of Pb in lipsticks at levels that would pose safety concerns were published in the media and on the internet [1]. A scientific paper, published in 2009 [2], also reported very high Pb levels (~ 2000 – 3700 ppm) in lipsticks (manufactured in China and available on the Saudi Arabia market). This led to FDA scientists to develop and validate an analytical procedure for Pb determination in lipstick [2] and, latter, to perform a survey of the U.S. market. This study, published in 2012 [3], found an average Pb content in the 400 lipsticks tested of 1.11 ppm, with results ranging from the detection limit (0.026 ppm) to 7.19 ppm. In 2013, the results of a European survey on the content of Pb in lipsticks were also published [4]. On average, the Pb content found was 0.75 ppm (max. 3.75 ppm).

At our Laboratory, during 2013, we performed a work aiming to determine the Pb content in lipsticks manufactured in Brazil⁵. Using the above-mentioned analytical procedure [2], combining a microwave-assisted acid digestion for samples mineralization with inductively coupled plasma-mass spectrometry (ICP-MS) for Pb determination, 54 lipstick samples, of 9 different brands (6 different colors of each), were analyzed. Results showed a low Pb level, quite similar to the content found in the US and the European surveys, which does not pose safety concerns under ordinary use. On average, the Pb content found in the Brazilian products analyzed was 1.09 ± 0.81 ppm (ranging from <0.03 to 3.90 ppm). Currently we are conducting a similar study on products manufactured in Portugal².

References:

- [1] <http://www.fda.gov/cosmetics/productandingredientsafety/productinformation/ucm137224.htm>
- [2] Al-Saleh, I., Al-Enazi, S., Shinwari, N. (2009), *Assessment of lead in cosmetic products*, Regulatory Toxicology and Pharmacology, 54(2), 105-13.
- [2] Hepp, N.M., Mindak, W.R., Cheng, J. (2009), *Determination of total lead in lipstick: development and validation of a microwave-assisted digestion, inductively coupled plasma-mass spectrometric method*, Journal of Cosmetic Science, 60(4), 405-14.
- [3] Hepp, N.M. (2012), *Determination of total lead in 400 lipsticks on the U.S. market using a validated microwave-assisted digestion, inductively coupled plasma-mass spectrometric method*, Journal of Cosmetic Science, 63(3),159-76.
- [4] Piccinini, P., Piecha, M., Torrent, S.F. (2013), *European survey on the content of lead in lip products*, Journal of Pharmaceutical and Biomedical Analysis, 76, 225-33.

⁵ Work conducted by Kainá Kiffer Paiva under the Brazilian scientific mobility programme “Ciência sem Fronteiras”, 2013. ² Under the Course Unit “Projeto I”, Master in Pharmaceutical Sciences, FFUP.

Celebrating the golden anniversary of Merrifield's Solid Phase Peptide Synthesis

L. Aguiar¹, N. Vale¹ and P. Gomes¹

¹ CIQ-UP, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal

Robert Bruce Merrifield reported in 1963 his invention of Solid Phase Peptide Synthesis (SPPS) [1], whose seminal and transversal importance in Science soon became clear and led to attribution of the Nobel Prize in Chemistry to its inventor, in 1984.

The geniality of this invention is outlined by its remarkable simplicity: the C-terminal amino acid (conveniently protected) is attached to an insoluble polymeric support ("resin"), and its N-protecting group is then removed to allow for attachment of the following amino acid in the growing peptide chain that is built in the C-N direction (opposite to peptide biosynthesis in ribosomes). Hence, in general, the peptide is assembled through a series of convenient coupling-deprotection cycles and, once the sequence is complete, it is detached from the resin while all protecting groups present are removed (Fig. 1).

The impact of Merrifield's invention is obvious: the ability to build peptides by strictly chemical routes opened a whole new world of possibilities, as researchers were no longer limited to peptide sequences bearing only natural amino acids; moreover, currently there are more than 60 peptide drugs on the market and over 500 peptides are in various stages of preclinical and clinical development [2], as bioactive peptides are usually highly selective and efficient, acting on their targets in low concentrations.

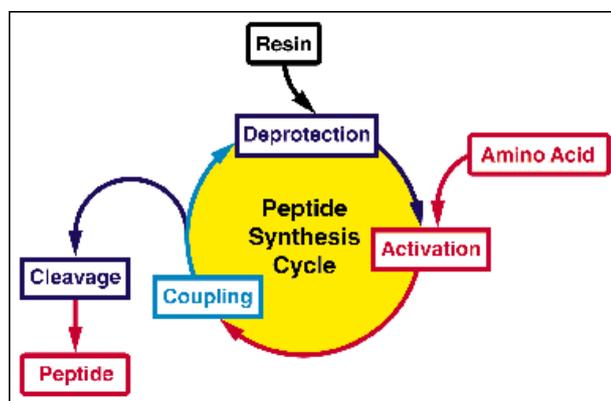


Figure 1

In this communication, we will present the current status and major applications of SPPS research in our group, at the Faculty of Sciences of the University of Porto.

References:

- [1] Merrifield R.B. (1963), *Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide*, Journal of American Chemical Society, 85 (14), 2149-2154
- [2] Vlieghe, P., Lisowski, V., Martinez, J., Khrestchatisky, M. (2010), *Synthetic therapeutic peptides: Science and market*. Drug Discovery Today, 15 (1-2), 40-56.

Stimuli-responsive lysine-based surfactants: synthesis and supramolecular self-assembly

Ana R. Fernandes, M. J. Araújo, Eduardo F. Marques

Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Novel amino-acid based surfactants have emerged as a viable alternative to conventional surfactants due to the presence of an amino acid polar headgroup, which allows the reduction of toxicity and the simultaneous increase of biodegradability. [1] These amino acid-based surfactants may be useful for a wide range of applications, namely in the pharmaceutical and biomedical field, as amphiphiles capable of forming nano-aggregates (e.g. micelles, liposomes, nanotubes) for drug delivery.

In this context, this work is part of a wider project comprising the synthesis and the assessment of the physicochemical properties of ionic amino acid-based surfactants. [2–4] Herein, we present the synthesis of a series of anionic lysine-based surfactants with two alkyl chains of different length. This molecular structure will impart structural asymmetry to the non-polar region of the compound and presumably induce different self-aggregation phenomena. The influence of this type of asymmetry on the aggregation behavior will thus be investigated, resorting to different techniques, viz. surface tension, conductivity, microscopy and light scattering.

The surfactants were obtained from the corresponding N,N-dialkyl lysine derivative by condensation with different fatty acids followed by saponification (Fig. 1). The structures of the synthesized surfactants were ascertained by spectroscopic methods (^1H and ^{13}C NMR, mass spectroscopy). Furthermore, the critical micelle concentration, *cmc*, for the different surfactants as well as the study of their thermal phase behavior are currently under course.

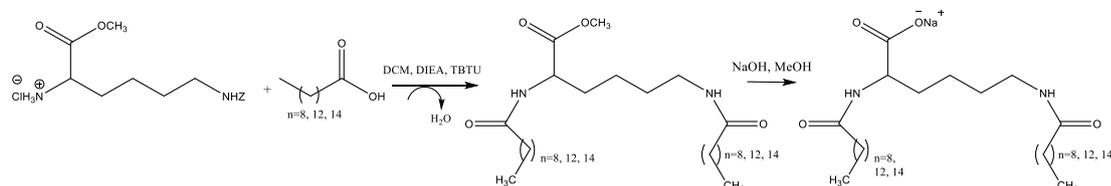


Fig. 2 – Synthetic path for the lysine-based surfactants.

References:

- [1] R. O. Brito, E. F. Marques, P. Gomes, M. J. Araújo, and R. Pons, *Structure/property relationships for the thermotropic behavior of lysine-based amphiphiles: from hexagonal to smectic phases*, J. Phys. Chem. B, 112, 14877–87, **2008**.
- [2] R. O. Brito, E. F. Marques, P. Gomes, S. Falcão, and O. Söderman, *Self-assembly in a catanionic mixture with an aminoacid-derived surfactant: from mixed micelles to spontaneous vesicles*, J. Phys. Chem. B, 110, 18158–65, **2006**.
- [3] R. O. Brito, I. S. Oliveira, M. J. Araújo, and E. F. Marques, *Morphology, thermal behavior, and stability of self-assembled supramolecular tubules from lysine-based surfactants*, J. Phys. Chem. B, 117, 9400–11, **2013**.
- [4] E. F. Marques, R. O. Brito, S. G. Silva, J. E. Rodríguez-Borges, M. L. do Vale, P. Gomes, M. J. Araújo, and O. Söderman, *Spontaneous vesicle formation in catanionic mixtures of amino acid-based surfactants: chain length symmetry effects*, Langmuir, 24, 11009–17, **2008**.

Synthesis and characterization of MIL-101-type metal-organic frameworks with different metallic centres

E. Correia¹, P. Pereira¹, V. Machado¹, C. M. Granadeiro¹, L. Cunha-Silva¹ and S.S. Balula¹

¹ Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Portugal.

Metal-organic frameworks (MOFs) are a new class of hybrid materials composed by metal ions that are linked by organic ligands giving rise to a very characteristic structure which has very high porosity and can present one-, two-, or three-dimensional networks.[1] This high porosity feature leads to an enormous internal surface area, which has revealed unique properties in technology applications, such as gas storage (for gases like H₂ and CO₂) and also in catalytic reactions which has been the subject of remarkable progress. These structures appeared with the high interest in zeolite-type materials with the advantage of accepting almost all types of metal cations in its structure, leading to various MOFs, including the ones used in this study: MIL-101(Cr) and NH₂-MIL-101(Al) (Fig. 1).

In this work, two approaches were used to prepare the two MOFs: hydrothermal and microwave-assisted methodology.[2,3] In the hydrothermal synthesis, the reactants were placed in a Teflon-lined autoclave and heated in an oven under static conditions. In the microwave-assisted synthesis, the reactants were placed in a glass tube under microwave radiation which allowed a significant reduction of the reaction time.

The structural and morphological characterization of the MIL-101 materials was performed by vibrational spectroscopy (FT-IR and FT-Raman), powder X-ray diffraction, scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). The characterization data obtained allowed to confirm the successful preparation of both the chromium- and aluminum-containing MIL-101 materials.

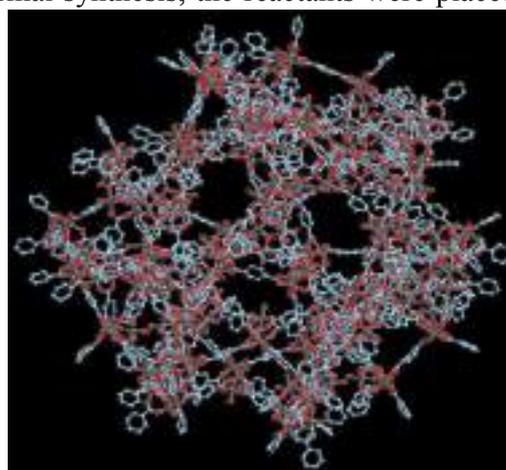


Fig. 1 – Representation of the MIL-101 framework.

References:

- [1] Corma, A.; Garcia, H. and Llabrés i Xamena, F.X. (2010), *Chem. Rev.*, 110, 4606.
- [2] Serra-Crespo, P.; Ramos-Fernandez E.V.; Jorge, G. and Kapteijn F. (2011), *Chem. Mater.*, 23, 2565.
- [3] Férey, G.; Mellot-Draznieks, C.; Serre, C.; Millange, F.; Dutour, J.; Surblé, S. and Margiolaki, I. (2005) *Science*, 309, 2040.

Farmed pheasant (*Phasianus colchius*) meat for human consumption: Amino acid profile characterization

I. Rocha¹, F.B. Pimentel¹, R.C. Alves^{1,2}, A.S.G. Costa¹, M.A.G. Quaresma³, M.B.P.P. Oliveira¹

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

³ Faculty of Veterinary Medicine, Technical University of Lisbon, Portugal.

Lean meats, especially poultry, are known to be rich in protein and low in fat. Therefore, they are recognized by the consumers as excellent protein sources. Not being a type of meat currently consumed in Portugal, pheasant can be an alternative and interesting protein source, as it has been presented like a high protein (25.61 g/100 g) and low fat (0.28 g/100 g) meat [1]. The aim of this work was to characterize the amino acid profile of farmed pheasant meat (breast and leg).

The amino acid characterization of the freeze-dried pheasant samples, breast ($n=3$) and legs ($n=3$), was performed after acidic hydrolysis, followed by derivatization with dansyl chloride, as described by Pimentel *et al.* [2]. The compounds were analyzed by HPLC with fluorescence detection. Each sample was analyzed in triplicate and results expressed in g of amino acid/ 100 g of freeze-dried sample.

The results show that the predominant essential amino acids in pheasant meat are lysine (99.0 mg/g breast meat and 81.1 mg/g leg meat) and leucine (69.5 mg/g of breast meat and 56.1 mg/g of leg meat) followed by isoleucine, valine plus methionine, phenylalanine and histidine. Regarding the non-essential amino acids serine stands out with higher values than the remaining (224.5 mg/g breast meat and 121.7 mg/g leg meat).

Although the general amino acid profiles were similar for breast and leg meat, higher contents of each amino acid were found in pheasant breast.

These preliminary data allow a better knowledge about the quality of this meat, obtained from farmed animals, and show that it can be a good protein source, with excellent potential to compete with others like poultry, pork or beef.

References:

[1] Ribeiro, A. P. F. (2013), *Perfil nutricional da fracção lipídica da carne de aves cinegéticas (perdiç, faisão, pato)*, Dissertation in Food Safety, Technical University of Lisbon – Faculty of Veterinary Medicine.

[2] Pimentel, F. B., Alves, R. C., Costa, A. S. G., Torres, D., Almeida, M. F., and Oliveira, M. B. P. P. (2014), *Phenylketonuria: Protein content and amino acids profile of dishes for phenylketonuric patients. The relevance of phenylalanine*. Food Chemistry, 149(0), 144-150.

Acknowledgments: The authors thank to the company CAÇABRAVA for providing samples. R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

Design of 3-hydroxy-4-pyridinone functionalized with hydrophilic ethoxylated chains

V. Gomes¹, T. Moniz², M. Rangel³ and J. E. Rodriguez-Borges¹

¹ Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4169-007 PORTO, Portugal

² REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4169-007 PORTO, Portugal

³ REQUIMTE, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 PORTO, Portugal

Metal ions are crucial for supporting all forms of life and alterations in their cellular homeostasis are finely related to many disorders thus implying the need of regulation of their concentration [1]. Our group has long been interested in the synthesis and solution properties of 3-hydroxy-4-pyridinone (3,4-HPO) ligands and their complexes with M(II) and M(III) metal ions for several applications [2-4]. In the sequence of our research, the improvement of water solubility of the ligands is very important and the development of more hydrophilic is being pursued.

Oligo(ethylene glycols) (OEGs) have been reported in the literature as compounds with high water solubility [5]. Herein we report the synthesis of the two types of amine-terminated OEGs with different chemical groups in the end of the chain: a methyl (**1**) or a hydroxyl group (**2**) (Fig.1A) for future functionalization of 3,4-HPO. The synthetic approach was based on the conversion of hydroxyl group of the OEGs to an azido group, which is subsequently reduced to form the amine by hydrogenation. The amino-terminated OEGs reacts with 3,4-HPO (**3**) where the oxygen atom of the ring was substituted by the nitrogen of the amine group of the chain (Fig. 1B).

The synthesis of compounds **1**, **2** and **4** was successfully achieved and is reported in this work. Other ligands are currently being synthesized.

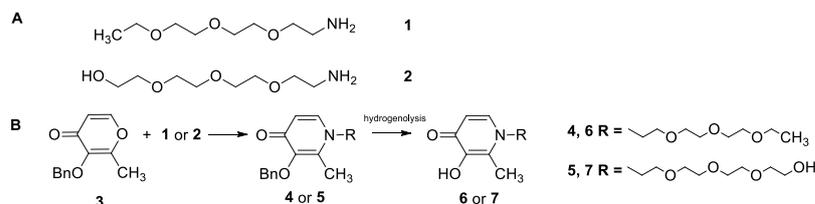


Figure 1: New amine-ethoxylated chains (A) and functionalized 3,4-HPO (B).

The conjugation of these amino-ethoxylated chains with 3,4-HPO will improve hydrophilic balance of these type of ligands and their metal ion complexes that will allow its use in biological and analytical applications.

Acknowledgements: Financial support from FCT through project PTDC/SAU-MET/113011/2009, PEst-C/EQB/LA0006/2011 and PhD grant SFRH/BD/79874/2011 (T. Moniz) is gratefully acknowledged.

References:

- [1] Dommaillie, D.W., *et al* (2008), *Nature Chemical Biology*, 4, 168-175.
- [2] Moniz, T., *et al* (2011), *Journal of Inorganic Biochemistry*, 105, 1675-1682.
- [3] Moniz, T., *et al* (2013), *Dyes and Pigments*, 98, 201-211.
- [4] Mesquita, R.B.R., *et al* (2013), *Talanta*, 108, 38-45.
- [5] Goswami, L. N., *et al* (2013), *Organic and Biomolecular Chemistry*, 11 (7), 1116-1126.

Enantioselective Synthesis and Biological Evaluation of Proline Mimetic of PLG based on (1*R*,3*S*)-2-azabicyclo[2.2.1]heptane

I. E. Sampaio-Dias^{1,2}, D. Neves¹, X. García-Mera², J. E. Rodríguez-Borges¹

¹CIQ-Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal

²Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, Spain

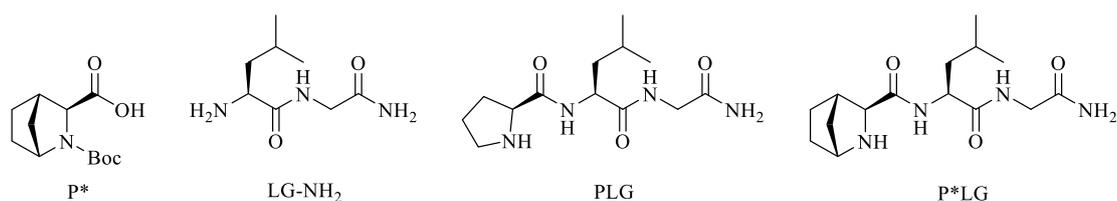
Peptides biological importance is linked to its broad range of functions in living organisms acting as hormones, neurotransmitters or neuromodulators, with straight intervention on respiratory, reproductive and immunological systems.[1]

In recent decades, investigators deal with synthesizing structurally modified peptides in order to improve the stability and biological activity of these compounds to obtain new potential pharmaceuticals with improved pharmacokinetics and dynamics scores.[2]

This project aims the synthesis of new class of mimetic compounds for neuropeptide **PLG**, L-prolyl-L-leucylglycinamide (Fig. 1), which perform important roles in central nervous system. Therefore, it is proposed the synthesis of new class of tripeptide structure-related to PLG containing proline mimetic derivative, **P*** (Fig. 1). This work might make an enormous contribution to the discovery of new potential therapeutic agents for the treatment of neurodegenerative diseases such Parkinson.

Summary:

- Synthesis of enantiopure proline mimetic **P*** by precursors obtained through aza-Diels-Alder reaction using (–)-8-phenylmenthol as chiral auxiliary to form the desired single cycloadduct in the presence of cyclopentadiene: the methodology in synthesizing this type of bicyclic compound is well known in our research group.[3]
- Synthesis of dipeptide **LG-NH₂**, L-leucylglycinamide (Fig. 1), through coupling reaction between the corresponding amino acids using TBTU as coupling reagent.
- P*LG** tripeptide synthesis by coupling the dipeptide **LG-NH₂** in b) with **P*** by the same methodology of condensation. Biological evaluation of the tripeptide obtained will be held at Faculty of Pharmacy, University of Santiago de Compostela, Spain.



Figure

1 – From left to right: chemical structures of proline mimetic (**P***), the dipeptide **LG-NH₂**, the natural **PLG** tripeptide and the desired PLG analogue (**P*LG**).

References:

[1] Fletcher, M. D.; Campbell, M. M. *Chem. Rev.* **1998**, *98*, 763-795.

[2] Trabochi, A.; Scarpì, D.; Guarna, A. *Amino Acids* **2008**, *34*, 1-24.

[3] García-Mera, X.; Rodríguez-Borges, J. E.; Vale, M. L. C.; Alves, M. J. *Tetrahedron* **2011**, *67*, 7162-7172.

Green synthesis of silver nanoparticles (AgNP) – an automatic approach

Marieta L. C. Passos, Diana Costa, José L. F. C. Lima and M. Lúcia M. F. S. Saraiva

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

This paper presents a new way to the synthesis of uniform and size-controlled silver nanoparticles by means of microreaction technology. It complies with the philosophy of green chemistry as it prevents pollution at source. The manipulations are automated by sequential injection analysis (SIA) system [1]. Besides it was used environmental friendly stabilizer and reducers, and a UV-light lamp that promoted the enhancement of the reduction of AgNO₃, and at the same time the use of less amounts of reagents. The SIA system, enables the precise and exact time control of fluidic manipulations leading to a repeatable and reproducible AgNP synthesis.

Effect of hydrodynamic parameters (linear flow rate, volume flow rate and reaction temperature) and concentrations (reducer and stabilizer), pH and UV irradiation time on morphology and size of nanoparticles was studied.

The characterization of obtained AgNP was performed by transmission electronic microscopy (TEM), electron diffraction x-ray spectroscopy (EDXS), UV-vis spectra analysis, dynamic light scattering (DLS) and zeta potential measurements. Particles are mostly spherical in shape and have average sizes between 7 and 20 nm. The particle size can be controlled by changing not only the flow rate and UV light time exposition but also the reducer/AgNO₃ concentration ratio.

Besides, the benign reaction conditions this automatic procedure, led to a sustainable and cost-saving methodology that guarantees not only reproducible synthesis, but also the changing of NPs characteristics at time by simple control of the fluid transport.

References:

[1] J. Ruzicka, J. and Marshall, G.D. (1990), *Sequential injection – a new concept for chemical sensors, processes analysis and laboratory assays*, Anal. Chim. Acta, 237 (2), 329-343.

Acknowledgments:

This work has been supported by Fundação para a Ciência e a Tecnologia through grant no. PEst-C/EQB/LA0006/2011. Marieta L. C. Passos thanks the Fundação para a Ciência e a Tecnologia, Pos-doc grant (SFRH / BPD /72378 / 2010) in the ambit of “POPH - QREN - Tipologia 4.1 - Formação Avançada” co-sponsored by FSE and national funds of MEC.

Ionothermal synthesis of transition metal based metal-organic framework materials

André D.S. Barbosa¹ and Luís Cunha-Silva¹

¹ REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

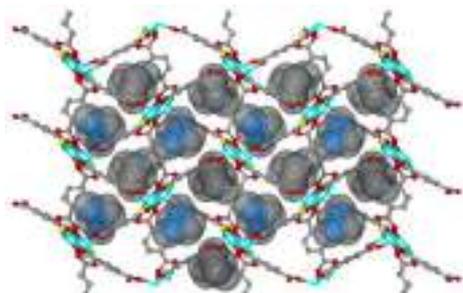


Fig. 1 - Structure representation of [PMI][Mn₃(bdc)₃Br₂].

Metal-Organic Frameworks (MOFs), also known as coordination polymers, have become one of the trending research fields in today's material chemistry and its development has provided novel functional materials with potential for gas storage/separation, detection and sensing, catalysis and drug delivery.[1] MOF materials consist of metal centres connected with each other by organic molecules, commonly known as ligands or linkers, and are usually obtained by conventional hydro/solvothermal methods. Ionothermal synthesis is a more recent preparation method of crystalline materials

and provides several advantages over the traditional reaction conditions, mainly that it can take place at ambient pressures because of ionic liquid's (ILs) very low vapour pressure, thus preventing the general necessity for high autogenous pressure of up to 15 atm under 473K temperature.[2] Moreover, ILs possess high chemical and thermal stability, non-flammability and high ionic conductivity.[3]

In the present work, several attempts were performed in order to synthesize MOF materials through the utilization of an ionic medium and variable stoichiometry between the ligand, terephthalic acid (H₂bdc) and Mn(II)-, Cu(II)-, Ni(II)- and Zn(II)-available salts. A Mn(II)-based MOF material was obtained with the use of 1-propyl-3-methyl-imidazolium bromide [PMIBr] at 140 °C during 7 days. The structure was determined by single-crystal X-ray diffraction, revealing a 2D MOF material (Fig. 1), formulated as: [PMI][Mn₃(bdc)₃Br₂]. All product materials were characterized by FT-IR and Powder X-ray diffraction and will be further characterized by FT-Raman, SEM-EDS, EA, TGA, and Solid-State NMR techniques.

Acknowledgments:

To the *Fundação para a Ciência e a Tecnologia* for financial support by the strategic project Pest-C/EQB/LA0006/2011 (to REQUIMTE), the R&D project PTDC/CTM/100357/2008.

References:

- [1] Meek, S. T.; Greathouse, J. A. and Allendorf, M. D. (2011), *Metal-Organic Frameworks: A Rapidly Growing Class of Versatile Nanoporous Materials*, *Advanced Materials*, 23, 249-267.
- [2] Parnham, E. R. and Morris, R. E. (2006); *Ionothermal synthesis using a hydrophobic ionic liquid as solvent in the preparation of a novel aluminophosphate chain structure*; *Journal of Materials Chemistry*, 16, 3682-3684.
- [3] Xu, L.; Kwon, Y. U.; de Castro, B. and Cunha-Silva, L. (2013); *Novel Mn(II)-Based Metal-Organic Frameworks Isolated in Ionic Liquids*; *Crystal Growth & Design*, 13, 1260-1266.

Chemometric discrimination of commercial squids based on fatty acids, vitamin E and cholesterol contents

A. Torrinha¹, R. Cruz², E. Mendes², F. Gomes¹, C. Delerue-Matos¹, S. Casal², S. Morais^{1*}

¹ REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal. sbm@isep.ipp.pt

² REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

Cephalopods are interesting marine organisms due to their nutritional, commercial and ecological importance. Among cephalopods, squids represent an important food source, with *Loligo duvaucelii*, *Loligo gahi*, *Loligo reynaudii*, *Loligo opalescens* and *Loligo vulgaris* as the major squid species in terms of world trade and landing [1]. It is then important to characterize and compare nutritional parameters such as fatty acids, cholesterol and vitamin E, as well as identify chemical descriptors to allow authenticity control of these species.

A total of 166 samples was collected. Fatty acids composition of the selected five species was analyzed by GC-FID while cholesterol and vitamin E were quantified by HPLC with diode-array and fluorescence detectors in series [2]. Canonical discriminant analysis (CDA; Statistica software v. 7, StatSoft Inc., USA) was applied as a pattern recognition technique to the obtained data. The basic strategy of canonical correlation analysis is to derive a linear combination from each of the sets of variables in such a way that the correlation between the two linear combinations is maximized.

Firstly, CDA was tested to distinguish the oceanic origin of the species via their individual fatty acids, cholesterol and vitamin E contents. A good separation between oceans was observed reflecting the marked influence of the environmental conditions in the levels of the determined compounds. The fatty acids C16:0, C20:1n9, C20:5n3 and C22:6n3 were the variables that contributed the most for the separation. In a second attempt, CDA was applied to group the different species and the results also provided satisfactory differentiation. The separation was mainly influenced by C16:0, C20:5n3, C20:1n9, C22:6n3 and C22:5n6. Clear discriminations among oceanic origins and squid species were reached based on their individual fatty acids, cholesterol and vitamin E contents. These compounds exhibited the potential to proof authenticity of commonly consumed and commercially relevant squid species while distinguishing them also from the nutritional point of view.

Acknowledgments:

This work was supported through the *FCT* project PTDC/AGR-AAM/102316/2008 (COMPETE and co-financed by FEDER), PEst-C/EQB/LA0006/2013.

References:

- [1] Gomes, F., Oliveira, M., Ramalhosa, M.J., Delerue-Matos, C. and Morais, S. (2013), *Polycyclic Aromatic Hydrocarbons in Commercial Squids from Different Geographical Origins: Levels and Risks for Human Consumption*, Food and Chemical Toxicology, 59, 46-54.
- [2] Cruz, R., Casal, S., Mendes, E., Costa, A., Santos, C., Morais, S. (2013), *Validation of a Single-extraction Procedure for Sequential Analysis of Vitamin E, Cholesterol, Fatty Acids, and Total Fat in Seafood*, Food Analytical Methods, 6, 1196-1204.

Novel methodology to immobilize efficiently polyoxometalates into silica nano sized spheres catalytically active for oxidation reactions

L. S. Nogueira¹, S. Ribeiro¹, C. M. Granadeiro¹, L. Cunha-Silva¹ and S. S. Balula¹

¹ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Over the past decades, several studies highlight the importance of using environmentally sustainable catalysts and oxidants in oxidation reactions due to their applications in the industry. The demand for active, selective and recyclable catalysts, as well as the optimization of the reaction conditions, is the key to practice an ideal process of oxidation [1]. The preparation of robust heterogeneous catalysts from the immobilization of homogeneous catalysts, based on polyoxometalates (POMs), on a solid support proved to be a new methodology for the efficient oxidation of organic compounds [1-3], where recycling is only possible after immobilization.

Metal transition monosubstituted and monovacant Keggin-type polyoxotungstates have been studied: $[PW_{11}O_{39}]^{7-}$ and $[PW_{11}M(H_2O)O_{39}]^{5-}$ where $M = Zn^{II}$ or Co^{II} . Metal transition monosubstituted polyoxotungstates (structure showed on top of Fig. 1) are special due to the reactive low valence transition metal coordinated to oxometalate ligands which have the capacity to transfer oxygen atoms [4]. In this study, heterogeneous catalytic systems were developed by immobilization of these POMs on silica. POMs immobilization was achieved by functional cross-linked organic/inorganic hybrid core (Fig. 1), which was obtained through successive spontaneous reactions in water [5]. POMs and silica nanoparticles were tested as homogeneous and heterogeneous catalysts for the oxidation of cis-cyclooctene, geraniol and styrene using hydrogen peroxide as oxidant. The POMs immobilized into silica nanoparticles proved to have good catalytic activity and to be recyclable catalysts. Inorganic complexes and silica nanoparticles were characterized by FT-IR, FT-Raman, ^{31}P RMN, SEM-EDS and TEM.

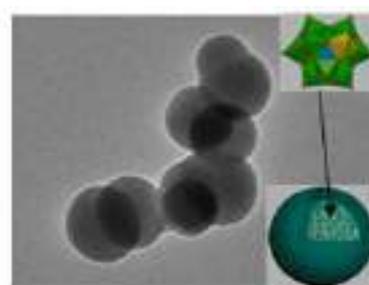


Fig. 1 – $PW_{11}M$ -APTES@SiO₂ nanoparticles.

References:

- [1] Balula, S.S.; Santos, I.C.M.S.; Cunha-Silva, L.; Carvalho, A.P.; Pires, J.; Freire, C., Cavaleiro, J.A.S.; Castro, B. and Cavaleiro, A.M.V. (2013), *Cat. Today* 203, 95.
- [2] Neves, C. S.; Granadeiro, C. M.; Cunha-Silva, L.; Ananias, D.; Gago, S.; Feio, G.; Carvalho, P.; Eaton, P.; Balula, S. S. and Pereira, E. (2013), *Eur. J. Inorg. Chem.* 2013, 2877.
- [3] Balula, S. S.; Cunha-Silva, L.; Santos, I. C. M. S., Estrada, A. C.; Fernandes, A. C.; Cavaleiro, J. A. S.; Pires, J.; Freire, C. and Cavaleiro, A. M. V. (2013), *New Journal of Chemistry*, 37, 2341.
- [4] Hill, C.L. and Prosser-McCartha, C.M. (1995), *Coord. Chem. Rev.* 143, 407.
- [5] Wang, X.; Cui, T.; Cui, F.; Zhang, Y.; Li, D. and Zhang, Z. (2011), *Chem. Commun*, 47, 6329.

Synthesis and characterization of μ -oxo-bridged Fe(III) complex of *meso*-tetrakis(pentafluorophenyl)porphyrin

**A. Aguiar¹, S. Ribeiro¹, A. M. N. Silva¹, L. Cunha-Silva¹, B. de Castro¹,
A. M. G. Silva¹ and S. S. Balula¹**

¹ REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências,
Universidade do Porto, 4169-007 Porto, Portugal.

The porphyrinic macrocycles are robust aromatic compounds that have tremendous biological importance in the transport and storage of oxygen (hemoglobin and myoglobin), electron transfer (cytochromes and chlorophylls) and biocatalysis (coenzyme B₁₂ and cytochrome P₄₅₀) [1]. These macrocycles, in particular the fluorinated analogues, can occur in diverse forms, such as porphyrins, 2,3-dihydroporphyrins (also named chlorins) and metal complexes, offering a very versatile platform for applications in photodynamic therapy, diagnosis, as models of naturally occurring compounds [2], and in catalysis [3].

In the present work we describe the synthesis and characterization of the binuclear μ -oxodiiron(III) complex of *meso*-tetrakis(pentafluorophenyl)porphyrin, μ -O(FeTPFPP)₂, which will be further applied as catalyst for the oxidative desulfurization of a model oil containing various benzothiophenes derivatives.

The structure of the porphyrin (Fig. 1) was unequivocally confirmed by single-crystal X-ray diffraction, UV/Vis spectroscopy and mass spectrometry. Experimental procedures and spectroscopic data of the new macrocycle will be presented and discussed.

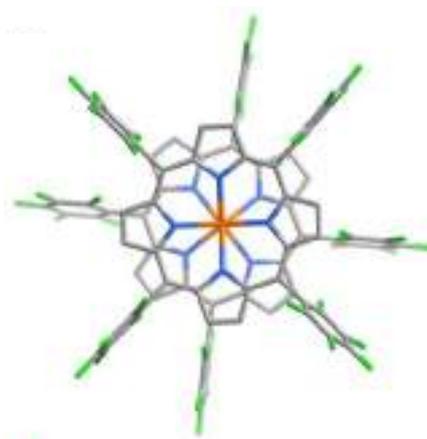


Fig 1

Acknowledgments: The authors acknowledge the Fundação para a Ciência e a Tecnologia (FCT, MEC, Portugal) for their general financial support through the strategic projects Pest C/EQB/LA0006/2011 and NORTE-07-0124-FEDER-000067-Nanochemistry (to Associated Laboratory REQUIMTE), and the R&D project PTDC/EQU-EQU/121677/2010.

References:

- [1] Wijesekera, T. P., Dolphin, D. (1985) *Some Preparations and Properties of Porphyrins*, *Advances in Experimental Medicine and Biology*, 193, 229-266.
- [2] Goslinski, T., Piskorz, J. (2011) *Fluorinated porphyrinoids and their biomedical applications*, *Journal of Photochemistry and Photobiology C: Photochemistry Reviews* 12, 304– 321.
- [3] Yi, W.-B., Ma, J.-J., Jiang, L.-Q., Cai, C., Zhang, W. (2014) *Synthesis and uses of fluorous and highly fluorinated macrocyclic and spherical molecules*, *Journal of Fluorine Chemistry* 157, 84–105.

Efficient oxidative desulfurization process catalysed by $PW_{11}Zn@MIL-101(Cr)$

D. Julião¹, B. Castro¹ and S. S. Balula¹

¹ REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal.

Sulfur content specifications in fuels are becoming increasingly stringent worldwide because upon combustion sulfur is converted to sulfur oxide (SO_x) which contributes to air pollution and acid rain. Therefore the desulfurization of fuels has become an important worldwide subject. One of the most promising desulfurization processes is the oxidative desulfurization (ODS) because it performed under very mild reaction conditions to remove the refractory sulfur compounds, dibenzothiophene (DBT) and its derivatives such as 1-benzothiophene (1-BT) and 4,6-dimethyldibenzothiophene (4,6-DMDBT). Generally, the ODS processes involve oxidizing sulfur compounds in a first step to transform them in the respective sulfone and/or sulfoxide, which are then easily removed with polar organic solvents. As alternatives to flammable and volatile organic compounds, the ionic liquids (ILs) that have been successfully applied as efficient “green” extracting solvents to ODS process. [1,2] The agent oxidant more commonly used in ODS is the hydrogen peroxide because is environmentally compatible. Polyoxometalates (POMs) have been attracting the worldwide due to its good results in ODS. Despite the good activity in ODS, these catalysts have as main obstacle their solubility, preventing their recovery. Therefore new strategies for converting soluble POMs to solid materials have been developed. Metal-organic frameworks (MOFs) such as MIL-101 exhibit an unique combination of properties, as high porosity combined with high thermal and chemical stability that make it an excellent candidate for supporting POMs by encapsulation, facilitating recovering of catalyst. [3]

In the present work, the efficiency of composite $PW_{11}Zn@MIL-101(Cr)$ formed by the encapsulation of $[PW_{11}Zn(H_2O)O_{39}]^{5-}$ into MIL-101(Cr) for the ODS process was studied using a model fuel oil containing various sulfur refractory compounds. The extracting solvent used was the IL (1-Butyl-3-methylimidazolium hexafluorophosphate, BMIPF₆) and H₂O₂ as oxidant. The composite was recycled for three consecutive cycles.

Acknowledgements:

The authors thank to FCT for the financial support through the strategic project Pest C/EQB/LA0006/2011 (REQUIMTE) and the R&D project PTDC/EQU-EQU/121677/2010.

References:

- [1] Pawelec, B, Navarro, R. M., Campos-Martin, J. M., Fierro, J.L.G., (2011), *Towards near zero-sulfur liquid fuels: a respective review*, Catalysis Science and Technology, 1, 23-4.
- [2] Ribeiro, S., Barbosa, A. D. S., Gomes, A. C., Pillinger, M., Gonçalves, I. S., Cunha-Silva, L., Balula, S.S., (2013) *Catalytic oxidative desulfurization systems based on Keggin phosphotungstate and metal-organic framework MIL-101*, Fuel Processing Technology, 116, 350-357.
- [3] Ribeiro, S., Granadeiro, C.M., Silva, P., Paz, F.A.A., Biani, F.F., Cunha-Silva, L., Balula, S.S., (2013), *An efficient oxidative desulfurization process using terbium-polyoxometalate@MIL-101(Cr)*, Catalysis Science and Technology, 3, 2404-2414.

Application of an Fe^{III} dimeric porphyrin as catalyst for oxidative desulfurization

Susana Ribeiro,¹ António Aguiar,¹ André M. N. Silva,¹ Luís Cunha-Silva,¹ Baltazar de Castro,¹ Ana M. G. Silva,^{1*} and Salete S. Balula,¹

¹ REQUIMTE & Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal

The oxidative desulfurization (ODS) is one of the most promising processes to meet the limit values of sulfur content in diesel. The presence of an efficient catalyst in the oxidation step of the ODS procedure is crucial for the success of this process. From the industrial point of view, it is also important to design novel efficient catalysts that could be recycled in successive cycles [1,2].

In this work, the porphyrin μ -O(FeTPFPP)₂ (TPFPP means *meso*-tetrakis(pentafluorophenyl)porphyrin) was used for the first time as efficient catalyst for the oxidative desulfurization of a model oil formed by the most refractory sulfur compounds present in fuels (dibenzothiophene, DBT, 1-benzothiophene, 1-BT, and 4,6-dimethyldibenzothiophene, 4,6-DMDBT), using a biphasic system model oil/extraction solvent. An optimization of different parameters was performed, namely the nature of the extraction solvent, amount of H₂O₂ as oxidant and reaction temperature. Acetonitrile and methanol revealed to be the best extraction solvents and a total desulfurization was achieved after 2 h. The best conditions, to reach the highest catalytic efficiency of μ -O(FeTPFPP)₂, were in the presence of a residual amount of oxidant (0.5 μ mol) at room temperature. The catalytic efficiency of the porphyrin was evaluated for each refractory sulfur compound following the order 1-BT > DBT > 4,6-DMDBT. Unpredictably, the porphyrin presents a remarkable catalytic performance for the desulfurization of 1-BT when compared with other catalysts already reported in the literature. Furthermore, the extracting phase containing the porphyrin could be reused in consecutive desulfurization cycles.

Acknowledgments

The authors thanks the FCT for the financial support through the strategic project Pest C/EQB/LA0006/2011 (REQUIMTE) and the R&D project PTDC/EQU-EQU/121677/2010.

References:

- [1] Ribeiro, S., Granadeiro, C. M., Silva, P.; Almeida Paz, F. A., de Biani, F. F., Cunha-Silva, L., Balula, S., (2013) *An efficient oxidative desulfurization process using terbium-polyoxometalate@MIL-101(Cr)*, Catal. Sci. Technol., 3, 2404-2014.
- [2] Zhou, X.R., et al., (2011) *Catalytic oxygenation of dibenzothiophenes to sulfones based on Fe-III porphyrin complex*, Applied Catalysis a-General, 396 (1-2), 101-106.

The use of a biosensor for the antioxidant determination in vegetables extract

Sofia Costa¹, Miguel S.B. Gonçalves¹, Helder Silva¹, Rita C. Alves^{1,2}, Manuela Correia¹, M. Fátima Barroso^{1,2}, M.B.P.P. Oliveira², Cristina Delerue-Matos¹

¹ REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal.

² REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Numerous epidemiological studies indicate that diet rich in phytochemicals and antioxidants execute a protective role in health and disease. Furthermore, fruit and vegetable based diet reduces the risk for development of chronic diseases, such as cancer, coronary heart disease, obesity, diabetes etc. [1]. The major groups of biologically active compounds that may contribute to the total antioxidant capacity (TAC) of vegetables include different group of polyphenols carotenoids and vitamins [2].

In the literature, several analytical methods are proposed for the quali-/quantitative evaluation of the TAC in different matrices. These methodologies are based on UV-vis spectrometry, chemiluminescence, fluorimetry, chromatography and electrochemistry techniques.

In this work, an electrochemical biosensor, was used in order to assess the TAC of vegetables extract. The performance of the developed biosensor consisted on the deoxyadenylic acid oligonucleotide (dA) immobilization onto carbon paste electrodes (CPE), followed by the study of the protective effect promoted by the vegetable extract in the presence of hydroxyl radical onto the dA-CPE. The electrochemical signal of the dA was measured by using square wave voltammetry (SWV).

Acknowledgments: Authors acknowledge financial support given by University of Porto through “Projetos Pluridisciplinares “PP-IJUP2011-276” and PEst-C/EQB/LA0006/2013”. M. F. Barroso is grateful for the pos-doc fellowship (SFRH/BPD/78845/2011) financed by POPH -QREN - Tipologia 4.1 - Formação Avançada, subsidized by Fundo Social Europeu and Ministério da Ciência, Tecnologia e Ensino Superior.

References:

- [1] Arts, I.C. and Hollman, PC. (2005), *Polyphenols and disease risk in epidemiologic studies*, American Journal of Clinical Nutrition, 81, 317S–325S.
- [2] Dragović-Uzelac, V., Kovačević, D.B., Levaj, B., Pedisić, S., Mezak, M. and Tomljenović, A. (2009), *Polyphenols and antioxidant capacity in fruit and vegetables common in the croatian diet*, Agriculturae Conspectus Scientificus, 74, 175-179.

Mexican four-eyed octopus species: nutritional value and metal contaminants

Marta Oliveira, Filipa Gomes, Vanessa Moreira, Cristina Delerue-Matos and Simone Morais*

REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal; *sbm@isep.ipp.pt.

Octopus species are highly distributed, living at various habitats from the coastline to the edge of the continental shelf and up to 200 m depth. They present no or very small capability of metabolization, feed mainly on small bivalves and crustaceans and live in direct contact with the substratum. Consequently, octopus may represent a significant source of toxic elements to high consumers of cephalopods species, such as the Portuguese and Mediterranean populations [1].

Thus, this study aimed to characterize the non-essential and toxic (Cd, Pb, As and Ni), and essential (Zn, Cu, Fe, Mn, Cr, Ca, Mg, Na, K and P) metallic elements in the edible tissues of Mexican four-eyed octopus (*Eledone cirrhosa*) from different geographical origins (Eastern and Western Central Atlantic Ocean), available in the Portuguese markets. Another goal of this study was to assess the potential health benefits/risks derived from octopus consumption.

The health benefits derived from octopus consumption were assessed based on daily minerals intake. The results indicated that the selected octopus species can contribute significantly to the intake of Cu, Na, Mg, K, P, Ni and Zn.

The high toxicity presented by Cd, Pb and inorganic As justified the assessment of their estimated weekly intake (EWI), target hazard quotients (THQ) and carcinogenic risks (TR) according to the methodology provided in the US EPA Region III Risk-based Concentration table [2]. Values were calculated based on Portuguese cephalopod consumptions [3], 4.1 kg per capita per year, since no specific data concerning octopus were found. Lower EWI values than PTWI were found for these three elements (PTWI of 7 µg/kg bw for Cd, 25 µg/kg bw for Pb and 15 µg/kg bw for inorganic As). Overall, THQ and TR values indicated that these species are safe for human consumption.

Acknowledgments:

This work was supported through the *FCT* project PTDC/AGR-AAM/102316/2008 (COMPETE and co-financed by FEDER), and by the IJUP project PP_IJUP2011 34 “Octopus: evaluation of food safety and pollution biomarkers”. M. Oliveira is grateful to FCT for her fellowship SFRH/BD/80113/2011.

References:

- [1] Gomes, F., Oliveira, M., Ramalhosa, M.J., Delerue-Matos, C. and Morais, S. (2013), *Polycyclic Aromatic Hydrocarbons in Commercial Squids from Different Geographical Origins: Levels and Risks for Human Consumption*, Food and Chemical Toxicology, 59, 46-54.
- [2] US EPA, 2010, Risk-Based Concentration Table (available from: <http://www.epa.gov/reg3hwmd/risk/human/index.htm>).
- [3] FAO (2012), 1961-2009 Fish and Fishery Products: World Apparent Consumption Statistics Based on Food Balance Sheets. FAO Yearbook, Fishery and Aquaculture Statistics, Food and Agriculture Organization of the United Nations, Rome.

Electrochemical study of the DNA damage induced by hypochlorous acid and its protection promoted by dietary antioxidants

Diana Cruz¹, Miguel S.B. Golçalves¹, M. Fátima Barroso¹, M. J. Ramalhosa¹, Abel J. Duarte¹, C. Delerue-Matos¹

¹ REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal.

The hypochlorous acid (HClO) belongs to the group of nonradical compounds that are produced in the living cell and presents high reactivity. The presence of the enzyme myeloperoxidase in the cell leads to production of HClO by interaction between hydrogen peroxides (H₂O₂) and chlorides (Cl⁻) (Eq. 1). Numerous pathologies and disease states serve as sources for the continuous production of free radicals and nonradicals [1].



Foodstuffs have an important external contribution to body defenses against oxidative stress. It provides cell specific antioxidants that are able to scavenge multiple types of oxidants contributing to maintain cellular health.

In this work, an electrochemical DNA-based biosensor was developed in order to assess the total antioxidant capacity (TAC) of beverages.

The design of this DNA-biosensor consisted in four steps: i) deoxyadenylic acid oligonucleotide (dA) immobilization onto carbon paste electrodes (CPE); ii) Damage of the dA by the immersion of the bioelectrode on HClO; iii) Protective effect promoted by antioxidants (ascorbic acid), in the presence of HClO, onto de dA-CPE; iv) Detection and measurement of the oxidation peak current of the dA using square wave voltammetry (SWV). This DNA-biosensor was used to assess the TAC of orange juices.

Acknowledgments: Authors acknowledge financial support “PEst-C/EQB/LA0006/2013”. M. F. Barroso is grateful for the pos-doc fellowship (SFRH/BPD/78845/2011) financed by POPH -QREN - Tipologia 4.1 - Formação Avançada, subsidized by Fundo Social Europeu and Ministério da Ciência, Tecnologia e Ensino Superior.

References:

[1] Kohen, R. and Nyska, A. (2002), *Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification*, Toxicologic Pathology, 30 (6), 620-650.

Brewer's spent grain valorisation: extraction and characterization of natural antioxidants

V. Castro¹, M. Moreira², S. Morais¹, C. Delerue-Matos¹ and L. F. Guido²

¹ REQUIMTE, Departamento de Engenharia Química, Instituto Superior de Engenharia do Porto, Portugal.

² REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Portugal.

Brewer's spent grain (BSG) is the major by-product of the brewing industry, representing approximately 85% of the total generated by-products. BSG is still underexploited, mainly used as cattle feeding, although it is available in large quantities all over the year. BSG real value as a source of a marketable product seems to be underestimated, since it is rich in oligo- and polysaccharides, as well as in phenolic compounds [1, 2]. Phenolic compounds, in particular, are of considerable interest to scientists, manufacturers and consumers due to their importance on food quality, as well as their protective and preventive roles in certain types of cancer and several other chronic diseases [3]. Such compounds are included in different major groups, among them phenolic acids, such as ferulic and *p*-coumaric acids, which are associated with cell wall constituents, especially with arabinoxylans and lignin [1].

The present work aimed at developing a microwave-assisted extraction (MAE) method for the extraction of phenolic compounds from BSG. The influence of the MAE operational parameters (extraction time, temperature, solvent volume and stirring speed) on the extraction yield of ferulic acid (FA) was investigated through response surface methodology. The results showed that the optimal conditions were 15 min extraction time, 100 °C, 20 mL of solvent, and maximum stirring speed. Under these conditions, the yield of FA was 1.31±0.04% (w/w), which was 5-fold higher than that obtained with conventional solid-liquid extraction techniques. The developed MAE method was applied to BSG obtained from light (*pilsen*, *melano*, *melano 80* and *carared*) and dark (*chocolate* and *black*) malts in order to evaluate their antioxidant properties and phenolic composition. The results obtained suggest that the type of malt used as well as the malting process may have important impact in terms of the phenolic composition and antioxidant features of BSG. Amongst the BSG studied, extracts from light types (malt kilning temperatures ≤ 160 °C) contained higher amounts of total and individual phenolic compounds, in particular *pilsen* BSG (20 ± 1 mg GAE/g DW BSG) [4].

Acknowledgments

This work has been supported by Fundação para a Ciência e a Tecnologia (FCT) through grant no. PEst-C/EQB/LA0006/634/2011, by QREN (NORTE-07-0124-FEDER-000069- Ciência do Alimento), as well as by Santander (PP_IJUP2011-51). The supply of the BSG is acknowledged to UNICER-Bebidas de Portugal, S.A. and Os Três Cervejeiros, Lda.

References

- [1] Meneses N.G.T., Martins, S., Teixeira J.A. and Mussatto S.I. (2013), *Separation and Purification Technology*, 108, 152–158.
- [2] Moreira M.M., Morais S., Barros A.A., Delerue-Matos C., Guido L.F. (2012), *Analytical and Bioanalytical Chemistry*, 403, 1019-1029.
- [3] Athanasios M., Georgios L., Michael K. (2007), *Food Chemistry*, 102, 606-611.
- [4] Moreira M.M., Morais S., Carvalho D.O., Barros A.A., Delerue-Matos C., Guido L.F. (2013). *Food Research International*, 54, 382-388.

Development of an automated analytical system for the calibration of micro-fiber electrodes sensitive to NO

Estudante: Patrícia Dias

Supervisores: Célia Amorim, Alberto Araújo, Conceição Branco

REQUIMTE/ Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal. R. Jorge Viterbo Ferreira, 228; 4050-313 Porto, Portugal

Nitric oxide (NO) mediates multiple molecular physiological processes including vasodilatation, neurotransmission, inflammation, thrombosis, and immunity. Moreover it can be considered as a potential marker for hepatocellular carcinoma. One of the major difficulties of measuring biological levels of NO is related with the calibration of the sensors in use. In fact the presence of O₂ *in vivo* and *in vitro* makes the NO molecule extremely unstable due to its rapid oxidation. *In vivo*, NO behaves as a diatomic free radical that is extremely short lived due to the presence of O₂. *In vitro* is extremely difficult to prepare calibrating solutions with low concentration in order to access the response of sensor.

In this project it is aimed the preparation of low concentrated NO calibrating solutions and sensor response evaluation in a full automatic fashion. To this a multi-task sequential injection system is being optimized where the Griess diazotization reaction is used to quantify spectrometrically NO₂ formed by oxidation of NO under physiological conditions.

*Estudante do Núcleo de Iniciação à Investigação

Quality evaluation of honey: diastase activity and 5-hydroxymethylfurfural

**D. M. D. Nascimento^{1,2}, L. P. M. Ribeiro^{1,2}, S. E. F. Soares², A. S. G. Costa²,
L. M. Cunha¹, R. C. Alves^{1,3}, M. B. P. P. Oliveira²**

¹ REQUIMTE, DGAOT, Faculty of Sciences, University of Porto, Portugal.

² REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

³ REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

Honey is an ancient valuable natural product due to its therapeutic potential and organoleptic properties. Its adulteration is a real problem with a significant economic impact and possible nutritional and organoleptic damages [1]. The content of 5-hydroxymethylfurfural (HMF) and diastase activity are the main parameters to be taken into account in the assessment of honey quality and freshness. In general, a high quality honey must have a low HMF content and a high diastase activity [2].

The present work aimed to evaluate these two quality parameters in 10 Portuguese honeys, produced by *Apis Mellifera* bees, from different floral and geographical origins. The HMF content was assessed by RP-HPLC-DAD [3] and the diastase activity was determined according to the AOAC method 958.09 [4].

The obtained results for the studied honey samples are within the limits established by legislation [5, 6]. The diastase activity ranged from 9.6 to 37.8 on the Gothe's scale, being these differences probably related to the geographical and floral origins of the samples. The HMF values ranged from 0.8 to 18.4 mg/ kg of honey.

The values attained for both parameters were typical of fresh and unprocessed honeys.

References:

- [1] Mehryar, L. and Esmaili, M. (2011), *Honey & Honey Adulteration Detection: A Review*, Department of Food Science and Technology, University of Urmia, Iran.
- [2] Tosi, E., Martinet, R., Ortega, M., Lucero, H. and Ré, E. (2008), *Honey diastase activity modified by heating*, Food Chemistry, 106, pp. 883-887.
- [3] Lemos, G. D. S., Santos, J. S. D. and Santos, M.L.P.D. (2010), *Validação de método para a determinação de 5-hidroximetilfurfural em mel por cromatografia líquida e sua influência na qualidade do produto*, Química Nova, 33, pp. 1682-1685.
- [4] AOAC, (2010), *Official Methods of Analysis*, Association of Official Analytical Chemists, Arlington, USA, 44, pp. 36.
- [5] Diretiva 2001/110/CE do conselho de 20 de Dezembro de 2001 relativa do mel. Jornal Oficial das Comunidades Europeias, 10, pp. 47-52.
- [6] Decreto de lei nº 131/ 85 de 29 de Abril, Diário da República Iª Série.

Acknowledgments: S. Soares and R. Alves are grateful to FCT for a PhD grant (SFRH/BD/75091/2010) and a post-doc grant (SFRH/BPD/68883/2010), respectively. This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069-CIÊNCIA DO ALIMENTO).

Polyoxometalates: synthesis, characterization and incorporation into multilayer films with organic dyes

A. Teixeira, H. Teixeira, D. M. Fernandes and C. Freire

REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto,
4169-007 Porto, Portugal

Polyoxometalates (POMs) are a group of metal-oxygen clusters with unique properties [1,2]. Among them, the ability to reversibly attract and release a large number of electrons under marginal structural rearrangement producing the mixed-valence coloured species makes them suitable materials for different applications [2,3]. Nevertheless, for practical applications, these must be processed as thin films. Layer-by-layer (LbL) self-assembly is based in electrostatic interactions between oppositely charged ions [4] and has proved to be a promising bottom-up method for the preparation of multilayer films because it is of simple execution and versatile from the point of view of thickness and composition control.

The potassium salts of cobalt-substituted sandwich-type polyoxometalate $K_{10}[Co_4(H_2O)_2(PW_9O_{34})_2] \cdot xH_2O$ ($Co_4(PW_9)_2$) and vanadium-substituted Keggin-type $K_5[PMo_{10}V_2O_{40}] \cdot xH_2O$ ($PMo_{10}V_2$) were prepared by adapted procedures from the literature [5,6]. Both compounds were fully characterized by several techniques such as elemental analysis, FT-IR spectroscopy, UV-Vis spectroscopy and ^{31}P and ^{51}V NMR (for $PMo_{10}V_2$), confirming the synthesis of the desired compounds.

The polyoxometalates were then incorporated into films by LbL method using cationic poly(ethylenimine) (PEI) as anchoring layer, the POMs as polyanions, and the organic dyes, Methyl Green (MG) and Pararosaniline (PR), as polycations. Two types of films were produced: $\{MG/Co_4(PW_9)_2\}_n$ and $\{PR/PMo_{10}V_2\}_n$. The film growth process was monitored by UV-vis spectroscopy. The characteristic absorption bands of POMs and dyes observed in the UV-vis spectra confirm the incorporation of both components into the LbL films. Furthermore the linear increase of *Abs* vs. number of layers suggested a regular growth process and also a strong interaction between the charged multilayers [4].

Acknowledgements The work was funded by FCT (Portugal) and FEDER, through proj. ref PTDC/CTM-POL/0813/ 2012, Operation NORTE-07-0124-FEDER-000067–Nanochemistry and to COST Action CM-1203 PoCheMoN. DMF also thanks FCT for her PD grant SFRH/BPD/74877/2010.

References:

- [1] Pope, M.T. (1983) Heteropoly and Isopoly Oxometalates, Springer Verlag, Berlin.
- [2] Sadakane, M., Steckhan, E. (1998) Chem. Rev. 98, 219-237.
- [3] Yamase, T. (1998), Chem. Rev. 98, 307-325.
- [4] Fernandes, D. M., Julião D., Pereira, C., Ananias, D., Balula, S. S., Freire C. (2012) Colloids and Surfaces A: Physicochem. Eng. Aspects 415, 302-309.
- [5] Finke, R.G., Droege, M.W., Domaille, P.J. (1987), Inorg. Chem. 26 (23), 3886-3896.
- [6] Himeno, S., Ishio, N. (1998) Journal of Electroanalytical Chemistry 451, 203-209.

Chiral xanthenes: Synthesis, biological evaluation and molecular docking as inhibitors of P-glycoprotein (Pgp), phospholipase A2 (PLA2) and cicloxygenases (COX-1 and 2)*

D. Torres^{1,2*}, **A. Marques**^{1,2}, **S. Monteiro**^{1,2}, **C. Carneiro**^{1,2}, **A. Santos**^{1,2},
C. Fernandes^{1,2,3}, **A. Palmeira**^{1,2}, **M.H. Vasconcelos**^{4,5}, **S. Reis**⁶ and **M. Pinto**^{1,2,3}

¹ Department of Chemistry, Laboratory of Organic and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

² CEQUIMED-UP, Center of Medicinal Chemistry, University of Porto, Portugal.

³ CIIMAR/CIMAR, University of Porto, Portugal.

⁴ Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

⁵ IPATIMUP, Cancer Biology Group, University of Porto, Portugal.

⁶ REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

* PROJETO PP_IJUP2011_19

The chiral derivatives of xanthenes (CDXs) present a pleiade of interesting pharmacological activities, namely anti-inflammatory, antitumor and anti-Pgp [1]. Considering that only few examples of chiral xanthenes were described, and our group has a vast experience in this field [2,3], we proposed to synthesize new CDXs, in an enantiomeric pure form, and to evaluate the biological/pharmacological activities of each enantiomer face to biotargets. The enantiomeric purity of the new CDXs was successfully established by HPLC using chiral chromatographic conditions.

Our library of CDXs was evaluated for antitumor activity, as well as inhibitors of P-glycoprotein (Pgp) and enzymes involved in inflammation, namely phospholipase A2 (PLA2), cicloxygenases (COX-1 and COX-2).

In order to understand the interactions of the CDXs with the active site of the biological targets and the structural features associated with the chiral recognition, molecular modeling studies were carried out by molecular docking technique.

The results allowed discover new CDXs with antitumor and anti-inflammatory activities, and clarify the molecular mechanisms of the recognition between the enantiomers and the targets. As a consequence, new CDXs could be designed by analogue-based design strategy.

[1] Pinto, M. and Sousa, M. (2005), *Xanthone Derivatives: New Insights in Biological Activities.* Curr. Med. Chem., 12 (21), 2517-2538.

[2] Fernandes, C., Masawang, K., Tiritan, M.E., Sousa, E., Lima, V., Afonso, C., Bousbaa, H., Sudprasert, W., Pedro, M., Pinto, M.M. (2013), *New chiral derivatives of xanthenes: synthesis and investigation of enantioselectivity as inhibitors of growth of human tumor cell lines*, Bioog. Med. Chem., DOI: 10.1016/j.bmc.2013.12.042.

[3] Fernandes, C., Oliveira, L., Tiritan, M.E., Leitão, L., Pozzi, A., Noronha-Matos, J.B., Correia-de-Sá, P., Pinto, M.M. (2012), *Synthesis of new chiral xanthone derivatives acting as nerve conduction blockers in the rat sciatic nerve*, Eur. J. Med. Chem., 55, 1-11.

Acknowledgments: FCT - Fundação para a Ciência e a Tecnologia under the project CEQUIMED-Pest OE/SAU/UI4040/2011, U. Porto, Santander Totta (Proj. PP_ijup2011_19), for financial support.

Influence of adipocytes in prostate cancer cells proliferation, invasion and migration profile

Â. Moreira^{1,2}, S. S. Pereira¹, M. Costa¹, T. Morais¹, R. Fernandes², M. P. Monteiro¹

¹Department of Anatomy and UMIB (Unit for Multidisciplinary Biomedical Research) of ICBAS, University of Porto, Portugal.

²Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, Portugal.

Introduction: Prostate cancer is the second most frequent cancer and the sixth leading cause of death from cancer in men worldwide [1]. Despite the high morbidity, its etiology is still unknown [2]. Obesity is associated with increased cancer incidence and mortality, though the association between prostate cancer and obesity is still controversial [3]. Changes in expression of adipokines associated with obesity have been one of the several mechanisms proposed to explain the association between obesity and prostate cancer, particularly in promoting the development and progression of the tumor cells[4].

Aim: The main goal of this study is to evaluate the effect of preadipocyte and adipocyte secretome in the proliferation, migration and invasion of androgen insensitive prostate carcinoma murine cells (RM1).

Methods: RM1 cells were co-cultured in transwells system with preadipocytes and adipocytes and cultured in their conditioned medium (CM). RM1 cell proliferation was assessed by flow cytometer and by performing XTT viability tests. An injury assay, Wound Healing was performed in RM1 cells cultured with conditioned media. Cellular invasion was assessed by using transwells systems, with matrigel membrane, when cultured with adipocytes and preadipocytes.

Results: Preadipocyte conditioned media slightly increased RM1 proliferation, while 1 and 2 days conditioned media of adipocytes significantly increased the RM1 cell proliferation ($p < 0.01$). For cell migration, there was a significant increase in migration of RM1 cells cultured with adipocyte (ACM) and preadipocytes (PACM) conditioned media when compared to control ($p < 0.01$). A significant increase in RM1 cell invasion was observed in cells cultured with adipocytes and preadipocytes ($p < 0.05$) and adipocytes significantly increased the RM1 cells proliferation in co-culture ($p < 0.01$).

Conclusion: Thus, prostate carcinoma RM1 cells seem to be influenced by factors secreted by adipocytes which are able to increase their ability to proliferate, migrate and invade.

UMIB is funded by FCT (Fcomp-01-0124-FEDER-015893).

1. Verma, M., P. Patel, and M. Verma, *Biomarkers in Prostate Cancer Epidemiology*. Cancers, 2011. **3**(4): p. 3773-3798.
2. Braun, D.P., D. Gupta, and E.D. Staren, *Predicting survival in prostate cancer: the role of quality of life assessment*. Support Care Cancer, 2012. **20**(6): p. 1267-74.
3. Ribeiro, A.M., et al., *Insulin prevents leptin inhibition of RM1 prostate cancer cell growth*. Pathol Oncol Res, 2012. **18**(2): p. 499-507.
4. Nieman, K.M., et al., *Adipose tissue and adipocytes support tumorigenesis and metastasis*. Biochim Biophys Acta, 2013.

Evaluation of hydration efficacy of a hand cream containing a coffee silverskin extract

C. Silva¹, M.H. Amaral¹, F. Rodrigues², A. C. Martins¹, A. Brito¹, A. S. Ribeiro¹, S. Silva¹, M.B.P.P. Oliveira², J.M. Sousa Lobo¹

¹ Departamento de Ciências do Medicamento, Laboratório de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

² REQUIMTE / Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

The skin of the hands is daily subjected to various types of damage caused by exposure to chemicals, low environmental temperature, and low humidity. The disruption of barrier function may lead to skin aging and irritation [1]. The coffee silverskin (SS) is a tegument of coffee beans which constitutes a by-product of the roasting procedure. Due to the antioxidant activity of coffee silverskin, this by-product can confer anti-aging action when included in a moisturizing hand cream [2].

The aim of this work was the *in vivo* evaluation of the hydration efficacy of a hand cream containing a coffee silverskin extract by measuring different skin surface parameters after cutaneous application of this cosmetic formulation during one month.

The *in vivo* assessment of the efficacy of the hand cream containing a coffee silverskin extract was performed after its application in ten healthy skin Caucasian volunteers, of both sexes. Volunteers were instructed to apply the formulation in one hand, once a day during one month. The hand with no formulation application was used as a control. To assess the effects on hydration after application of the hand cream the following skin surface parameters were evaluated: skin hydration, using a Multi Probe Adapter[®] System (Courage-Khazaka, Germany); and micro-relief (scaliness, smoothness, roughness and energy), using Visioscan[®] VC98 (Courage-Khazaka, Germany). All the measurements were performed in triplicate on days 0 (before the cream application) and 30 (after the cream application).

The study of the efficacy of the hand cream containing silverskin extract did not show significant improvements in the skin parameters analyzed after application of the product for 30 days. However, to draw more reliable conclusions it would be necessary to perform these *in vivo* studies in a larger number of volunteers and with a more advanced age, so that in addition to hydration can also be proven the anti-aging effect.

References:

[1] Lóden, M. (2003), Role of Topical Emollients and Moisturizers in the Treatment of Dry Skin Barrier Disorders, *American Journal of Clinical Dermatology*, 4 (11), pp. 771-788.

[2] Yusaku N., Kuniyo I. (2012), High antioxidant activity of coffee silverskin extracts obtained by the treatment of coffee silverskin with subcritical water, *Food Chemistry*, 135, pp. 943-949.

Acknowledgments to Projeto Pluridisciplinar PP_IJUP2011_239

“Nos Copains les Légumes!” – Effects of an intervention program on the consumption and appreciation of vegetables in preschool and primary school French children

K. Dias¹, R. Poínhos², B. Pereira³

¹ Graduated in Nutritional Sciences, Faculty of Nutrition and Food Sciences, University of Porto, Portugal

² Nutritionist, guest assistant at the Faculty of Nutrition and Food Sciences, University of Porto, Portugal

³ Guest assistant at the Faculty of Nutrition and Food Sciences, University of Porto, Portugal

Results from European studies show that vegetable intake is low in the population, namely in children [1]. Adequate consumption of vegetables seems to be associated with a reduced risk of many chronic diseases [2]. Since children's preferences and food habits will define their choices when adults [3], it is important to promote healthy choices in youngsters, namely through nutrition education. The present work consisted in the implementation of an intervention project, aiming to raise awareness for the consumption of vegetables in preschool and primary school children. The effects of the intervention on the consumption and the appreciation of vegetables were evaluated.

Nutrition education sessions were held in the school. Children (or their caregivers) answered baseline and follow-up questionnaires to assess the average frequency of consumption and the appreciation of vegetables. Final sample consisted of 71 children (20 preschoolers and 51 3rd graders). Data were analyzed on SPSS[®] version 21.0 for Macintosh.

There were no significant differences in the mean frequencies of consumption and in the appreciation of the studied vegetables in the preschoolers. Among 3rd graders, there was a significant increase in the frequency of consumption of tomato. The appreciation of broccoli and vegetables in general has increased significantly in this group, and the appreciation of the remaining vegetables has shown no differences.

Albeit the short period of intervention, these results are promising and an incentive to the continuity of the project, with a bigger sample and more time available for intervention.

References:

[1] Yngve, A., Wolf, A., Poortvliet, E., Elmadfa, I., Brug, J., Ehrenblad, B. et al (2005), *Fruit and vegetable intake in a sample of 11-year-old children in 9 European countries: The Pro-Children Cross-sectional Survey*, Annals of Nutrition and Metabolism, 49:236-45.

[2] World Health Organization (2003), *Diet, nutrition and the Prevention of Chronic diseases*, WHO Technical Report Series, 916.

[3] Perez-Rodrigo, C. and Aranceta, J. (2001), *School-based nutrition education: lessons learned and new perspectives*, Public Health Nutrition, 4(1A):131-39.

Mutation analysis of genes involved in sperm motility: A study in patients with total sperm immotility

R. Pereira^{1,2}, J. Oliveira³, R. Santos³, A. Alves², E. Oliveira², L. Ferraz⁴, A. Barros⁵, M. Sousa²

¹Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Portugal;

² Departamento de Microscopia, Laboratório de Biologia Celular, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), UMIB-FCT, Universidade do Porto, Portugal;

³ Unidade de Genética Molecular, Centro de Genética Médica Dr. Jacinto Magalhães, Centro Hospitalar do Porto (CHP), Portugal;

⁴ Departamento de Urologia (Diretor), Centro Hospitalar de Vila Nova de Gaia, Portugal;

⁵ Centro de Genética da reprodução Prof. Alberto Barros, Porto, Portugal

Reduced sperm motility represents one of the major male causes of infertility. The axoneme (Ax) is the flagellar motor of the sperm cell and several mutations in genes involved in the assembly and regulation of the Ax have been proved to be responsible for certain cases of infertility associated with severe sperm immotility. For instance, mutations in the genes *CCDC39*, *CCDC40*, *DNAI1* and *DNAH5* (1,2) are associated with primary ciliary dyskinesia (PCD), that is a disease whose typical diagnostic features include infertility (3). Fibrous Sheath Dysplasia (FSD) is a flagellar pathology, which causes total sperm immotility (4) and studies suggest that mutations in *AKAP3* and *AKAP4* genes might be associated to FSD (5,6). In a group of five Portuguese patients from Assisted Reproductive Medicine centers that presented totally sperm immotility, transmission electron microscopy revealed several structural defects in sperm flagellum. Given the importance of *CCDC39*, *CCDC40*, *DNAH5*, *DNAI1*, *AKAP3* and *AKAP4* genes in sperm motility, we decided to screen these genes in our patients. To identify genetic alterations that could explain their phenotype, we initiated the analysis of the exonic regions of these 6 genes by Sanger sequencing. We have already sequenced five genes and *DNAH5* analysis is still ongoing. Seven variants in *CCDC39*, seventeen in *CCDC40*, two in *DNAI1* and five in *AKAP3* have been identified. Seven of these thirty-one variants have not been previously described in the literature or in gene mutation databases. With this work we expect to be able to offer a differential diagnosis to the patients and find potential genetic markers for individuals with this kind of problem.

References

1. Geremek M, Witt M. (2004), *Primary ciliary dyskinesia: genes, candidate genes and chromosomal regions*. J Appl Genet 2004;45(3):347–61.
2. Antony D, et. al. (2013), *Mutations in CCDC39 and CCDC40 are the Major Cause of Primary Ciliary Dyskinesia with Axonemal Disorganization and Absent Inner Dynein Arms*. Hum Mutat;34(3):462–72.
3. Boon M, et. al. (2013), *Primary ciliary dyskinesia, an orphan disease*. Eur J Pediatr;172(2):151–62.
4. Chemes HE, Rawe VY. (2010), *The making of abnormal spermatozoa: cellular and molecular mechanisms underlying pathological spermiogenesis*. Cell Tissue Res;341(3):349–57.
5. Brown PR, et. al. (2003), *A-kinase anchoring protein 4 binding proteins in the fibrous sheath of the sperm flagellum*. Biol Reprod;68(6):2241–8.
6. Baccetti B, et. al. (2005), *Gene deletions in an infertile man with sperm fibrous sheath dysplasia*. Hum Reprod;20(10):2790–4.

Gene screening for gluten detection in food

B. Martín-Fernández^{1,2,3}, J. Costa¹, M.B.P.P. Oliveira¹, B. López-Ruiz², N. de-los-Santos-Álvarez³, I. Mafra^{1*}

¹ REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal. ² Department of Analytical Chemistry, Faculty of Pharmacy, Complutense University of Madrid, Spain. ³ Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, Spain *
E-mail: isabel.mafra@ff.up.pt

Celiac disease is the most frequently autoimmune enteropathy that occurs in genetically predisposed individuals as the result of an abnormal immune response to gluten [1]. The only known treatment is a lifelong gluten-free diet that requires the avoidance of wheat, rye, and barley [2]. Since the correct labelling of foods is the only effective way of protecting the celiac patients, it is essential to have easy and reliable methodology to verify the content of gluten in foods. So far, most of the available methods are based on protein detection, namely ELISA assays, but false negative results or cross-reactions are frequently observed. DNA analysis is an attractive alternative due to its high stability when compared to proteins. Besides this, the application of DNA-based methods for the detection of gluten in foods, like conventional polymerase chain reaction (PCR) or real-time PCR, could improve the sensitivity and specificity of the assays.

The aim of the present work was the development of three real-time PCR methods to trace toxic cereals for celiac patients in food products. Model mixtures of wheat flour in soybean flour were prepared in different known percentages (50% to 0.0001%, $n=13$). DNA was extracted using the NucleoSpin Food kit method. Yield and purity of DNA were assessed by UV spectrophotometry. The presence of amplifiable DNA was evaluated by targeting a universal eukaryotic gene. For the specific detection of gluten in foods, three nucleotide sequences encoding the allergenic proteins Tri a 18 and Tri a 25 and an alpha-type gliadin were selected from NCBI database. The targets enabled the successful development of TaqMan real-time PCR systems to detect minute amounts of wheat, barley and rye in foods. The most sensitive results were obtained with the system targeting the gene encoding gliadin, which enabled an absolute limit of detection of 2 pg of wheat DNA and a relative sensitivity down to 0.005% of wheat flour in soybean material. These results highlight the adequacy of the developed PCR systems for the detection of cereals containing gluten in processed foods.

Acknowledgments:

This work was supported by FCT grant no. PEst-C/EQB/LA0006/2013 financed by POPH-QREN (subsidised by FSE and MCTES), bilateral project E-38/12 Acções Integradas Luso-Espanholas 2012, University of Porto "Projectos Pluridisciplinares" IJUP2011-176 and Ministerio de Ciencia e Innovación (Spain) (Programa Nacional de Internacionalización de la I+D, PRI-AIBPT-2011-0769). B.M.F and N.S.A are grateful to Complutense University PhD grant (BE45/10) and Spanish Government for a Ramón y Cajal contract, respectively.

References:

- [1] Green, P.H.R. and Jabri, B. (2006), *Celiac disease*. Annual Review of Medicine, 57, 207-221.
- [2] Green, P.H.R. and Cellier, C. (2007), *Celiac disease*. The New England Journal of Medicine, 357, 1731-1743.

Evaluation of school snacks and breakfast after the implementation of the project "Lancheirinhas Saudáveis"

A. Ferreira¹, R. Poínhos², P. Bárbara²

¹ Bachelor Degree in Nutrition Sciences, University of Porto, Portugal.

² Nutritionist, Invited Assistant, Faculty of Nutrition and Food Sciences, University of Porto, Portugal.

Intervention programs in the school environment which aim to promote healthy eating habits in children are of extreme importance [1, 2]. However, their efficacy is variable [3]. Programs with multiple components that involve teachers, healthcare professionals, other community professionals, parents and other family members are, generally, more efficient [4]. However, with the cultural particularities inherent to the several regions of the country and specific characteristics of the schools, it is necessary to conduct an individual evaluation of their effectiveness [5].

This study aimed to evaluate a nutrition education program named "Lancheirinhas Saudáveis", that lasted 4 months, performed in preschool children of four schools in Trofa Municipality, through the evaluation of school snacks and breakfast before and after the intervention.

A cross-sectional study was performed. The project consisted of four educational sessions delivered in the classroom, and in a debriefing session for the parents. Data collection was performed by means of a written questionnaire delivered to the parents and by a written and photo record of the school snacks brought to school by children in one school day. The different foods were categorized into groups. Statistical analysis was conducted using SPSS© version 20.0 for Windows.

The program "Lancheirinhas Saudáveis" yielded positive results in school snacks and breakfast composition. Although the program didn't last for a long period, their results are promising and emphasize the importance of its maintenance and subsequent evaluations.

[1] Nicklas TA, Demory-Luce D, Yang SJ, Baranowski T, Zakeri I, Berenson G. (2004), *Children's food consumption patterns have changed over two decades (1973-1994): The Bogalusa heart study*. Journal of the American Dietetic Association, 104(7):1127-40.

[2] Birch LL, Fisher JO. (1998), *Development of eating behaviors among children and adolescents* [Comparative Study Review]. Pediatrics, 101(3 Pt 2):539-49.

[3] Van Cauwenberghe, E., Maes, L., Spittaels, H., van Lenthe, F. J., Brug, J., Oppert, J. M., & De Bourdeaudhuij, I. (2010), *Effectiveness of school-based interventions in Europe to promote healthy nutrition in children and adolescents: systematic review of published and 'grey' literature*. Br J Nutr, 103(6), 781-797.

[4] Summerbell CD, Moore HJ, Vogeles C, Kreichauf S, Wildgruber A, Manios Y, et al. (2012), *Evidence-based recommendations for the development of obesity prevention programs targeted at preschool children* [Research Support, Non-U.S. Gov't Review]. Obesity reviews: an official journal of the International Association for the Study of Obesity, 13 Suppl 1:129-32.

[5] Rosário H. (2011), *Excesso de peso e obesidade em crianças: implementação e avaliação de um programa de intervenção na escola*. Universidade do Minho.

The role of oocyte-secreted factors in the human granulosa cell line - GC1a

SA Reis¹, A Ribeiro^{1,2}, JL Silva-Carvalho^{1,2,3}, AM Gouveia^{1,4}, H Almeida^{1,3}

¹ Departamento de Biologia Experimental, Faculdade de Medicina, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto.

² CETI – Centro de Estudo e Tratamento da Infertilidade, Porto.

³ Hospital CUF, Porto.

⁴ Faculdade de Ciências da Nutrição e Alimentação, Universidade Do Porto.

The success of assisted reproductive techniques (ART) is partly dictated by the oocyte quality, achieved during its development. A major contribution for oocyte quality is provided by the follicular micro environment that includes the follicular fluid and the oocyte adjacent cells. These comprise granulosa cells (GCs) that multiply along oocyte growth and development and differentiate into two structurally and functionally distinct types: the mural GCs and cumulus cells (CCs). The oocyte is an indirect regulator of itself since it can modulate follicular cells function. In fact, it produces the oocyte-secreted factors (OSFs), which are TGF β superfamily growth factors that promote follicular cells survival that, in turn, modulate oocyte growth.

In order to increase our understanding on the role of OSFs in GCs, and also aiming to uncover molecules with potential properties of clinical biomarkers, we employed an immortalized human granulosa cell line, the GC1a type. This appears to circumvent considerably the lesser progress attained when primary culture of human granulosa cells are employed instead.

In this work, we aimed to characterize the dynamic process between GCs and OSFs, in GC1a cells. To investigate the presence of OSFs and their receptors in GC1a, the total mRNA was extracted and converted to cDNA to analyze gene expression by real-time PCR. To study the effects of OSFs, present in the follicular fluid, GC1a cells were incubated with different concentrations of this fluid. Firstly cells viability was determined by MTT assay and the study of cell morphology was established by microscopic analysis, assessed by actin staining.

The experiments made so far evidenced OSFs transcripts, namely GDF7, GDF9, and also the OSFs receptors, such as BMPRI, BMPRII, TGF β RI, TGF β RII, ACVR II, ALK3, reported for the first time in this cell line. Concerning the effects of follicular fluid in cell culture, the results suggest that concentrations higher than 50% of follicular fluid in the cells medium interfere with cell viability. Additionally, the follicular fluid seems to interfere with cellular morphology, since cells lose their epithelial like morphology and acquire a more closed fibroblastic shape, with cellular protrusions, suggesting that follicular fluid may induce cytoskeleton reorganization.

The data suggest that this cell line mimics CCs cells, regarding the presence of OSFs and their, and may thus be employed in the study of OSFs signaling. As a whole, the study establishes a consistent ground for future work in the TGF β transductive pathways.

Resistance to biocides in *Salmonella* from Portugal: a multilayered approach

J. Mourão¹, P. Ramos², S. Marçal², C. Novais¹, L. Peixe¹, P. Antunes^{1,2}

¹ REQUIMTE. Microbiology Laboratory, Faculty of Pharmacy, University of Porto, Portugal.

² Faculty of Nutrition and Food Science, University of Porto, Portugal.

Background. Antibiotic resistant (ABR) *Salmonella* clones and the contributions of diverse environmental stressors (e.g. metals) widely used in food animal production (feed/disinfectants/antiseptics) for their selection/persistence are a current public health concern. We aimed to characterize metal tolerance and its associated genetic elements in clinical relevant ABR *Salmonella* clonal lineages. **Methods.** Portuguese *Salmonella* non-typhoid isolates (n=121/2000-2011) from human/non-human sources belonging to 16 serotypes were selected. The isolates included the 2 most frequent serotypes, *S. Enteritidis* (n=4) and *S. Typhimurium* (n=12), and the emergent *S. 4,[5],12:i:-* (n=64) and *S. Rissen* (n=26). They are representative of different PFGE and Sequence Types (ST) and associated with particular ABR phenotypes/genotypes. Genes associated with ABR/integrations (Int), Cu^R (*pcdD*), Ag/Cu^R (*silA-silE*), Hg^R (*merA*), As^R (*arsB*) or Te^R (*terF*) were searched by PCR/sequencing. MICs to CuSO₄ and AgNO₃ were determined in aerobic/anaerobic atmospheres by agar dilution method and susceptibility to 10 antibiotics by disk diffusion methods. Conjugation assays, genomic location (I-CeuI/S1-PFGE/hybridization) and plasmid (PL) analysis (PBRT/sequencing) were done. **Results.** Metal tolerance genes *silA-silE* (69%), *pcdD* (51%), *merA* (50%), *terF* (2%) or *arsB* (1%) were found in different serotypes/clones. *S. Rissen* (ST469; 62%-*bla*TEM-*aadA2-sul1/sul3-tetA-dfrA12*) carried *pcdD+silA-silE* in chromosome (Ch) as *S. 4,[5],12:i:-* from European clone (n=22/ST34), which also have co-located ABR genes (*bla*TEM-*strA-strB-sul2-tetB*) and the majority *merA*. In contrast, *S. Typhimurium* monophasic variant of the Spanish (n=5/ST19) and Portuguese (n=5/ST19) MDR clone carried *merA* and/or *silA-silE* on large non-transferable IncA/C (130-170Kb) or IncR PL (110-140Kb) respectively. MDR *S. Typhimurium* (n=12; 5 clones; ST19/ST313) carried *silA-silE* (n=3/DT104 clone) with atypical type I-*sul3* integron on IncN PL (135Kb) or *merA* (n=4) with *int11-oxa30-aadA1/int11-aadA1* on transferable IncFII PL (120-140Kb). In *S. Enteritidis* (ST11) only *merA* (n=2/4) was detected on transferable IncP PL (80Kb) along with *int11-dfrA1-aadA1*. In isolates of other MDR clones (n=15/12 serotypes), *merA* (n=13) and/or *silA-silE+pcdD* (n=6) were co-located with different Int on large plasmids (>120Kb; IncHI1/IncP/IncI1). The *arsB* (n=1) and/or *terF* (n=3) were located on transferable IncHI2 (220Kb) with *bla*CTX-M-9 or IncP (265kb) (250kb) PL with *int1-aadA1* or *dfrA1-aadA1/pcdD/silA/merA*. Higher CuSO₄ MIC values were obtained in aerobiosis in contrast with anaerobiosis, where a correlation between MICs and Cu^R genes was found (MIC₅₀=28mM *pcdD/silA-silE*⁺ vs 1mM *pcdD/silA-silE*⁻). MICs values for AgNO₃ were higher in anaerobic than aerobic conditions and a slightly difference between *silA-silE*⁺ (MIC₅₀=0,32/0,25mM) and *silA-silE*⁻ (MIC₅₀=0,25/0,16mM) was observed in both atmospheres. **Conclusions.** High prevalence of metal tolerance genes associated with increased copper/silver tolerance, and the co-location with ABR genes in different plasmids, suggests that besides antibiotics, metals used in the animal production setting may have contributed for the selection/maintenance of *Salmonella* clinical relevant clonal lineages. **Acknowledgments:** This research was supported by UP/Santander Totta “Projectos Pluridisciplinares 2011” and FCT-PEst-C/EQB/LA0006/2011.

Hub and non-Hub Proteins in protein-protein interaction networks

Fátima Martins¹, Rute Gonçalves¹, João Oliveira¹, Sofia Serodio¹, E. Tejera², I. Rebelo³

¹ Department of Biochemistry, Faculty of Pharmacy, University of Porto, Portugal.

² Institute for Molecular and Cell Biology, Porto, Portugal.

³ Department of Biochemistry, Faculty of Pharmacy, University of Porto, Portugal and Institute for Molecular and Cell Biology, Porto, Portugal.

Most proteins interact with only a few other proteins while a small number of proteins (hubs) have many interaction partners. Hub proteins and non-hub proteins differ in several respects; however, sequence complexity is an important factor to consider when protein structure or interactions are analyzed.

Recent proteome-wide screening approaches have provided a wealth of information about interacting proteins in various organisms but also strategies for quantification and analysis of the sequence complexity and/or repetition as well as its effect in protein evolution. We create a protein-protein interaction networks (PPI) for *Homo sapiens* to understand the relationship of protein sequence and their centrality in PPI. This study was possible because we used several protein centrality indexes and we divided the proteins in hubs and non-hubs considering several criteria and the complexity variation were analyzed such as protein length and protein mean solvent accessibility. Our results indicate that there are several trends between complexity and specific biological processes. Moreover, hubs proteins are more ordered, therefore, less complexes than no-hubs proteins, however, the effect of the protein length is considerably as well as the solvent accessibility profile.

D-Galactose aging model and antioxidant treatment effects in anxiety, learning and memory

S. Magano, J.P. Andrade and A. Cardoso

Department of Anatomy, Faculty of Medicine, University of Porto, Portugal.

In the last years the industrialized countries have seen an increase in average life expectancy. This has led to an increased incidence of pathologies linked to aging. In search of a way to minimize the alterations associated with neurodegenerative diseases researchers have sought to find compounds that confer effective neuroprotection. The green tea with high content of polyphenols, with marked anti-inflammatory properties and antioxidant compounds, is one of the most studied beverages. The main goal of the present work was to analyze the potential neuroprotective capabilities of the most abundant catechin in green tea, (-)-epigallocatechin-3-gallate (EGCG), in an accelerated aging model using D-galactose. The study was focused in the hippocampus and in behavioral performance dependent of its integrity.

Male Wistar rats were maintained throughout the experiment under room temperature and a daily photoperiod of 12 h and were fed with standard laboratory chow. After weaning animals were randomly separated in three groups. In one group we applied a model of accelerated aging using D-galactose (300mg/kg) administered intraperitoneally (i.p.) during 8 weeks and animals had free access to water. The second group was treated in the same way and simultaneously with EGCG in an oral solution (2g/L). The control animals were injected i.p. with saline and had free access to water. After treatments, animals were submitted open-field, elevated plus-maze and Morris water maze behavioral tests. The handling and care of the animals followed the Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985) and the European Communities Council Guidelines in Animal Research (86/609/UE). All efforts were made to minimize the number of animals used and their suffering.

In the Morris water maze test all animals decreased the distance to find the platform during the 14 days of acquisition. However there were no statistically differences between groups. In the open-field test all animals pass more time in the outer zone than in the inner zone. The animals treated with D-galactose pass more time in the inner zone than controls, however this difference was not statistically significant. In the plus-maze test all animals pass more time in the closed arms than in the other areas. However there were no significant differences between groups in the all areas of the plus-maze.

D-galactose has been reported to cause deterioration of cognitive functions that are similar to aging. However we did not find alterations in the anxiety and in the learning and memory. Treatment with the antioxidant EGCG did not result in any changes in the behavioral parameters tested. In conclusion, these results suggest that D-galactose treatment may not be an appropriate model for inducing age-related neurobehavioral alterations in rat.

This work was supported by Projectos IJUP (PP_IJUP2011 73) and National Funds through FCT – Fundação para a Ciência e a Tecnologia within the scope of the Strategic Project Centro de Morfologia Experimental (CME/FM/UP) – 2011-2012 and Project PEst-OE/SAU/UI0121/2011.

Chromium and vanadium in the human brain: A post-mortem study of anatomical region differences and age-related changes

P. Ramos¹, N. Pinto², R. Mendes², A. Santos² and A. Almeida¹

¹ REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

² National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal.

Although its causes are not yet well defined, neurodegenerative diseases (ND) have been subject of intensive research in last decades in an attempt to understand the mechanisms underlying the neurodegeneration processes. The etiology of these diseases is multifactorial, being assumed that it involves a complex interaction between the (natural) ageing, genetic predisposition and environmental factors. Neuronal resistance to insulin has been identified as potentially responsible for cognitive impairment and development of some ND [1].

Chromium (Cr) and vanadium (V) are essential trace elements and play a key role in insulin function, supporting normal blood sugar levels. While Cr is a key component of glucose tolerance factor and participates in glucose metabolism by enhancing the effects of insulin, V supports these vital metabolic processes because of its ability to mimic insulin actions. Imbalances in these trace elements levels may lead to impaired glucose homeostasis and consequently to the formation of advanced glycation end products, accelerating the ageing process [2].

Based on this background, the goals of this work were to study, directly in human brain samples: 1) the regional anatomic differences of Cr and V levels within the brain tissue and 2) the changes on Cr and V levels in relation to age.

From neurologically and psychiatrically healthy individuals submitted to autopsy ($n=44$) the following 14 areas were sampled: frontal cortex; superior and middle temporal; caudate nucleus, putamen and globus pallidus; cingulate gyrus; hippocampus; inferior parietal lobule; occipital lobe; midbrain; pons; medulla; cerebellum. After microwave-assisted acid digestion of the samples, Cr and V levels were determined by Inductively Coupled Plasma-Mass Spectrometry.

Considering the mean values for the 14 regions, Cr was present at ca. 3.8-fold higher levels than V (mean \pm sd: 1.79 ± 0.68 $\mu\text{g/g}$ vs. 0.47 ± 0.25 $\mu\text{g/g}$). Vanadium distribution within the brain showed to be fairly heterogeneous: highest levels were found in the frontal cortex (0.62 ± 0.42 $\mu\text{g/g}$) and caudate nucleus (0.62 ± 0.47 $\mu\text{g/g}$), while lowest levels were found in the cerebellum (0.23 ± 0.21 $\mu\text{g/g}$) and pons (0.30 ± 0.27 $\mu\text{g/g}$). A tendency for V levels to increase with age in some of the brain regions was found, namely in frontal cortex and putamen. On the contrary, Cr presented a quite homogeneous distribution and seems to remain quite unchanged irrespective of ageing.

References:

[1] Shubert, M. et al. (2004), *Role for neuronal insulin resistance in neurodegenerative diseases*, Proceedings of the National Academy of Sciences, 101(9): 3100–3105.

[2] Praveena, S. et al. (2013), *Trace Elements in Diabetes Mellitus*, Journal of Clinical and Diagnostic Research, 7(9): 1863–1865.

Acknowledgments: The authors thank to Universidade do Porto and Santander Totta for financial support through the project “TRAIN : Trace elements in human brain: age-related changes and anatomic region specific differences” (PP_IJUP 2011 342).

The threat of multidrug resistance bacteria to public health: when aquacultures raise more than fish

J. Campos¹, J. Mourão¹, J. Pereira², A. Mesquita², M. Marques², C. Novais¹,
P. Antunes^{1,2} and L. Peixe¹

¹ REQUIMTE. Department of Microbiology, Faculty of Pharmacy, University of Porto, Portugal

² Faculty of Nutrition and Food Sciences, University of Porto, Portugal

Background. Aquaculture is currently one of the fastest growing food production sectors and is associated with 50% of the fish consumed worldwide. The occurrence of food safety threats, as resistance to antibiotics (ABR) relevant to the treatment of human infections (e.g. fluoroquinolones), is of concern to public health. Our goal was to assess the presence of pathogenic bacteria and clinically relevant ABR genes in aquacultures receiving water from secondary rivers. **Methods.** Samples were collected from water/sediments upstream (13) and downstream (13) of farms, water/sediments from juvenile/adult fish ponds (15) and feed (6) from 2 Portuguese trout aquacultures (TR-A/TR-B; winter/summer; 2010-2012). They were analysed for *Salmonella* (standard methods), *Aeromonas* and other Gram negative in selective medium with/without AB after an enrichment step. Genes conferring resistance to β -lactams (*bla*_{TEM}/*bla*_{CTX-M}/*bla*_{SHV}), fluoroquinolones [*qnr/qepA/aac(6')*-*Ib-cr/oqxAB*] and other AB were searched by PCR/sequencing. ABR was studied by agar diffusion/Etest (CLSI/EUCAST). Species were identified by ID32GN/16SrDNA sequencing. Clonality (XbaI PFGE/MLST), characterization of plasmid (PL) and integron backbones (PCR, rep-PCR and/or sequencing), conjugation assays and genomic location (I-CeuI/S1 PFGE hybridization) were done. **Results.** *Salmonella* was detected in 30% (14/47) of samples from both TR during the summer. They belonged to 4 uncommon serotypes (Linguere, Guerin, Muenchen and Budapest), 4 PFGE clones and 3 MLST types (ST118, ST508, ST1672). We also detected a MDR *E.coli* clone (ST3595) carrying *bla*_{SHV-12} (on transferable IncI1 PL) disseminated in TR-A (upstream/downstream river water/ juvenile/adult ponds water). PMQR genes were found in 9% (14/160) of the isolates: *Aeromonas* spp (3; upstream/downstream river water/adult ponds water/TR-A), *E.coli* (6; ST423-CC23, ST641, ST661, ST1049, ST2739; upstream/ downstream river water/TR-A, juvenile/adult ponds water or sediment/TR-B), *Klebsiella* spp (2; downstream river sediment/TR-A, feed/TR-B) and *Citrobacter freundii* complex (3; sediment from adult pond/TR output/TR-B; feed/TR-A) with MIC to ciprofloxacin (0.25-1 mg/L) above ECOFF for *Enterobacteriaceae*. The *qnrS1* was found in an untypable (UN) PL of *E.coli* (2); *qnrS2* in UN PL of *Aeromonas*; *qnrS3* in PL (2 UN/1 IncN) of *E.coli* and in an IncN PL of a *C.freundii* complex. The *oqxAB* in IncN-F hybrid or UN PL of 2 MDR *Klebsiella* spp and *aac(6')*-*Ib-cr* in IncU PL in *Aeromonas*. Different *qnrB* alleles, including a new variant, were chromosome located in *E.coli* (1), *Klebsiella* spp (1) and *C.freundii* complex (1). Transferable PL were found only in *E. coli* carrying *qnrS1* (2) or *qnrS3* (2). Most strains carried other ABR genes, class 1 integrons and/or IS26. **Conclusions.** The presented results place trout aquacultures within the ecological niches that might constitute reservoirs/vehicles for pathogenic bacteria and ABR bacteria/genes of relevance for human and animal health which can contaminate fish for human consumption or the aquatic surrounding environments. **Acknowledgments.** This research was supported by Universidade do Porto/Santander Totta “Projectos Pluridisciplinares 2011” and Fundação para a Ciência e a Tecnologia-PEst-C/EQB/LA006/2011.

Street-vending foods: a vehicle of pathogenic bacteria and clinically relevant antibiotic resistance genes

J. Campos¹, J. Gil², J. Mourão¹, L. Peixe¹ and P. Antunes^{1,2}

¹ REQUIMTE. Department of Microbiology, Faculty of Pharmacy, University of Porto, Portugal

² Faculty of Nutrition and Food Sciences, University of Porto, Portugal

Background. Street-vending food has grown exponentially worldwide, representing in some countries a significant proportion of the food consumed by the population. However, microbiological food safety hazards of vending units in industrialized countries are scarcely evaluated. The aim of this study was to analyse the microbiological quality and safety of street-vended foods as well as the hands hygiene of those vendors. **Methods.** We analysed samples of hot dogs (n=10), hamburgers (n=10) and hands of food handlers (n=9) from ten street vending units in Porto region. We screened for *Salmonella*, *Listeria monocytogenes*, *Escherichia coli*, *Enterobacteriaceae*, coliforms, coagulase-positive *Staphylococcus* and aerobic mesophilic counts (standard methods). The bioindicator *E. coli* was identified by API/MALDI-TOF/16S rDNA PCR and clonal relatedness by PCR for phylogenetic groups (PhG) and Pulsed Field Gel Electrophoresis (PFGE). Those isolates were characterized for antibiotic resistance (ABR) by disk diffusion (CLSI/EUCAST) and ESBL expression by double disk synergy test. ABR genes, class 1 integrons and virulence factors were searched by PCR/sequencing. **Results.** *Salmonella* and coagulase-positive *Staphylococcus* were absent, but *L. monocytogenes* was detected in 4 food samples (20%; 3 vending units). All food samples were unsatisfactory for *Enterobacteriaceae* and coliforms (>10³ UFC/g). *E. coli* was detected in 11 (55%) food samples of both types, with 4 (20%) from 4 units (40%) presenting unsatisfactory levels (≥10 UFC/g). The food handler's carried *Enterobacteriaceae* and coliforms (100%), coagulase-positive *Staphylococcus* (44%) and/or *E. coli* (11%). *E. coli* isolates (n=30) detected in the food/vendors samples belonged to 3 PhG (A0-9, A1-8, B1-13) and 8 clones, with 3 of them spread in different samples from the same vending unit and/or different units, pointing out for cross-contamination or common source of contamination. 33% (5 clones) presented resistance to tetracycline (30%; *tetA/tetB*), ampicillin (23%; *bla*_{TEM}), streptomycin (20%; *strA-strB/aadA*), sulfamethoxazole (20%; *sul1/sul1*), chloramphenicol (20%; *catA/catB3/floR*), trimethoprim (13%; *dfrA1*); nalidixic acid (17%), ciprofloxacin (13%). Multidrug resistance (MDR) profiles were observed in 23% (n=7; 3 clones; food/vendor samples), including to the clinically relevant antibiotic ciprofloxacin in one clone/PhG B1 (food), carrying a 3000 bp integron (*intI1-aacA4-catB3-dfrA1-sul1*), *tetB/bla*_{TEM}/*strA-strB/aadA/sul2/catA* but absence of plasmid-mediated quinolone resistance determinants and ESBL genes. Screening of intestinal pathogenic *E. coli* virulence factors found *astA* (vendor) or *eaeA* (food) genes in two MDR PFGE-types. **Conclusions.** Ready-to-eat street foods and food handlers analysed in this study had a poor microbiological quality and seems to be vehicles of pathogenic bacteria (*L. monocytogenes*) and/or *E. coli* carrying virulence/antibiotic resistance genes with potential clinical impact. Those data highlights the need to establish specific regulations/measures and training to improve food safety in the street food sector in industrialized countries.

Selection of antibiotic resistant bacteria: a role for mercury?

E. Silveira¹, A. R. Freitas¹, M. Barros¹, H. Pinto¹, P. Antunes³, S. Marçal³, P. Ramos³, T. M. Coque⁴, L. Peixe¹, C. Novais^{1,2}

¹REQUIMTE. Faculdade de Farmácia. Universidade do Porto. Portugal

²CEBIMED. Faculdade de Ciências da Saúde. Universidade Fernando Pessoa. Porto. Portugal.

³Faculdade de Ciências da Nutrição e Alimentação. Universidade do Porto. Portugal.

⁴Servicio de Microbiología. Hospital Ramón y Cajal. Madrid. Spain.

Background: Diverse environmental stressors (e.g. metals) can participate in the selection and maintenance of antibiotic resistance (AB^R) strains and/or genetic elements. Mercury (Hg) is present in environment, in part associated with anthropogenic contamination. The occurrence of *mer* operon among *Enterococcus* was scarcely analyzed, although it has been linked to mobile genetic elements with AB^R. Our goal was to assess the occurrence of known Hg^R genes among *Enterococcus* spp from several sources, and to evaluate their genetic context.

Methods: We analyzed 231 *E. faecalis*-*Efl*, 252 *E. faecium*-*Efm*, and 205 *Enterococcus* spp-*Ep* from hospitalized (H, n=47), healthy humans (HV, n=68), poultry (P, n=155), piggeries environment/swine PE, n=220), aquacultures (A, n=167) and sewage (S, n=52) (Portugal; 1997-2011). Genes linked to AB^R (*vanA*, *tetM*, *tetL*, *ermB*, *aac6''-aph2*, *blaZ*), Hg^R (4 *merA* sequences) and Tn6009 were searched (PCR). Mating assays, clonal relatedness (PFGE/MLST) and analysis of the plasmid carrying Hg^R genes (S1-PFGE, rep typing, hybridization) were done.

Results: Genes encoding Hg^R (*merA1*-2%; *merA2*-3%;, *merA3*-0,5%) were identified in 16 *Efm*, 2 *Efl*, and 2 *Ep*, and linked to 8 PFGE types (10 *Efm*, 1 *Efl*) from PE/H/HV (9%) and S (8%). Representative isolates corresponded to *Efm* CC17 (ST132, ST393 and ST431; PE and H), *Efm* CC5 (ST185; PE), STnew (S) and to *Efl* ST159 (H). The *merA3* and *tetM* were located at chromosome (*Efl*-H) while other Hg/AB^R genes were detected on plasmids: i) *merA2* (185Kb, 190Kb; *Efm*-2PE); ii) *merA2+tetM* (250kb, 270Kb; *Efm*-1S, 1PE); iii) *merA2+tetM+ermB* (200kb; *Efm*-1HV); iv) *merA2+merA3+ermB+vanA* (75kb; *Efl*-1H). AB^R (erythromycin, tetracycline, ampicillin, HLR-streptomycin, HLR-gentamicin) was co-transferred with Hg^R genes at different rates. The *repA*-pLG1 was only identified in *Efm* plasmids tested which carry only Hg^R (n=1) or Hg^R+AB^R genes (n=2). The *merA* were not linked to Tn6009 or plasmids carrying *blaZ* as described.

Conclusion: This work represents the first study addressing the diversity of *merA* genes among *Enterococcus* from different ecological niches. Their co-location with AB^R genes on widespread large pLG-like conjugative plasmids of *E. faecium* may favoured their persistence under different selective pressures linked to antibiotic use or environmental Hg contamination. The diversity of *merA* genes also suggests frequent lateral gene transfer between bacteria sharing common ecological settings.

Acknowledgments. This research was supported by Universidade do Porto/Santander Totta "Projectos Pluridisciplinares 2011" and Fundação para a Ciência e a Tecnologia-PEst-C/EQB/LA006/2011.

Balanced and vegetarian diets: comparison in macro and micronutrients composition

Ana Catarina Fernandes Vieira, Cátia da Cunha Carvalho, Carolina Alexandra de Sousa Monteiro, Daniela Belova de Magalhães, Sofia da Silva Moreira, Raquel Carneiro da Mota, I.M.P.L.V.O. Ferreira

Departamento de Ciências Químicas, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto

Every day we hear a lot of advices concerning the intake of a Healthy Diet. Eat only the calories we need. Make a diet rich in fruits and vegetables. Choose whole grains and high-fiber foods. Limit the saturated fat and sugars. Select foods with little salt. On the other hand, we also hear that Vegetarianism (a diet that excludes meat and fish, or shellfish, with several variants, some of which also exclude eggs and dairy products) has been shown to reduce the risk of heart disease and obesity. Thus, the challenge we received in the classes of Human Nutrition of Pharmacy course was to investigate the composition of macro and micronutrients of an example of a real diet, learn how to make the appropriate changes to transform it in a balanced diet according to the rules of World Health Organization and compare with the composition of a vegetarian diet.

The example described in this work was the one day diet of a 20 years old male, with 60 kg weight and 1.80 meters height. His Body mass index was 18.52 and the Estimated Daily Caloric Needs were 2397 kcal. According to his description of the diet from the previous day and using appropriate manuals and tables his diet was studied concerning macro nutrients, vitamins and minerals. The described diet presented an excessive amount of calories, fat and proteins, low intake of fiber and folate and high intake of sodium. However, with slight substitutions of some foods or changes of the cooking method an example of a balanced diet in macronutrients and presenting increased content of the micronutrient lacking is given. Additionally, a proposal of vegetarian diet that replaces meat by tofu and soya is presented. The analyses of macro and micronutrients in this last diet showed that all the parameters under evaluation were in agreement with WHO recommendations except vitamin B₁₂. The Recommended Intake per day of this vitamin is 2.4 µg, although some people appear to maintain health on as little as 0.5 µg per day [1]. Evaluation of the balance of vegetarian diets was a difficult task due to the lack of micronutrients composition of those products.

References:

[1] Wiseman, G. Vitamin B₁₂ in Nutrition & Health, Published by Taylor & Francis, London 2002.

Antioxidant activity and total phenolic compounds of leaf lettuce (*Lactuca sativa* L., var. *crispa*) and cape gooseberry (*Physalis peruviana*) mix

M. Arantes¹, A. Aires¹, A.S.G. Costa¹, R.C. Alves^{1,2}, M.A. Nunes¹, M.B.P.P.Oliveira¹

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

The consumption of fruit and vegetables is important to the prevention of chronic diseases, such as cancer and cardiovascular dysfunction. These benefits are related to the content of phytochemicals in food matrices, which can interact and even result in higher antioxidant activity together [1].

In this study, we evaluated the antioxidant capacity and total phenolics content of a fruit/vegetable mixture of leaf lettuce/cape gooseberry in different proportions: 75:25 and 25:75. Antioxidant activity was determined by the ferric reducing antioxidant power (FRAP) [2], and 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH•) assays [3]. The total phenolic content was evaluated by Folin-Ciocalteu method [4].

Quantitative differences were observed in the two different combinations used. The leaf lettuce (75%) and cape gooseberry (25%) mix showed a lower content of total phenolic compounds and antioxidant activity (lower percentage of inhibition) than the leaf lettuce (25%) and cape gooseberry (75%) mix.

Data show that the antioxidant activity is strongly correlated with the antioxidants content of the samples. Cape gooseberry seems to be a better antioxidant source than lettuce.

References:

- [1] Iiu, R. (2004). *Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action*, The Journal of Nutrition 134: 3479S–3485S.
- [2] Benzie, I. and Strain, J. (1996). *The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay*. Analytical Biochemistry. 239 (1), 70-76.
- [3] Harini R, Sindhu S, Gurumoorthi P, Sagadevan E, Arumugam P. (2012). *Characterization of in vitro antioxidant potential of Azadirachta indica and Abutilon indicum by different assay methods*. Journal of Pharmacy Research 5: 3227-3231.
- [4] Vinha, A.F., Alves, R.C., Barreira, S.V.P., Castro, A., Costa, A.S.G., Oliveira, M.B.P.P. (2014). *Effect of peel and seed removal on the nutritional value and antioxidant activity of tomato (Lycopersicon esculentum L.) fruits*. LWT – Food Science and Technology, 55(1), 197-202.

Acknowledgments: R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

Phylogeny of OAS genes and association with dengue fever

Joel Ribeiro^{1,2}, Petr Triska¹, Pedro Soares¹, Luísa Pereira^{1,3}

¹ IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Portugal

² FCUP – Faculty of Sciences of the University of Porto, Portugal

³ FMUP – Faculty of Medicine of the University of Porto, Portugal

The World Health Organization estimates that one of the main consequences of global warming will be an increased burden of vector-borne diseases. Among these, dengue appears to be particularly problematic, with tens of millions of cases occurring annually, including up to 500,000 life-threatening cases [1]. The outbreak last year in Madeira constitutes the proof of the risk of dengue spreading to uninfected areas, especially Southern Europe. It becomes essential to confirm associations of candidate genes conferring susceptibility to dengue and to estimate its allele and haplotype frequencies across worldwide populations. One identified candidate is the gene cluster 2'-5'-oligoadenylate synthetase (OAS1, OAS2, and OAS3) which maps to human chromosome 12q24 and encodes a family of enzymes pivotal to innate antiviral defence [2]. Interestingly, the analyses of ancient DNA from extinct humans revealed an OAS haplotype extending for ~185 kb which introgressed from Neanderthals, being restricted to Europeans and Asians [3].

In this work, we performed a haplotype analysis for OAS genes in dengue fever patients and controls from GWAS studies performed in Cuba [unpublished] and Vietnam [4], and contextualised this information in a worldwide background by using information from the 1000 Genomes Project database. We used Dnasp and Network Softwares to estimate the parameters of gene and haplotype diversities and to infer the phylogenetic relation between OAS haplotypes.

We confirmed that the allele and haplotype diversities for the three OAS genes are higher for Europeans and Asians than for Africans, due to the presence of the divergent Neanderthal haplotype and its derivatives in the former populations. At least for Cuba, we have shown that the Neanderthal haplotype is not associated with dengue fever, refuting previous claims of association with dengue of some of its polymorphisms in the Indian population [2]. So far, no OAS haplotype has been identified in Cuba as conferring susceptibility or resistance to dengue.

We are still performing the analysis for the Vietnam dataset, and will present these results, enabling in that way to provide information for the OAS possible contribution to dengue fever susceptibility across the European, African (as Cuba is an admixed population made of both these two genetic components) and Asian population groups.

References

[1] World Health Organization (2009) Dengue: guidelines for diagnosis, treatment, prevention and control. 160 pp. Geneva.

[2] Alagarasu K et al. (2013). Polymorphisms in the oligoadenylate synthetase gene cluster and its association with clinical outcomes of dengue virus infection. *Infect Genet Evol.* 14:390-395.

[3] Mendez FL et al. (2013) Neandertal Origin of Genetic Variation at the Cluster of OAS Immunity Genes. *Mol. Biol. Evol.* 30:798–801.

[4] Khor CC et al. (2011) Genome-wide association study identifies susceptibility loci for dengue shock syndrome at MICB and PLCE1. *Nat Genet.* 43:1139-1141.

Expression Regulation of Rho GTPases family members during oligodendrocytes differentiation and myelination

S. Braz^{1,3}, A. Cruz^{1,2}, J. Relvas² and A. Moreira¹

¹Gene Regulation Group of IBMC – Instituto de Biologia Molecular e Celular, University of Porto, Portugal.

²Glial Cell Biology Group of IBMC – Instituto de Biologia Molecular e Celular, University of Porto, Portugal.

³Department of Biology, University of Aveiro, Portugal.

Cell–cell signalling relies on the ability of cells to adjust their local proteome with high spatiotemporal precision in response to extracellular signals. In axons and dendrites, local proteomic homeostasis is controlled by local mRNA translation [1]. Axons exhibit a large number of mRNAs, yet, only some are translated into protein upon stimulation, requiring an abundant level of local translation control. In central nervous system (CNS), myelination of axons is carried out by oligodendrocytes (OL), this process can need to be regulated in a temporal and localised manner. Thereby, this suggests that local control of translation can be a mechanism of regulation in OL, similar to neurons [1]. In the nervous system, Rho GTPases are essential regulators of neuronal growth cone motility, axonal migration, and dendritic spine morphogenesis [2]. In glia, RhoA acts as a negative regulator inhibiting process extension and differentiation, whereas the expression and activity of Cdc42 and Rac1 increases as differentiation progresses, acting as positive regulators of OL morphological differentiation, process extension and branching [3]. Alternative polyadenylation (APA) occurs in 79% of the mammal genomes and controls mRNA translation [4] however it is still unknown if these mechanisms are relevant for the expression of Rho GTPases family members. Our results, using *in silico* analysis of Expressed-Sequence-Tag (EST), indicate the existence of several ESTs with different 3'UTRs, for the Rac1 gene. To validate these findings, we mapped the 3'ends of the Rac1 mRNAs by 3'RACE and confirmed that Rac1 generates at least 4 mRNAs due to APA in the 3'UTR during OL differentiation. Furthermore, for the two splicing-isoforms of the Cdc42 gene (Cdc42iso1 and Cdc42iso2), we observed the existence of several ESTs with different 3'UTRs. Moreover, we mapped the 3'ends of the Cdc42 mRNAs and we observed the existence of 5 mRNAs due to APA of the Cdc42iso1 and 2 mRNAs in the Cdc42iso2. By performing RT-qPCR analysis we observed an increase in Rac1 expression levels and a differential expression pattern of Cdc42 isoforms during the OL differentiation. This work will unravel the molecular mechanisms of gene expression in OL differentiation and myelination, which might give useful insight into new therapeutic strategies to treat demyelinating diseases, such as Multiple Sclerosis in the future. This work was funded by FEDER funds through the Operational Competitiveness Programme–COMPETE and by National Funds through FCT–Fundação para a Ciência e a Tecnologia under the project FCOMP-01-0124-FEDER-037277 (PEst-C/SAU/LA0002/2013)". Personal Grants: ON2-201304-CTO-I to AC.

References:

- [1] Jung, H., B.C. Yoon, and C.E. Holt. 2012. Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair. *Nat Rev Neurosci* 13:308-324.
- [2] Feltri, M.L., U. Suter, and J.B. Relvas. 2008. The function of RhoGTPases in axon ensheathment and myelination. *Glia* 56:1508-1517.
- [3] Thurnherr, T., Y. Benninger, X. Wu, A. Chrostek, S.M. Krause, K.A. Nave, R.J. Franklin, C. Brakebusch, U. Suter, and J.B. Relvas. 2006. Cdc42 and Rac1 signaling are both required for and act synergistically in the correct formation of myelin sheaths in the CNS. *J Neurosci* 26:10110-10119.
- [4] Pinto PAB, *et al.* (2011) RNA polymerase II kinetics in polo polyadenylation signal selection, *The EMBO Journal*, 30: 2431–2444

Uncoated tablets: influence of the technological excipients and compaction force on “in vitro” thiamine hydrochloride release

J. Conceição, M. Estanqueiro, M.H. Amaral, J.P. Silva, J.M. Sousa Lobo

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

A medicine is a physical system whose properties depend, among others, on the individual contributions of drug(s) and excipients [1]. The advantages of high-precision dosing, manufacturing efficiency and patient compliance make tablets the most popular dosage forms [2].

The major objectives of this work were: (i) to produce and characterize uncoated tablets containing 10 mg of thiamine hydrochloride (vitamin B1); (ii) and to study the influence of the technological excipients, which behave differently during compaction, and the compression force on the release/dissolution of vitamin B1, used as a model of a very soluble drug. Pure excipients and binary mixtures, whose composition varied between 20% (w/w) and 80% (w/w) at intervals of 20% (w/w), were used. The studied excipients were Avicel-PH 200 (microcrystalline cellulose) which is an insoluble diluent with plastic behaviour, and Emcompress[®] (dibasic calcium phosphate dihydrate), an insoluble diluent with fragmentable behaviour. The mixtures of the components (100 g samples) were made in WAB Turbula[®] mixer for 15 minutes. Then, samples were directly compressed and the study of their physical compression behaviour (n = 10) was performed in an instrumented alternative machine DOTT. Bonapace model CPR-6 (Italy) coupled to a computer. Weight variation (n = 10), thickness (n = 10), hardness (n = 10) and “in vitro” drug release (n = 3) of the manufactured tablets were evaluated. The “in vitro” drug release study was performed in a dissolution apparatus Sotax AT7 (Switzerland) according to the paddle method (European Pharmacopoeia 7) at 50 rpm [3]. The dissolution medium used was 900 mL of a solution of hydrochloric acid 0.1M at 37.0 ± 0.5 °C. Aliquots of 5.0 mL were collected after 5, 15 and 30 minutes (without volume replacement). Samples were filtered and drug concentration was determined using a UV-VIS spectrophotometer (Jasco, model V-650, Japan) at 273 nm (isosbestic point of vitamin B1). In addition, the dissolution profiles of the different tablets were compared through the calculation of the similarity factor (f_2) [4]. A f_2 value between 50 and 100 suggests that the dissolution profiles are similar [4].

In this study, uncoated tablets with acceptable physical properties were produced. However, it was not possible to prepare tablets with Emcompress[®] and vitamin B1, maintaining the same volume of the compression chamber and the same upper punch displacement. Through the results obtained in dissolution tests for all tablets, it was not possible to establish a relationship between the technological excipients (and proportions) and the compaction force with the “in vitro” release of thiamine hydrochloride.

References:

- [1] Prista LN, Alves AC, Morgado R. *Tecnologia Farmacêutica*. III Volume. 6. ^a ed: Fundação Calouste Gulbenkian; 2009, pp. 2125-2150.
- [2] Patel S, Kaushal AM, Bansal AK. *Compression physics in the formulation development of tablets*. Crit Rev Ther Drug Carrier Syst. 2006;23(1):1-65.
- [3] *European Pharmacopoeia 7*: EDQM; 2011.
- [4] *Guideline on the investigation of bioequivalence*. Committee for medicinal products for human use (CHMP). European Medicines Agency (EMA). 2010.

Evaluation of adhesion and biofilm producing abilities of worldwide spread *Escherichia coli* uropathogenic clonal complexes

Manuel Lascasas¹, Carla Rodrigues¹, Elisabete Machado¹, Luísa Peixe¹, Ângela Novais¹

¹ REQUIMTE; Laboratório de Microbiologia; Faculdade de Farmácia da Universidade do Porto, Portugal

Introduction: Antibiotic resistant *Escherichia coli* isolates belonging to A and B1 phylogroups are increasingly recovered from extraintestinal human infections. The diversity of isolates belonging to representative A and B1 *E. coli* (EC) clones from different settings, and their ability to adhere on abiotic surfaces is investigated.

Methods: Fifty-one A (22 ST10, 14 ST23 complexes) and B1 (8 ST155 complex, 7 ST359) isolates from different countries (6 EU, 2 South American) were characterized (1997-2008). They exhibited variable antibiotic resistance profiles (including ESBL or AmpC production) and were identified in nosocomial (52%) and community (12%) infections, healthy volunteers (14%) or animals (22%). Clonal relatedness was established by PFGE and MLST. Screening for 38 ExPEC virulence factors (VFs) was performed by PCR. Biofilm formation was investigated by a modified quantitative assay.

Results: The number of virulence traits for ST23 isolates (median 9/range 2-14) was higher than for ST10 (median 5/range 1-14), ST155 (median 6/range 2-9) or ST359 (median 6/range 3-12) isolates. Extra-intestinal pathogenic *E. coli* (ExPEC) features were identified among ST23 (42.9%), ST359 (28.6%), ST155 (25%) and ST10 (9.1%), although all other human isolates also caused extraintestinal infections. Whereas *fimH* (71-100%), *iss* (57-100%), *iutA* (38-100%), and *iroN* (38-86%) were common in all STs, a few VFs were significantly associated with ST23 (*ompT*, *cvaC*, *traT*, *fyuA*, 57-71%), ST10 (*traT*, *usp*, 52-76%), ST155 (*ompT*, *cvaC*, *sfa/focDE*, 43-57%) or ST359 (*usp*, 71%). Clusters containing isolates from diverse origins were identified within each clone, although they contained a high diversity of PFGE and virulence profiles. Presumptive biofilm formation was detected among ST10 (n=12, 0.35<O.D.<2.2), ST23 (n=9, 0.39<O.D.<2.0), ST155 (n=3, 0.6<O.D.<1.1) and ST359 (n=1, O.D.=0.39), which corresponded to moderately or strongly adherent strains from diverse origins exhibiting variable virulence profiles.

Conclusion: Despite isolates of A and B1 EC isolates exhibited a lower VF profile than those of phylogroups B2 and D, they were frequently biofilm producers. A high diversity of PFGE, virulence profiles and adhesion abilities was identified among each clonal group, suggesting a remarkable genome plasticity. The identification of ST10 and ST23 complex isolates particularly enriched in VFs and/or with abiotic adhesion abilities might explain their emergence as extraintestinal pathogens.



P

POSTERS

**THURSDAY
13TH**

**POSTER
SESSIONS**

Florence case: violation of privacy of the body and the State of Exception

F. P. Carvalho¹

¹ Faculty of Law, University of Brasília, Brazil; exchange student at the University of Porto.

This work can be divided into three distinct parts. The first is a narrative of events that led to Albert W. Florence [1], the black American finance executive, to be wrongly imprisoned in institutions Burlington County Detention Center and Essex County Correctional Facility and be subjected to an invasive search on your body. The arguments used by the judges to admit his arrest and "inspection" as constitutional, and the arguments of opposing votes were analyzed. The second part of the work aims to question the naturalness with which the violation of rights was treated in the social sphere, including questioning the reader's own impressions about the case: would we be in a state of emergency, where violations of the law are not questionable? Where violations do not cause great discomfort among citizens? Finally, the third part is an exposition and comparison between the figure of Homo Sacer [2] and the Petitioner. Would they have in common? The aim is to approach from the present case, at which point the state of emergency today is really an exception, and we as citizens are lawfully related to the figure of Homo Sacer, subject to the sovereign will as killable bodies.

References:

[1] Supreme Court of The United States (Argued October 12, 2011—Decided April 2, 2012). *Florence V. Board of Chosen Freeholders Of County Of Burlington Et Al. Certiorari To The United States Court Of Appeals For The Third Circuit* No. 10-945. 41pgs.

[2] Agamben, Giorgio (2007). *Homo Sacer: sovereign power and bare life I* / Giorgio Agamben translation of Henry Burigo. - Belo Horizonte: Editora UFM. Reprinting. 207 p. - (Humanitas)

The Portuguese textile and clothing industry: How foreign events influenced its performance since 1973

A. Soares¹, D. Silva¹

¹ BSc in Economics, Faculty of Economics, University of Porto, Portugal.

The Portuguese textile and clothing industry has a strong exporting culture. Therefore, not only internal but also foreign shocks affect this industry's performance. This paper presents an overview of those events since 1973 and aims to relate them with this sector's historical evolution.

In order to provide a clear and objective view of our insights, we developed an extensive statistical analysis of important economic variables.

Through this analysis, we found that the process of joining the EEC and the resulting market integration was the most important shock affecting the domestic textile and clothing industry. This event changed the specialization of the Portuguese economy and this specific sector's competitiveness. Thenceforth, textile and clothing exports became more dependent on technology and innovation, rather than in cheap and unqualified labor force.

In addition, the liberalization of world trade and the Multifibre Arrangement also played an important part in this process. The abolition of quantitative restrictions in 2005 opened the Portuguese market to the exports of emerging countries, placing pressure on the Portuguese industry.

As a result of this major shift on global trade, the Portuguese textile and clothing sector was forced to establish a strategy based on innovation and differentiation in order to outlast in an extremely competitive worldwide market.

By anchoring our assessment to data collected in various statistic sources, we aim to stand out by developing a purely quantitative and objective analysis on this topic.

The efficiency of Portuguese Technology Transfer Offices and the importance of university characteristics

A. Monteiro¹ and A.A.C. Teixeira²

¹ Faculdade de Economia, University of Porto, Portugal.

² Faculdade de Economia, University of Porto; INESC Porto; OBEGEF.

University-Industry (U-I) relationships have been the focus of a growing number of studies, particularly since the 1990s [1]. The relevance of cooperation between University and Industry has been highlighted by several authors [2] who stress, for instance, that universities can contribute to regional development through the production and transfer of knowledge [3].

Given that one of the most noticeable structural weaknesses of the Portuguese economy is the virtual lack of linkages between universities and industry [4], following the creation of the GAPI (*Gabinetes de Apoio à Propriedade Intelectual*) network, in March 2007, the Portuguese Science and Technology Foundation (FCT) launched the University Technology Enterprise Network (UTEN), in conjunction with the IC2 Institute, The University of Texas at Austin (UTEN, 2011). Formally, the UTEN network includes all the public Portuguese universities and a private one, their associated Technology Transfer Offices (TTOs), research centers and, in some cases, technology parks. It focuses on capacity building for the accelerated commercialization of Portuguese Science and Technology (S&T).

Despite the political and economic relevance of such a program, to date, an assessment of the efficiency of these TTOs has yet to be conducted and there is, consequently, no account of what may comprise its main determinants. The issue of efficiency in technology transfer, although to some extent neglected in the innovation literature, has been addressed in a few important studies. Most of these studies focus their analysis on more advanced countries, where U-I relations are mature and well developed, namely the USA [5], the UK [6], Spain [7], and France [8].

This study therefore intends to complement the research that has been conducted in the field of technology transfer, by comparing the efficiency of several Portuguese universities' TTOs, assessing the evolution of their efficiency in technology transfer processes in the last ten years. Specifically, the main research question of the present study is: Do the characteristics of universities impact on the efficiency of the associated TTOs?

Using the Data Envelopment Analysis (DEA) approach, which is a multiple-measure evaluation tool, the performance of several organizations is evaluated over a five-year period (2007-2012) when there are multiple inputs and multiple outputs to the system. Additionally, the main determinants of TTO efficiency are econometrically assessed through panel data estimations.

References:

- [1] Teixeira, A.A.C.; Mota L. (2012). "A bibliometric portrait of the evolution, scientific roots and influence of the literature on university-industry links". *Scientometrics*, 93(3): 719-743.
- [2] Bekkers, R.; Bodas Freitas, I.M. (2008). "Analyzing knowledge transfer channels between universities and industry: to what degree do sectors also matter?" *Research Policy*, 37: 1837-1853.
- [3] Bergman, E.M. (2010). "Knowledge links between European universities and firms: A review". *Papers in Regional Science*, 89(2): 311-333.
- [4] Teixeira, A.A.C.; Costa, J. (2006). "What type of firm forges closer innovation linkages with Portuguese Universities?". *Notas Económicas, FEC*, 24: 22-47
- [5] Anderson, T.R.; Daim, T.U.; Lavoie, F.F. (2007). "Measuring the efficiency of university technology transfer". *Technovation*, 27: 306-18.
- [6] Chapple, W.; Lockett, A.; Siegel, D.; Wright, M. (2005). "Assessing the relative performance of U.K. university technology transfer offices: Parametric and non-parametric evidence". *Research Policy*, 34: 369-384
- [7] Caldera, A.; Debande, O. (2010). "Performance of Spanish universities in technology transfer: an empirical analysis". *Research Policy*, 39(9): 1160-73.
- [8] Curi, C.; Daraio, C.; Llerena, P. (2012). "University technology transfer: How (in)efficient are French universities?" *Cambridge Journal of Economics*, 36: 629-654.

Corporate Ecological Footprint – Methodological Discussion and Application to STEF

L. Soares¹

¹ Master in Economics, Faculty of Economics, University of Porto, Portugal.

In today's world, where we talk more and more about environmental impacts and climate change, it is essential to find ways to measure those impacts and monitor the influence that different actions produce in the ecosystems.

In this context, taking into account that we all have our share of responsibility for the damages caused to the planet, it is essential that not only governments but also companies have effective tools to manage their environmental impacts.

This paper begins by presenting the Ecological Footprint in general and then focuses on the Corporate Ecological Footprint.

Within the Corporate Ecological Footprint, the present work focuses on the MC3 method, developed by Doménech from the concept of families' Footprint, created by Wackernagel and his colleagues. For the MC3, the consumption data is obtained mainly from the company's accounts, therefore it can be applied to any organization at any scale.

Having explained and discussed the MC3 method, we move on to its application to a company in the food logistics sector – STEF Portugal, namely calculating STEF Porto's Footprint, twice over a five year period, and also making a comparative analysis with STEF Lisbon's Footprint.

In the final part simulations are carried out, testing, in particular, the hypotheses of STEF's existence being an asset that exceeds its participation in the Portuguese economy, extending its benefits to the environment preservation. In addition, several propositions are made to reduce the Footprint, both at the company's and at the global level.

The main conclusions are that the Ecological Footprint of STEF Porto remains constant in the period analyzed and is less than half of the Footprint of STEF Lisbon. However, the stability of the Footprint in STEF Porto may be due not only to factors internal to the company but also to external factors that result of market changes.

A questionnaire adjustment and validation for Portuguese language about the perceptions of pre-service student teachers about the educational process: final results

J. Oliveira¹, D. Gomes¹, T. Sousa², M. Cunha² and P. Batista²

¹ Faculty of Sport, University of Porto, Portugal.

² CIFI²D, Faculty of Sport, University of Porto, Portugal

The teacher education should recognize the importance of the transition from an academic to a professional setting in the development of the future teacher [1]. In this sense, the practicum seems to be a critical moment where the pre-service teachers can perform this transition. As consequence, initial teacher training has been (re)configured in order to respond to these concerns.

This study aims to illustrate the results of a linguistic and cultural translation of a questionnaire originally presented and adapted by Ezer, Gilat and Sagee [2] to the Portuguese language and context. Specifically, this instrument aims to examine the pre-service student-teacher perceptions about the training and profession. In the process we followed the principles of functional equivalence and literal translation [3], ending in a constant review of language and conceptual equivalence.

To verify equivalences, we followed the seven-step methodological approach of Vallerand [4] although we only present the information from the top four in this study: (i) creation of the preliminary version, (ii) review and adjustment of the preliminary version, (iii) assessment of the clarity of the questions by public members through a pre-test, (iv) assessment of the validity and content of the questionnaire.

The results show the necessity to adapt some terms and concepts, and in the process of translations some terms that were changed in the initial step returned to the original one, for example: «*Teaching promotes self-realization*», firstly translated as «*I fulfil myself through teaching*» but then reconverted into Portuguese's literal significance in the final form of the questionnaire.

Finally, the process of questionnaire translation and cultural adjustment was successfully achieved. Nonetheless, in future studies it's important to assess the psychometric equivalence (functional reliability and validity) so that this instrument can be used in the context of the Portuguese language.

Acknowledgements

This study is part of a project funded by IUJP, multidisciplinary projects with the following reference: PP_IJUP2011 69

References

- [1] Azevedo, E., Pereira, B. & Sá C. (2011). *Percepções docentes acerca da formação inicial na atuação pedagógica: estudo de caso dos professores de EF*. Revista Iberoamericana de Educación, 56, 201-226.
- [2] Ezer, H., Gilat I. & Sagee R. (2010). *Perception of teacher education and professional identity among novice teachers*, European Journal of Teacher Education, 33(4), 391-404.
- [3] Geisinger, F. (1994). Cross-Cultural Normative Assessment: Translation and Adaption Issues Influencing the Normative Interpretation of Assessment Instruments. *Psychological Assessment*, 6(4), 304-312.
- [4] Vallerand, R. (1989). Vers une méthodologie de validation transculturelle de questionnaires psychologies: implications pour la recherche en langue française. *Canadian Psychology/Psychologie Canadienne*, 30(4), 662-680.

Systematic characterization of attack coverage formations in high-level men's volleyball

L. Laporta¹, J. Afonso², I. Mesquita³

¹ Department of volleyball, Faculty of sport, University of Porto, Portugal.

² Department of volleyball, Faculty of sport, University of Porto, Portugal.

³ Department of volleyball, Faculty of sport, University of Porto, Portugal.

Volleyball is a non-invasive team sport characterized by two fundamentally different games complex (Ks) [1]. The KI or side-out consists in the attack organization after serve-reception, while the KII refers to the attack after the defense (i.e., the counter-attack). Within each of the game complexes, the moment of attack implies, most of the times, opposition by the adversary's blockers. When facing such blocks, the attacking team may engage in active anticipation and prepare for countering a blocked attack.

However, a large gap is apparent in the existent literature concerning the attack coverage or KIV and the logic of the current top-level volleyball. The literature mentions only a few formations or systems that are highly structured in each attack zone, thus being incompatible with the diversity of offensive maneuvers of the teams, which present direct implications for the possible structures of attack coverage. Such constraints are mainly related to attackers changing their attack zones, as well as very quick attacks, all impacting of the final formation of coverage.

We analyzed matches of the Finals of the Volleyball World League 2011. Overall, 45 sets totaling 4544 actions were analyzed (1579 KI and 2965 KII).

The occurrence of KIV was verified in 177 plays (99 related with the KI and 78 to KII), wherein 27 different types of coverage formations, each with two to four lines of defense. Six different types of coverage were particularly frequent, four of which are different from those shown in the existent literature (1//2//2, 2//1//2, 2//2, 2//2//1, 2//3, 3//2). Results clearly show that literature concerning attack coverage in volleyball is outdated and solid research should be conducted in this topic.

The type of ball, quality of the first action, maneuvers of the attackers, game velocity, among others, can change the collective availability for coverage, thus resulting in the diversified formations for attack coverage, adjusting to the constraints that the modern game needs.

References:

[1] Castro, J.M. and Zouza, A. and Mesquita, I. (2011), Determinants of the attack efficacy in volleyball's complex II – study on elite male teams, *Perceptual and Motor Skills*, United States.

[2] Hileno, R. and Buscà, B. (2012), *Observational tool for analyzing attack coverage in volleyball*, *Revista INterncaional de Medicina y Ciencias de la actividad Fisica y el deporte*, 12 (47), 557-570.

Firing distance estimation through the detection of gunshot residues in blowfly larvae (*Calliphoridae*): an experimental study

M. Duarte¹, P. Ramos², L. Cainé³, A. Almeida² and A. Santos^{1,3}

¹ Faculty of Medicine, University of Porto, Portugal.

² REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

³ National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal.

The detection and quantification of gunshot residues (GSR) has an essential role in criminal investigation. Sometimes, this is the only evidence that confirms the occurrence of a firearm discharge incident [1]. Furthermore, the GSR distribution pattern around the bullet entrance hole can be useful to estimate the distance from which the weapon was discharged. [2].

However, the identification of firearm injuries, namely in corpses in an advanced state of decomposition, can be very difficult since some arthropods may colonise the corpse, lay their eggs on the moist body parts (such as the eyes, mouth and wounds) and, consequently, soft tissues will be consumed and degraded. Therefore, the ability to detect and identify the presence of GSR around a lesion which is suspected to have resulted from the passage of firearm bullet can be challenging for the forensic pathologists.

Given this difficulty and taking into account the above-mentioned usual colonization of the corpse by insect larvae, our study aimed to evaluate the correlation between the firing distance and the concentration of the most common metallic components of the GSR [lead (Pb), antimony (Sb) and barium (Ba)] in blowfly larvae (*Calliphoridae* family).

Using a 9-mm Glock pistol, test shots were made against a target made of porcine tissue (15 x 6 cm) at different distances (3, 20, 40, 60, 80 and 100 cm). Blowfly larvae (n=10), in the second stage of development, were collected after their feeding on the GSR exposed porcine tissue. Samples were digested with nitric acid and hydrogen peroxide in a closed-vessel microwave digestion system and the solutions were analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for Sb, Ba and Pb quantification.

In these experimental conditions, Sb, Ba and Pb content on the larvae was negatively correlated with the firing distance, and it was possible to approximately estimate the firing distance on the interval [20-80] cm. These results raise the possibility, in mortal events by firearm discharge, of using this approach to estimate the firing distance in bodies found in an advanced state of decomposition.

References:

[1] Saverio Romolo, F., P. Margot, P. (2001), *Identification of gunshot residue: a critical review*, Forensic Science International, 119 (2), 195-211.

[2] Santos, A., Magalhaes, T., Vieira, D.N., Almeida, A.A., Sousa, A.V. (2007), *Firing distance estimation through the analysis of the gunshot residue deposit pattern around the bullet entrance hole by inductively coupled plasma-mass spectrometry: an experimental study*, American Journal of Forensic Medicine and Pathology, 28 (1), 24-30.

Three-dimensional craniofacial reconstruction – An art or a reliable forensic method?

T. Quaresma¹, M. Ferreira¹ and S. Silva¹

¹ Post-graduation on Forensic Sciences, Institute of Biomedical Science Abel Salazar, University of Porto, Portugal.

Three-dimensional (3D) craniofacial reconstruction presents a growing factor of importance in a forensic context. This method can be applied for the recognition of individuals in a diversity of situations such as advanced decomposition, mass disasters, mutilations or calcifications, where the recognition turns impossible [1]. When other forensic methods of identification do not succeed, 3D craniofacial reconstruction can lead to positive identification assisting in criminal investigations [2]. However, it is not a method of identification; instead it is a method of recognition that allows recreating an *in vivo* profile of an individual without compromising the integrity of the physical remains [3].

This work is a review of some case-studies from the forensic published literature which aims to present the different methodologies used in 3D craniofacial reconstruction. For that matter case-studies of manual and computerized techniques of 3D craniofacial reconstruction were accessed for their presented accuracy, advantages and limitations. The results show that the cranial bones are the key for the facial appearance. They are the base structure where the tissues are disposed. The appearance of an individual relies on the conjugation of several factors such as different thickness of tissues (dermic, muscular and adipose tissues), population affinities, gender and age. The reconstruction process based on the mentioned factors uses referenced tables from data bases [1,3].

Although facial characteristics follow scientific procedures the physiognomic aspects (ears, soft tissues surrounding the mouth and cheeks) need a degree of artistic interpretation [4].

Forensic facial anthropology can be a powerful legal tool that can contribute significantly for recognition. An integrated approach of forensic anthropology and computerized sciences that are in constant evolution can originate unique and reproducible protocols that diminish the artistic subjectivity associated and constitute an excellent auxiliary method when identification is an obstruction for the Law.

References:

- [1] Greef, S.D. & Willems, G. (2005) *Three-dimensional Cranio-Facial Reconstruction in Forensic Identification: Latest Progress and New Tendencies in the 21st Century*, Journal of Forensic Sciences, 50 (1).
- [2] Stephan, C.N. and Henneberg, M. (2005), *Recognition by Forensic Facial Approximation: Case Specific Examples and Empirical Tests*, Forensic Science International, 156, 182-191.
- [3] Wilkinson, C. (2010), *Facial Reconstruction - anatomical art or artistic anatomy?*, Journal of Anatomy, 216, 235-250.
- [4] Decker S., Ford, J., Davy-Jow, S., Faraut, P., Neville, W. and Hilbelink, D. (2013), *Who is this person? A comparison study of current three-dimensional facial approximation methods*, Forensic Science International 229, 161.e1–161.e8.

Acoustic emission experiments in different types of rocks

T. Queiroz,^{1,2*} **A. F. P. N. Martins**,² **H. G. Silva**,^{2,3} **M. Moreira**⁴, **T. M. Seixas**^{1,5}, **M. A. Salgueiro da Silva**^{1,5}, **M. Tlemçani**^{2,3} and **M. Bezzeghoud**^{2,3}

¹ Departamento de Física e Astronomia, Faculdade de Ciências, Universidade do Porto, Portugal

² Centro de Geofísica da Universidade de Évora, Évora, Portugal

³ Departamento de Física, Escola de Ciências e Tecnologia, Universidade de Évora, Évora, Portugal

⁴ Instituto Superior de Engenharia de Lisboa (ISEL), Lisboa, Portugal

⁵ Centro de Geofísica da Universidade de Coimbra, Coimbra, Portugal

*tiagorfq@gmail.com

The analysis of acoustic emissions (AE) in different types of rocks, has proven to be quite useful in studying the mechanisms involved in the fracture process. The knowledge of rock structure and parameters like density and porosity, complemented with the study of the P and S waves speed, enables us to determine the elastic dynamic parameters. This investigation main objective is to provide information that will be helpful to understand the mechanisms of generation of seismo-electromagnetic signals occurring during the preparatory phase of major seismic events [1, 2]. The research includes the study of P- and S-waves speed, analysis of b-value, measurements and localization of AE events, in rocks of different types, under uniaxial mechanical tension, until fracture occurs. Fractal dimension coefficient (d-value) and density of AE events are some of the analysis that are expected to be done later. In this presentation, experimental measurement devices will be described, as well as the results of experiments performed in samples of different classes.

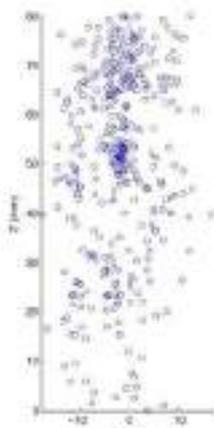


Fig 1: Acoustic emission event localization.

References:

[1] FREUND, F., M. A. Salgueiro da Silva, B. W. S. Lau, A. Takeuchi, and H. H. Jones (2007). Electric currents along earthquake faults and the magnetization of pseudotachylite veins, *Tectonophysics* 431, 131.

[2] AREIAS, P., H. G. Silva, N. Van Goethem, and M. Bezzeghoud (2013). Damage-based fracture with electromagnetic coupling, *Comput Mech* 51, 629–640, DOI 10.1007/s00466-012-0742-6.

Antioxidant analysis of green-leaf vegetables: comparison between several preservation methods used on vegetable samples

**M. Almeida¹, J. Santos¹, R. Alves², M.F. Barroso², M. Correia², C. Delerue-Matos² and
M.B.P.P Oliveira¹**

¹ REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² REQUIMTE/Instituto Superior de Engenharia do Porto, Politécnico do Porto, Portugal

The antioxidant capacity of vegetables results mainly from the synergistic action of a wide variety of antioxidant compounds such as some polyphenols, vitamins, carotenoids and other compounds. The presence of phenolic compounds has been correlated with the antioxidant capacity of plants and it is believed to be beneficial to the human health, demonstrating an active role in preventing the progress of certain degenerative diseases. In plants, the phenolic compounds have an important role in defense mechanisms and other metabolic reactions. Green-leaf vegetables are normally recognized for their richness in antioxidant compounds, especially in phenolic compounds [1]. To study the chemical composition of a plant the use of fresh tissues is often recommended, as preservation methods and storage can cause changes in the sample composition. However, preservation of the samples for later extraction and analysis is normally necessary. Samples are usually frozen or dried. These methods are combined in freeze-drying process, considered as an ideal drying method for phytochemical studies [2]. Thus, the objective of this study was to evaluate the effect of different preservation methods on the phenolic content and on their antioxidant capacity of vegetable samples.

In this study, the effect of the preservation process was evaluated in two varieties of lettuce, green and ruby red. Fresh, frozen (2 and 3 months) and freeze-dried samples were analyzed. The chemical compounds were extracted by an ultrasound assisted extraction with 80% methanol. The phenolic and flavonoid contents were assessed in all extracts by spectrophotometric assays. The antioxidant capacity was assessed through the determination of ferric ion reducing antioxidant assay (FRAP) and by monitoring the reduction of the DPPH• radical. The extracts obtained from freeze dried samples showed a higher content of phenolic compounds, flavonoids and antioxidant capacity. Fresh and frozen samples revealed more similar results between both extracts.

Acknowledgments: This work was supported by FCT grant no. PEst-C/EQB/LA0006/2011 and University of Porto “Projectos Pluridisciplinares” IJUP2011-317. Joana Santos is grateful to FCT PhD grant (SFRH/BD/66476/2009) financed by POPH-QREN (subsidized by FSE and MCTES).

References:

[1] Pérez-Jiménez, J., Arranz, S., Taberner, M., Díaz- Rubio, M.E., Serrano, J., Goñi, I., Saura-Calixto, F. (2008), *Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results*. Food Research International, 41 (3), 274–285.

[2] Keinäne, M, Julkunen-Tiitto, R. (1996). *Effect of Sample Preparation Method on Birch (Betula pendula Roth) Leaf Phenolics*. Journal of Agricultural and Food Chemistry, 44, 2724-2727.

Assessment of nitrite and nitrate contents and antioxidant capacity of Portuguese fresh vegetables

C. Saraiva¹, A. Coelho¹, M. Almeida², J. Santos², Rita C. Alves^{1,2}, M.F. Barroso^{1,2}, M. Correia¹, C. Delerue-Matos¹ and M.B.P.P Oliveira²

¹ REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Portugal.

² REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

In the past few years there has been a renewed interest in the study of the relationship between food and health. Plant phenolic compounds have received considerable attention owing to their antioxidant capacity and potential protective effects.

Besides being important sources of biologically active compounds, vegetables may be a major source of dietary exposure to nitrate, which are implicated in the endogenous formation of carcinogenic *N*-nitroso compounds and in methaemoglobinemia. Therefore, several studies aim to assess the nitrate contents of fresh vegetables and their compliance to maximum levels [1].

After being accumulated in the saliva, nitrate can be reduced to nitrite by several bacterial organisms. Under acidic conditions both in the oral cavity and in the stomach, further reduction of nitrite to NO may occur. Additionally, several studies have indicated that this bioconversion is stimulated by polyphenols, namely red wine polyphenols, and that it is associated to health-promoting effects [2].

The purpose of this study was to assess the antioxidant capacity and the nitrite and nitrate contents of 20 fresh vegetable samples purchased in Oporto region (Northern Portugal). As regards the antioxidant capacity, total phenolic content ranged between 10 and 63 µg gallic acid/g, DPPH radical scavenging activity was in the range 17 – 39 µg Trolox/g and the ferric reduction activity power (FRAP) ranged between 0.7 – 27 µg ascorbic acid/g. Lettuce samples presented the lowest values, followed by cabbage “couve coração”. Vegetables with a more intense green colour, such as kale, wild rocket, cabbage “couve portuguesa”, spinach, watercress and ready-to-eat salad showed higher antioxidant capacity. All nitrate contents (28.8 – 2768.5 mg NO₃⁻/kg) respected the EU maximum levels. As expected, wild rocket, watercress and lettuce samples presented the highest nitrate contents. Nitrite levels were below the detection limit (0.01 mg/kg) for 85% of the samples.

Acknowledgments

This work was supported by FCT grant no. PEst-C/EQB/LA0006/2013 and University of Porto “Projectos Pluridisciplinares” IJUP2011-317. Joana Santos (SFRH/BD/66476/2009) and M. Fátima Barroso (SFRH/BPD/78845/2011) thank FCT for their grants financed by POPH-QREN (subsidized by FSE and MCTES).

References:

- [1] Correia, M., Barroso, A., Barroso, M. F., Soares, D., Oliveira, M.B.P.P. and Delerue-Matos, C. (2010), *Contribution of different vegetable types to exogenous nitrate and nitrite exposure*, Food Chemistry, 120, 960-966.
- [2] Nadtochiy, S.M. and Redman, E.K. (2011), *Mediterranean diet and cardioprotection: The role of nitrite, polyunsaturated fatty acids, and polyphenols*, Nutrition, 27, 733–744.

ANTIOXIDANT CAPACITY OF TROPICAL FRUITS

Mário S.O.Paz¹, Valentina F. Domingues², M. Fátima Barroso², A. P. Carvalho², Cristina Delerue-Matos², Helena Becker¹, Elisane Longhinotti¹.

¹Departamento de Química Analítica e Físico-Química - Universidade Federal do Ceará (UFC) - Cx. Postal 12200 CEP 60455-960 Fortaleza-CE, Brasil.

²REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal.

The consumption of fruits has been highly recommended for its nutritional value, high-fiber and vitamins. Recent studies have been demonstrated these foods as sources of compounds with antioxidant activity. These are products of metabolism vegetable and constitute a diverse group of phytochemicals, highlighting polyphenols and flavonoids. Therefore, they are free radical scavengers, with a protective effect against injuries or degenerative processes that lead to chronic diseases. The aim of this study was to evaluate the total phenolic and flavonoids content and the antioxidant activity of tropical fruits. Considered as a methods of great applicability, 1,1- diphenil-2-picrilhydrazil radical (DPPH•) and ferric reducing antioxidant power (FRAP) were used to determine the antioxidant activity; the Folin-Ciocalteu method was used to determine the total phenolic content (TPC) and the AlCl₃ method to measure the total flavonoid content (TFC). Ethanol and water (1:1) was the extractor solvent. The selected fruit were: acai (*Euterpe oleracea*), acerola (*Malpighia emarginata DC.*), caja (*Spondias mombin L.*), guava (*Psidium guajava*), graviola (*Annona muricata*), mango (*Mangifera indica L.*), pineapple (*Ananas comosus L.*) and tamarind (*Tamarindus indica L.*). The levels of TPC found exhibited relevant quantities of polyphenols, specifically for the acerola, acai and guava, with 5870, 904 and 561 µg gallic acid/g, respectively. The fruits tamarind and pineapple showed the lowest levels of TPC. For TFC the acai exhibited the best result (346 µg epicatechin/g), after graviola (134 µg epicatechin/g) and guava (106 µg epicatechin/g). The other fruits showed a value below of 100 µg/g. Regarding the antioxidant activity, the best results were found for aqueous and ethanolic of fruits acerola, acai e guava. It was observed a direct correlation between the amount of the TPC and antioxidant activity in the analyzed fruits.

Key-words: antioxidant activity, DPPH, FRAP and fruit.

Acknowledgments

The authors are thankful to FCT through the project PEst-C/EQB/LA0006/2013 and CAPES through the project BEX 1998/13-7.

Evaluation of isoflavones content in hydro-alcoholic extracts of *Medicago* spp

F. Rodrigues¹, I. Almeida¹, N. Braga¹, B. Sarmento^{2,3}, M.H. Amaral⁴, M.B.P.P. Oliveira¹

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

² IINFACTS - Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Instituto Superior de Ciências da Saude-Norte, Department of Pharmaceutical Sciences, CESPU, Gandra-PRD, Portugal

³ INEB, Institute of Biomedical Engineering, University of Porto, Portugal

⁴ Department of Drug Sciences, Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Portugal

Introduction: This study focuses on the isoflavones composition of hydro-alcoholic extracts of seven *Medicago* species (*M. minima*, *M. tornata*, *M. truncatula*, *M. rigidula*, *M. scutelata*, *M. segitalis* and *M. sativa*) used in animal feeding. As leguminous crop they contain isoflavones in amounts scarcely described [1-2].

The aim of this study is to evaluate the isoflavones profile of hydro-alcoholic extracts of these *Medicago* species, regarding their incorporation in cosmetics-based products with anti-aging properties.

Experimental description: In this work, two glycosides (daidzin and genistin) and six aglycones (daidzein, glycitein, genistein, formononetin, prunetin and biochanin A) were analyzed by high performance liquid chromatography (HPLC-DAD). Chromatographic separation of compounds was achieved with a Luna 5U C18 (5 µm, 150 x 4.60 mm) column (Teknokroma, Spain) operating at 40 °C. Analytes were monitored at 254 nm, and quantified on the basis of the internal standard method. Chromatographic data were analyzed using the Borwin-PDA Controller Software (JMBS Developments, Le Fontanil, France).

Discussion: In all samples, genistin, daidzein and genistein were the most abundant, being the highest concentration of genistin observed in *M. scutelata* extract (5.16 ± 0.56 mg/kg db), in amounts 2 – 4.5 fold higher than the other samples evaluated. Daidzein is also present in high amounts in *M. rigidula* (12.21 ± 0.1 mg/kg db) and genistein in several species at concentrations ranging from 1.72 to 7.08 mg/kg db. In terms of total isoflavones content per species, *M. rigidula* and *M. sativa* showed the highest and the lowest amounts, respectively, 29.06 mg/kg db and 2.29 mg/kg db.

Conclusion: This study demonstrates that *Medicago* extracts possess an excellent potential as a source of several health promoting bioactive compounds.

References:

[1] Farag, M.A., et al. (2007), *Metabolic profiling and systematic identification of flavonoids and isoflavonoids in roots and cell suspension cultures of Medicago truncatula using HPLC–UV–ESI–MS and GC–MS*, Phytochemistry, 68(3), 342-354.

[2] Nunes, C., et al. (2008) *Physiological responses of the legume model Medicago truncatula cv. Jemalong to water deficit*, Environmental and Experimental Botany, 63(1–3), 289-296.

Acknowledgments: F. Rodrigues is grateful to FCT for a PhD grant (SFRH/BDE/51385/2011). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069-CIÊNCIA DO ALIMENTO).

Castanea sativa by-products: a new sustainable source of bioactive compounds

N. Braga¹, F. Rodrigues¹, MBPP. Oliveira¹

¹ REQUIMTE / Dep. of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

Introduction: *Castanea sativa Mill.* is a species of Fagaceae family abundant in south Europe and Asia and their fruits, chestnut, are an added value resource in producing countries. The chestnut economic value is increasing not only for their nutritional qualities (energy, antioxidants, calcium, minerals, fatty acids and vitamin contents) but also for the beneficial health effects related with its consumption. Chestnut nutritional composition is influenced by seasonal variability, environmental conditions and storage time [1].

Experimental description: To perform this review, bibliographic references from international journals and official documents, related with the composition of *Castanea sativa* by-products and its possible uses in a sustainable society were consulted.

Discussion: During chestnut processing a large amount of waste material is generated namely inner shell, outer shell, leaves and bur. Studies on chestnuts by-products revealed a good profile of bioactive compounds like polyphenols, flavonoids and tannins related with antioxidant, anticarcinogenic and cardioprotective properties [2].

These agro-industrial residues, as extracts, can be explored as a sustainable source of bioactive compounds, and probably used in food, cosmetic and pharmaceutical industries. Recent articles have focused the interest of chestnut leaf and shell extracts, for use in topical formulations and as metal ion adsorbent respectively, showing great potential [3,4]. In the future, chestnut by-products can provide more profits and business opportunities, promoting efficiency and reducing the pollution management costs.

Conclusion: The present review highlights the recent information on the functional properties of chestnut by-products, environment benefits and future applications.

References:

[1] Fernandes, A. et al. (2011). *Assessing the effects of gamma irradiation and storage time in energetic value and in major individual nutrients of chestnuts.* Food and Chemical Toxicology 49 (2011) 2429–2432.

[2] Vasconcelos, MCBM. et al. (2010). *Evaluating the potential of chestnut (Castanea sativa Mill.) fruit pericarp and integument as a source of tocopherols, pigments and polyphenols.* Industrial Crops and Products 31 (2010) 301–311.

[3] Almeida, IF. et al. (2010). *Evaluation of Functional Stability and Batch-to-Batch Reproducibility of a Castanea sativa Leaf Extract with Antioxidant Activity.*

AAPS PharmSciTech, Vol. 11, No. 1, March 2010.

[4] Vazquez, G. et al. (2009). *Chestnut shell as heavy metal adsorbent: Optimization study of lead, copper and zinc cations removal.* Journal of Hazardous Materials 172 (2009) 1402–1414.

Acknowledgments: F. Rodrigues is grateful to FCT for a PhD grant (SFRH/BDE/51385/2011). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069-CIÊNCIA DO ALIMENTO).

Quantification of Biogenic Amines in Liquors by a DLLME based method

M. R. Lopes, J. O. Fernandes and S. C. Cunha

REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal.

Biogenic amines are organic bases of low molecular weight with an aliphatic, aromatic or heterocyclic structure that may be found in various foods, mainly produced by microbial decarboxylation of amino acids [1]. The presence of these compounds in food is a critical point of food safety due to their implication in food poisoning phenomena. Furthermore, it is considered a marker of the level of microbiological contamination in food [2], so their detection and quantification in food is very important.

The aims of this work were to develop and validate a simple, reliable and sensitive chromatography-mass spectrometry method enabling the simultaneous determination of 18 biogenic amines in liqueurs and other alcoholic beverages. The method is based in an advanced dispersive liquid-liquid microextraction (DLLME) procedure that provides the simultaneous extraction and derivatization of all the amines under study in a simple and fast mode, and enabling a great enrichment factor [3]. Shortly, 5 mL of sample were added with a mixture of acetonitrile (dispersive solvent; 1.0 mL), toluene (extractive solvent; 325 μ L), and isobutyl chloroformate (derivatizing reagent; 25 μ L). After a briefly swirling and a centrifugation step, 1 μ L of the toluene phase was used to analyse the amines under the form of chloroformates derivatives. The method showed a good analytical performance in what linearity, precision, recovery and limit of detections were respected. Preliminary results in the several samples analyzed indicated that low amounts of ethylamine, diethylamine, morpholine, 2-phenylethylamine, 1,3-diaminopropane and cadaverine are common to all samples. These results are important taking into account the need to assess the total income of biogenic amines into the human diet.

References:

- [1] Marques, A.P., Leitão, M.C. and San Romão, M.V. (2008), *Biogenic amines in wines: Influence of oenological factors*, Food Chemistry, 107, 857-860.
- [2] Önal, A. (2007), *Analytical, Nutritional and Clinical Methods - A review: Current analytical methods for the determination of biogenic amines in foods*, Food Chemistry, 103, 1475-1486.
- [3] Almeida C., Fernandes J.O. and Cunha S.C. (2012), *A novel dispersive liquid-liquid microextraction (DLLME) gas chromatography-mass spectrometry (GC-MS) method for the determination of eighteen biogenic amines in beer*, Food Control, 25, 380-388.

Acorns as a food resource

R. Pacheco¹, A. F. Vinha^{1,2}, M.B.P.P. Oliveira¹

¹ REQUIMTE, Dep. of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² Faculty of Health Sciences, University Fernando Pessoa, Portugal.

The oaks (*Quercus spp.*) comprise the largest genus Fagaceae, an important family of evergreen or deciduous trees of temperate and tropical climatic areas. The acorn is a one-seeded nut, the hard, dry and indehiscent fruit of oaks, derived from a unilocular ovary of fused carpels [1]. Acorns are an interesting source of carbohydrates, fats and fibers and also contain protein, amino acids and vitamins, mostly A and C. Additionally, it has been proven its antioxidant effects [2]. Appreciation of acorn has been increasing, because of its potential as a food resource. The goal of the present review is to compile the published information about nutritional, phytochemical and bioactive composition of acorns.

Analytical data gathered from a limited number of acorn samples show a considerable variation in nutrient composition, particularly for crude proteins and fat contents. The most significant difference between acorns of different species is their fat amounts [3]. Acorns of some species are more astringent than others what is related with the tannin content. Significant differences in constituents of acorns are dependent of harvest time, storage period, and consequently from the metabolic condition of the acorn (depending upon, for example, factors relating to ripeness, dormancy or germination). Acorns have high fat and starch contents, but low protein amounts. In what concerns fat composition high contents of oleic, palmitic and linoleic acids were described. Acorns have a considerable amount of antioxidants, but show different behavior with thermal treatment [4]. All of these data encourage the idea of using acorns as a potential sustainable food resource, with positive economical impact.

By studying the nutritional and phytochemical composition of autochthones acorns or its extracts, aiming their application as a food ingredient, we expect to obtain data that support the valorization of this by-product. This approach wishes to answer to food security without forgetting the environmental and sustainable impacts.

References:

- [1] Mason, S. R. L (1992), *Acorns in human subsistence*, Ph.D. thesis, Deposited: Institute of Archaeology, University College, London.
- [2] Šálková T., Divišová M., Kadochová S., Beneš J., Delawská K., Kadlčková E., Němečková L., Pokorná K., Voska V., Žemličková A. (2011), *Acorn as Food Resource. An Experiment with Acorn Preparation and Taste*, Interdisciplinaria Archaeologica, Natural Sciences in Archaeology Volume II, Issue 2, 139-147.
- [3] Nieto R., Rivera M., García M.A., Aguilera J.F. (2002), *Amino acid availability and energy value of acorn in the Iberian pig*, Livestock Production Science 77, 227-239.
- [4] Cantos E., Espin J.C., López-Bote C., De La Hoz L., Ordóñez J.A. and Tomás-Barberán F.A. (2003), *Phenolic Compounds and Fatty Acids from Acorns (*Quercus spp.*), the Main Dietary Constituent of Free-Ranged Iberian Pigs*, J. Agric. Food Chem. 51, 6248-6255.

Acknowledgments: This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

Persimmon and Passion Fruit Cultures on Vale do Ave: Agronomic, Economic and Financial Analysis

Inês Anacleto¹, Mário Cunha²

¹ Department of Geosciences, Environment and Spatial Planning, Faculty of sciences, University of Porto, Portugal.

² Department of Geosciences, Environment and Spatial Planning, Faculty of sciences, University of Porto, Portugal.

At a time when many young farmers resort to funds aimed supporting investment projects in the area of primary production, the activity of fruticulture in Northern Portugal, which has hitherto been insignificant, becomes extremely important for the region's economic development.

A study of the agro climatic potential of the different agricultural produce for specific regions, such as Vale do Ave, is almost nonexistent, especially with regard to less conventional cultures. In the region of Vale do Ave, following a decade of decrease of the cultivated land area in general and fruticulture in particular, there is now a growing interest in agricultural activity. In this context, this work aims to analyze the different possibilities which exist for regional fruticulture, namely persimmon and passion fruit, both from an agricultural and economical perspective.

This study describes the climatic conditions required for the production of passion fruit and persimmon, and analyzes the feasibility of such production within the context of the Vale do Ave region. From the agro climatic analysis presented herein, the aforementioned region is found to be suitable for such production.

This work looks into two specific Proder projects concerning passion fruit and persimmon cultivation respectively, and assists potential investors by identifying where investment is needed and, subsequently, the value of returns for a useful life period of nine years. Although both projects are forecast to yield positive returns the passion fruit project is the most promising as it shows full recovery of the invested amount at the end of the second year.

In addition to the conclusions which follow from the specificities of the analyzed projects, the data were extrapolated to different scenarios, and it was found that both cultures yield positive returns on the investment, resulting in IRR values as high as 11% for persimmon and 39% for passion fruit.

Epiphytic fungal communities in vineyards: characterization by culture and molecular techniques

C. Pinto da Silva¹, N. Zilhão², J. Guerner-Moreira³, R. Araújo², M. Cunha⁴, J. Bondoso¹, O. Lage^{1,5} and M. Oliveira²

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² Institute of Molecular Pathology and Immunology of the University of Porto, Portugal.

³ Regional Directorate of Agriculture and Fisheries of the North, Porto, Portugal.

⁴ Department of Geosciences, Environment and Spatial Planning, Faculty of Sciences, University of Porto, Portugal.

⁵Centre of Marine and Environmental Research (CIIMAR), Porto, Portugal.

Fungal spores are an omnipresent group of biological particles in the atmosphere. It is widely known that the bioaerosol composition presents spatio-temporal variations, being highly influenced by meteorological conditions such as temperature, rainfall, and relative humidity. Moreover, these bioparticles are often responsible for the development of plant diseases resulting in enormous productivity losses in crops with high commercial interests such as the grapevine. Therefore, the aim of this work was to identify the main fungal spore types presents in grapevine leaf surface from different demarcated wine regions from Portugal.

Samples of vine leaves and berries were collected, from September to December 2012, in several Demarcated Wine Regions of northern Portugal: Bairrada, Dão, Douro and Vinhos Verdes. Leaves and berries pieces were placed on Sabouraud dextrose agar (SDA) dishes and grown for 3 days. Single colonies presenting the typical morphology of fungi were isolated. Monospore isolates were cultured on SDA, for 5 days, and conidia were harvested for DNA extraction. Amplification and sequencing was performed using universal specific fungal primers pairs. Internal-transcribed spacer region 1, the 5.8S rRNA gene, and ITS region 2 were amplified using the primer pair ITS1 and ITS4. 18S rDNA was amplified with the primers NS1 and NS6. Sequences were identified by similarity searches against other sequences from available databases (NCBI GenBank), using BLASTn search algorithm. Sequences obtained were aligned per locus, and the maximum likelihood trees were generated using the Mega5 software. Bootstrap replication (1,000 replications) was used for statistical support for the nodes in the phylogenetic tree.

A total of 74 fungal isolates were retrieved from environmental samples belonging to the genera *Alternaria* (31%), *Penicillium* (23%), *Cladosporium* (20%), *Aspergillus* (4%), and *Epicoccum* (3%).

A better knowledge on the components of the epiphytic fungal communities is crucial for crop management strategies, namely when a wiser use of fungicides is required.

This research was supported by Reitoria da Universidade do Porto (PP_IJUP2011_54), the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology, under the projects PEst-C/MAR/LA0015/2013.

Phylogeography and population structure of the powdery mildew fungus (*Erysiphe necator*) from diverse grapevine (*Vitis vinifera*) cultivars grown in Portugal

N. Zilhão¹, C. Pinto da Silva², J. Guerner-Moreira³, R. Araújo¹, M. Cunha⁴ and M. Oliveira¹

¹ Institute of Molecular Pathology and Immunology of the University of Porto, Portugal.

² Faculty of Sciences, University of Porto, Portugal.

³ Regional Directorate of Agriculture and Fisheries of the North, Porto, Portugal.

⁴ Department of Geosciences, Environment and Spatial Planning Faculty of Sciences, University of Porto, Portugal.

Grapevines, as many other cultures, are highly susceptible to several fungal phytopathogens that cause economically devastating diseases and require intensive application of fungicides for its prevention and control. Powdery mildew, caused by *Erysiphe necator*, is considered the most important disease since it attacks several plant organs being responsible for enormous losses in productivity. The main objective of this work was to describe the diversity and population structure of the Portuguese isolates from *E. necator*.

Samples of vine leaves and grapes were collected, from September to December 2012, in main Demarcated Wine Regions of northern Portugal: Bairrada, Dão, Douro and Vinhos Verdes. DNA was extracted from leaves and berries using a sodium hydroxide based method. Amplification and sequencing was performed using specific primers pairs for *E. necator*. Five nuclear loci were PCR-amplified and sequenced from each isolate. The gene regions we sequenced included: CYP51, ITS/IGS, β -TUB2, EF1- α , and MAT1. Sequences were identified by similarity searches against other sequences from available databases (NCBI GenBank and EMBL), using BLASTn search algorithm. Sequences obtained were aligned per locus, and the maximum likelihood trees were generated using the Mega5 software. Bootstrap replication (1,000 replications) was used for statistical support for the nodes in the phylogenetic tree.

Amplification was only successful using ITS and IGS primers. The resulting phylogenetic trees obtained from the sequences of these regions showed that the population of *E. necator* do not present genetical diversity. Despite of its geographical provenance, all samples were grouped in a single cluster together with other sequences retrieved from available databases.

A better knowledge on this phytopatogen will undoubtedly contribute to a efficiently management of the grapevine disease and increase production levels.

This research was supported by Reitoria da Universidade do Porto (PP_IJUP2011_54). M. Oliveira (SFRH/BPD/66071/2009) is recipient of Fundação para a Ciência e a Tecnologia (FCT) fellowship.

Perceptions of risk associated with the consumption of honey

Diana M.D. Nascimento^{1,2}, Liliana P.M. Ribeiro^{1,2}, Sónia E.F. Soares², Anabela S.G. Costa², M. Beatriz P.P. Oliveira² and Luís M. Cunha^{1*}

¹ REQUIMTE, DGAOT, Faculty of Sciences, University of Porto, Portugal.

² REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

*lmcunha@fc.up.pt

Food health and safety is an increasing concern for the population since food products are inherently associated with multiple hazards, which may be classified as lifestyle, technological, microbiological and production [1]. This concern arose when most Western European countries faced various food safety incidents in the mid-1980s, leading to a public unease about safety of modern methods of food production [2]. This study sought to assess the perception of risk associated with the consumption of honey from the perspective of Portuguese consumers, adopting the psychometric paradigm by considering a Portuguese version of the Perceived Food Risk Index (PFRI) [3], previously developed by Fife-Schaw and Rowe [4]. The questionnaire was administered to a convenience sample of 100 subjects from the Districts of Bragança, Guarda and Porto, during June and July 2013. Subjects were asked to choose the five most relevant hazards out of a list of twelve. Risk perception was evaluated over ten risk characteristics, for each of the following hazards: i) residues of pesticides; ii) bacteria and fungi, iii) high level of sugars; iv) residues of antibiotics, and v) presence of 5-hydroxymethylfurfural (HMF). Additionally, subjects were asked to rate the probability of each hazard occurring in Protected Designation of Origin (PDO) honeys, other commercial honeys or in homemade honeys. Sixty-nine percent of the respondents were female, with an average of 32.8 (\pm 11.6) years old. Over 60 % of the respondents had consumed honey during the last semester. Main hazards associated with consumption of honey were: residues of pesticides, labelling, excess of sugars, country of origin and residues of antibiotics. Regarding the perception of risk towards the consumption of honey, most dreaded hazards were the presence of residues of both pesticides and antibiotics, and least dreaded was the presence of high levels of sugars, with an inverse effect on knowledge. Perception of probability of occurrence of the hazards was negative for PDO honeys and significantly higher for homemade honeys. In conclusion, this work adds to knowledge about the perceptions of risk associated with honey, and how consumers minimize risk when addressing the consumption of PDO honeys.

References:

- [1] McCarthy, M., Brennan, M., Ritson, C. and De Boer, M. (2006), *Food hazard characteristics and risk reduction behaviour: the view of consumers on the island of Ireland*, British Food Journal, Vol. 108 No. 10, pp. 857-891.
- [2] Knowles, T., Moody, R. and McEachern, M.G. (2007), *European food scares and their impact on EU food policy*, British Food Journal, Vol. 109 No. 1, pp. 43-67.
- [3] Cunha, L. M., de Moura, A. P., Lopes, Z., do Céu Santos, M., and Silva, I. (2010), *Public perceptions of food-related hazards: an application to Portuguese consumers*, British Food Journal, Vol. 112, No. 5, pp. 522-543.
- [4] Fife-Schaw, C. and Rowe, G. (2000), *Extending the applications of the psychometric approach for assessing public perceptions of food risk: some methodological considerations*, Journal of Risk Research, Vol. 3 No. 2, pp. 167-79.

Safety concerns regarding Plant Food Supplements

T. Rocha¹, J.S. Amaral^{1,2} and M.B.P.P. Oliveira¹

¹ Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² ESTiG, Instituto Politécnico de Bragança, Bragança, Portugal.

Introduction: In the last years, plant food supplements (PFS) consumption has been increasing in developed countries, in part reflecting the growing acceptance and use of alternative/traditional medicine but also due to the common idea that PFS are natural products posing no risks to human health. Being legally considered as foods under Directive 2002/46/EC, PFS are under control of the European Food Safety Agency (EFSA) and do not require the same requisites as traditional herbal medicines for legal authorization. In practice, this means that several products are being sold under the umbrella of PSF, being easily available in supermarkets, TV shops and the internet, and not under the supervision and control of the health authorities.

Experimental Description: To perform this review, several bibliographic references from international journals and official agencies documents were consulted, allowing the gathering of sufficient and reliable information supporting the facts presented.

Discussion: The gaps in regulation and guidelines regarding PFS can result in insufficient quality control, therefore allowing the possibility of intentional adulteration of these products [1]. Among the issues that can affect PFS safety, the illegal addition of pharmaceutical substances is of major concern. One of the most popular type of PFS are weight-loss products. Hoping to increase sales, unscrupulous producers can dope PFS with pharmaceutical drugs to provide for quick effects. These drugs can include appetite suppressors (e.g., sibutramin), stimulants (e.g., ephedrine), antidepressants, anxiolytics, diuretics and laxatives (e.g., phenolphthalein) [2]. It should be emphasized that some of these compounds have been banned due to its highly adverse side effects [2,3]. In the last years, the presence of banned appetite suppressors and laxatives have been detected by FDA in the US. Nevertheless, studies regarding this issue in the EU are almost inexistent.

Conclusion: This review highlighted the urgent need for improvement regarding PFS regulation and the need to conduct studies including the development of multitarget methodologies that would allow for the simultaneous screen of several drugs in PFS.

References:

[1] Silano V., Coppens P., Larrañaga-Guetaria A., Minghetti P. and Roth-Ehrang R. (2011). Regulation applicable to plant food supplements and related products in the European Union, *Food & Function*, 2 (2011), 710-719.

[2] Sanzini E., Badea M., Dos Santos A., Restani P. and Sievers H. (2011). Quality control of plant food supplements, *Food & Function*, 2 (2011), 740-746.

[3] Petroczi P., Taylor G., Naughton D.P. (2011). Mission impossible? Regulatory and enforcement issues to safety of dietary supplements, *Food and Chemical Toxicology*, 49 (2011) 393-402.

Acknowledgments: to FCT (PEst-C/EQB/LA0006/2013 and EXPL/DTP-SAP/1438/2013) and QREN (NORTE-07-0124-FEDER-000069-CIÊNCIA DO ALIMENTO)

Study of the regulation of a zinc transporter in *Leishmania infantum*

A. Ramos^{1,2,3}, S. Carvalho³, A. M. Tomás^{2,3}

¹ Department of Biochemistry, Faculty of Sciences, University of Porto, Portugal.

² Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

³ Molecular Parasitology Group, Institute for Molecular and Cell Biology, University of Porto, Portugal.

Leishmania are trypanosomatid parasites and the causative agents of leishmaniasis, a group of neglected diseases for which no effective prevention and treatments are available. Zinc is a crucial element for biological processes and disruption of its homeostasis may cause cell death. Thus, investigating the process by which parasites regulate metal homeostasis is important not only to better understand their biology but also because it can lead to the discovery of new drug targets. Previous work from the group identified and characterized the first Zn transporter in *Leishmania infantum*, *LiZIP3* [1], a protein encoded by two identical genes arranged in tandem. *LiZIP3* expression was shown to decrease when Zn is in excess due to the destabilization of its mRNA. This process appears to require the involvement of a short-lived protein that binds to Zn-responsive element(s) in the gene's untranslated regions (UTRs). The objective of this work was to map the Zn-responsive element(s).

Regulatory elements in trypanosomatid genes have been mapped mainly to the 3'UTR [2]. Therefore, to identify the Zn-responsive sequence(s) in the *LiZIP3* transcript, we made use of vectors expressing luciferase (a reporter gene) under the control of the *LiZIP3* 3'UTR or fragments of it. In order to construct these vectors, we first determined the correct sequence between the two *LiZIP3* genes and identified the polyadenylation site. The intergenic region was observed to be longer than what is annotated in the genome database and the first nucleotides are identical to the region downstream the second *LiZIP3* gene. The poly-A addition site was determined, demonstrating that both *LiZIP3* genes have identical 3'UTRs. After having defined the 3'UTR, the Zn-responsive element was mapped by measuring the activity and expression of luciferase in parasites transfected with the constructs carrying the complete 3'UTR or portions of it and grown in normal and in Zn-supplemented media. This strategy allowed us to locate the *cis*-acting element to a short region within the 3'UTR.

This work, besides elucidating the *LiZIP3* intergenic region and the identification of the gene's 3'UTR, has as its major contribution the delimitation of the Zn-responsive element. This facilitates the identification of the protein that binds to it and directs *LiZIP3* transcript to degradation.

References:

[1] Carvalho, S., *Studies on metal acquisition in Leishmania infantum*, in *Instituto de Ciências Biomédicas Abel Salazar* 2012, University of Porto: Porto. p. 158.

[2] Requena, J.M., *Lights and shadows on gene organization and regulation of gene expression in Leishmania*. *Front Biosci*, 2011. **16**: p. 2069-85.

Tetralogy of Fallot: a cardiopathy with different physiopathology and evolution

M. Campos¹

¹ Faculty of Medicine of University of Porto, Portugal.

Background: Tetralogy of Fallot is the most common cyanotic congenital heart disease [1,2] and perioperative cares' progress allowed achieving survival rates to adulthood about 90% [3]. However, its wide anatomic-clinical spectrum and resulting hemodynamic changes, contribute to a different evolution of these children [4]. The aim of this study is to describe the clinical outcome of newborns admitted to the Neonatology Service of Centro Hospitalar de São João, diagnosed with Tetralogy of Fallot.

Methods: The clinical files of 15 neonates, admitted to the Neonatology Service of Centro Hospitalar de São João, from January 1, 2010 to December 31, 2012, with the diagnosis of tetralogy of Fallot, were retrospectively reviewed.

Results: Of the 15 children, two were excluded for not confirming the diagnosis. The remaining (N=13) were followed for 28.23 ± 9.80 months. Seven (53.8%) underwent palliative surgery, three of which (42.9%) subsequently performed corrective surgery to 23.67 ± 3.51 months. Six (46.2 %) underwent only surgical definitive repair with a mean age of 14.33 ± 8.02 months. There were two deaths (15.4%). Two children were reoperated after palliative surgery and only one after complete repair. Major complications during hospitalization included respiratory infections, hemothorax, seizures, cardiac right bundle branch block and junctional ectopic tachycardia. There was moderately to severe residual pulmonary insufficiency and right ventricular dilatation in 77.8 % of children and tricuspid insufficiency in 66.7 %. Psychomotor evaluation, conducted in eleven children with 21.55 ± 2.88 months, highlighted changes mainly in the motor and language areas.

Conclusions: In this study, these children, admitted in the first days of life in the Neonatal Intensive Care Unit, underwent definitive reparative surgery later, relatively to timing described in the literature. Despite many complications, there was a survival rate of 84.6 % . However, the resulting cardiac, psychomotor and ponderal changes evidence the need for a continuous and multidisciplinary follow-up of these children .

References:

- [1] Hoffman, J.I., Kaplan, S. (2002), *The incidence of congenital heart disease*, Journal of the American College of Cardiology, 39(12), 1890-1900.
- [2] Duro, R.P., Moura, C., Leite-Moreira, A. (2010), *Anatomophysiologic basis of tetralogy of Fallot and its clinical implications*. Revista Portuguesa de Cardiologia, 29(4), 591-630.
- [3] Polat, S., Okuyaz, C., Hallioglu, O., Mert, E., Makharoblidze, K. (2011), *Evaluation of growth and neurodevelopment in children with congenital heart disease*, Pediatrics international: official journal of the Japan Pediatric Society, 53(3),345-9.
- [4] Bailliard, F., Anderson, R.H. (2009), *Tetralogy of Fallot*. Orphanet journal of rare diseases, 4:2.

Lupin: Nutritional composition and applications in the food industry

L. Oliveira¹, D. Torres^{1,2} and O. Pinho^{1,3}

¹ Faculty of nutrition and food science, University of Porto, Portugal.

² Department of Biochemistry (U38 - FCT), Faculty of Medicine, University of Porto, Porto, Portugal.

³ REQUINTE - Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

In Mediterranean region, lupin is a widely grown food and is frequently consumed as an aperitif, while in other European regions its cultivation remains far behind that of other pulses[1].

Recently, interest in lupin was increased worldwide [1, 2]. Its production increased from 757,647 tons in 2006 to 1,290,656 tons in 2012 [3]. This increase was not only due to the agronomical traits of lupin, such as its strong capacity to fix nitrogen, but also due to its biological characteristics allowing its use as a source of food or feed proteins, in pharmaceuticals, as green manure or as a source of natural pesticides [1, 2].

In the present work, nutritional characteristics and possible applications of lupin seeds in food industry were reviewed.

According to published data, lupin seeds show high protein (30%) and high dietary fiber content (16%)[1]. Lupin flour, made from whole lupin or lupin proteins isolates, proves to be an excellent choice for the enrichment of various food products. For example, lupin flour is rich in lysine and poor in methionine and cysteine, therefore, may be a good complement of wheat flour, as it is lysine-poor and sulfur amino acids-rich[4].

Moreover, lupin proteins have technological and functional properties with great interest to food formulation, such as emulsifying and foaming ability, development of animal-free protein foods, or food products suitable for specific population groups, such as celiac patients[5]. Although, in 1997, when lupin was approved as a food ingredient, it was also clear that allergenic proteins exists in this pulse [6].

In this way, it becomes relevant to extend lupin applications in food industry, not only due to its nutritional value, but also due to its technological characteristics making it suitable to the development of new food products.

References:

- [1] Sujak, A., A. Kotlarz, and W. Strobel, *Compositional and nutritional evaluation of several lupin seeds*. Food Chemistry, 2006. **98**(4): p. 711-719.
- [2] Gulewicz, P., et al., *Effect of germination on the protein fraction composition of different lupin seeds*. Food Chemistry, 2008. **107**(2): p. 830-844.
- [3] FAO. 2013; Available from: <http://faostat.fao.org/>.
- [4] Kohajdová, Z., J. Karovičová, and S. Schmidt, *Lupin Composition and Possible Use in Bakery—A Review*. Czech J. Food Sci, 2011. **29**: p. 203–211.
- [5] Martínez-Villaluenga, C., J. Frías, and C. Vidal-Valverde, *Functional lupin seeds (Lupinus albus L. and Lupinus luteus L.) after extraction of α -galactosides*. Food Chemistry, 2006. **98**(2): p. 291-299.
- [6] Goggin, D.E., et al., *Proteomic analysis of lupin seed proteins to identify conglutin β as an allergen, Lup an I*. Journal of Agricultural and Food Chemistry, 2008. **56**(15): p. 6370-6377.

Does environmental exposure to xenoestrogens contribute to cardiometabolic risk during obesity in menopause?

D. Teixeira¹, R. Silva^{1,2}, D. Pestana¹, G. Faria³, S. Norberto¹, C. Sá¹, C. Marques¹, M. Meireles¹, A. Faria^{1,2,4}, L. Correia-Sá^{1,5}, S. Sousa⁵, J.T. Guimarães¹, V. F. Domingues⁵, C. Delerue-Matos⁵, A. Taveira-Gomes⁶, C. Calhau^{1,7}, R. Monteiro¹

1 Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Porto, Portugal

2 Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal

3 General Surgery Department, Oporto Hospital Center, Faculty of Medicine at University of Porto, Porto, Portugal

4 Chemistry Investigation Centre (CIQ), Department of Chemistry, Faculty of Sciences, University of Porto, Porto, Portugal

5 Requimte – Instituto Superior de Engenharia, Instituto Politécnico do Porto, Porto, Portugal

6 General Surgery Department, Pedro Hispano Hospital, Faculty of Medicine, University of Porto, Porto, Portugal

7 CINTESIS - Center for Research in Health Technologies and Information Systems, Porto, Portugal

Some chemicals used in consumer products or manufacturing (e.g. plastics, pesticides) have estrogenic activities; these xenoestrogens (XE) may affect immune responses and have recently emerged as a new risk factor for obesity and cardiovascular disease.

In this regard, we evaluated XE levels in plasma in a sample of Portuguese obese patients that underwent bariatric surgery. Its putative association with pre-operative metabolic parameters and 10-year cardiovascular disease (CVD) risk was assessed, according to menopausal status.

Plasma samples from obese (BMI \geq 35) premenopausal (n=73) and postmenopausal (n=48) women were collected and the levels of XE were determined by gas chromatography with electron-capture detection. Anthropometric and biochemical data were collected at the time of surgery.

Our data shows that XE are pervasive in this obese population. Postmenopausal women had higher prevalence of metabolic syndrome and lower median concentration of the xenoestrogen hexachlorocyclohexane-lindane in plasma. In premenopausal women XE in plasma seems to be a predictor 10-year CVD risk.

Our findings point toward the influence of XE on the development of metabolic abnormalities in women according to menopausal status, when considering an obese population. Therefore, this study underlines the need for human biomonitoring to assess exposure to environmental pollutants

This work was supported by FCT (Fundo Social Europeu, Programa Operacional Potencial Humano da EU (POPH); PEst-OE/SAU/UI0038/2011; SFRH/BPD/40110/2007, SFRH/BD/46640/2008, SFRH/BD/64691/2009, SFRH/BPD/75294/2010, SFRH/BD/78367/2011 and PEst-C/EQB/LA0006/2013), and Projectos de Investigação na Pré-graduação 2011, Universidade do Porto (IJUP).

Alpha1-antitrypsin deficiency caused by rare alleles: haplotype characterization and history

D. Silva^{1,2} and **S. Seixas**²

¹ Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

² Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal.

Alpha-1-Antitrypsin deficiency (AATD) is one of most common genetic disorders among individuals of European descent characterized by reduced serum levels of SERPINA1. AATD is associated to an increased risk of developing pulmonary disease at early ages, due to the unopposed activity of neutrophil elastase in the lower lung. An increased risk of acquiring liver disorder in childhood or adulthood is also attributed to AATD, as a result of SERPINA1 polymerization in hepatocytes. More than 100 variants have been described for *SERPINA1* including rare deficient alleles associated to different levels of protein polymerization and secretion into bloodstream [1,2]. To portrait the spectrum of rare alleles leading to AATD in Portugal and to characterize their haplotype background, we covered ~ 8kb of *SERPINA1* sequence and genotyped two flanking microsatellites in 58 unrelated AATD patients. We identified a total of 14 deleterious variants, including 5 novel and 9 mutations described elsewhere in Europe. The novel variants are 2 missense mutations predicted to affect protein structure (E162G and L263P), 2 frameshifts mutations (R281fsX297 and M374fsX392) and 1 splice donor mutation expected to impair SERPINA1 translation in hepatocytes (IVS1C+3Tdel) [3]. Among the previously described variants, the F52del [4] mutation (M_{Malton} and M_{Palermo} alleles) was the most frequent (N=27), followed by R39C [4] (I allele; N=8) and D256V [4] (P_{Lowell}; N=4). In our dataset the F52del mutation was associated to 2 major haplotypes suggesting an independent origin of M_{Malton} and M_{Palermo} alleles and codon 52 as potential mutational hotspot. Notwithstanding, other rare alleles (T and Z_{Augsburg}) [4] are proposed to have arisen by recombination due to the haplotype sharing with common variants of *SERPINA1*. Our work provides evidence for a larger repertoire of rare variants underlying AATD in which older alleles are likely to be dispersed throughout Europe while recent ones are expected to be confined to specific geographic regions.

[1] American Thoracic S, European Respiratory S (2003) *American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency*. Am J Respir Crit Care Med 168: 818-900.

[2] Janciauskiene SM, Bals R, Koczulla R, Vogelmeier C, Kohnlein T, et al. (2011) *The discovery of alpha1-antitrypsin and its role in health and disease*. Respir Med 105: 1129-1139.

[3] Seixas S, Mendonca C, Costa F, Rocha J (2002) *alpha1-Antitrypsin null alleles: evidence for the recurrence of the L353fsX376 mutation and a novel G-->A transition in position +1 of intron 1C affecting normal mRNA splicing*. Clin Genet 62: 175-180.

[4] Dickens JA, Lomas DA (2011) *Why has it been so difficult to prove the efficacy of alpha-1-antitrypsin replacement therapy? Insights from the study of disease pathogenesis*. Drug Des Devel Ther 5: 391-405.

Dysphagia in the elderly and nutritional status

A. M. Dias¹, C. M. Morais¹

¹ Faculty of Nutrition and Food Sciences of the University of Porto, Portugal.

Dysphagia is an emergent swallowing disorder in the elderly population, which is characterized by a difficulty or impairment in swallowing liquids, solids or semi-solids elements [1,2]. Age related physiologic changes and pathologies make elderly people more suitable to present dysphagia [2]. This swallowing disorder has an impact in the nutritional status, and can lead to undernutrition and/or dehydration, by reducing the food and liquid intake [3,4].

The aim of this study is to evaluate the prevalence of dysphagia in hospitalized elderly people (≥ 65 years) and the relation between dysphagia, demographic factors and nutritional status. This study took place in the Hospital de Sant Joan Despí Moisès Broggi, Barcelona (HSJDMB), where we applied a survey to 70 random patients (aged between 66-96 years, 50% females).

The presence of dysphagia was evaluated by the Volume-Viscosity Swallow Test (V-VST), and 20% of the patients presented dysphagia. We found a significant relation between older age and dysphagia.

The nutritional status was evaluated by the application of the Mini Nutritional Assessment Short Form (MNA-SF). Although the differences between the two groups were not statistically significant, we found a higher association between malnutrition (37,5%) and risk of malnutrition (50%) in patients with dysphagia, when compared with the patients without dysphagia (23,2% and 44,6%). We also found lower Body Mass Index (BMI) and higher percentage of recent weight loss (%WL) in patients with dysphagia (n.s.).

We consider dysphagia to be an underdiagnosed problem in the elderly. To prevent nutritional complications in this population, the knowledge about the diagnostic and the nutritional approach of dysphagia must be enhanced. Further studies are also needed in order to understand the determinants of dysphagia in older people.

[1] Sura L, Madhavan A, Carnaby G and Crary MA. (2012) *Dysphagia in the elderly: management and nutritional considerations*, Clinical interventions in aging; 7, 287-98.

[2] Forster A, Samaras N, Gold G and Samaras D. (2011), *Oropharyngeal dysphagia in older adults: A review*, European Geriatric Medicine, 2 (6), 356-62.

[3] Taylor KA and Barr SI. (2006), *Provision of small, frequent meals does not improve energy intake of elderly residents with dysphagia who live in an extended-care facility*, Journal of the American Dietetic Association, 106 (7), 1115-8.

[4] Ney DM, Weiss JM, Kind AJ and Robbins J., (2009), *Senescent swallowing: impact, strategies, and interventions*, Nutrition in clinical practice: American Society for Parenteral and Enteral Nutrition, 24 (3), 395-413.

Homozygosity for the c.1486T>C polymorphism in *toll-like receptor 9* gene is associated with a decreased inflammatory response in hemodialysis patients

Miguel Santos-Martins¹, Sandra Ribeiro^{2,3}, Petronila Rocha-Pereira^{3,4}, Flávio Reis⁵, Leonilde Amado⁶, Maria do Sameiro-Faria⁶, Vasco Miranda⁶, Alexandre Quintanilha^{1,3}, Luís Belo^{2,3}, Alice Santos-Silva^{2,3}, Elsa Bronze-da-Rocha^{2,3}, Elísio Costa^{2,3}

1- Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto; 2- Departamento de Ciências Biológicas, Laboratório de Bioquímica, Faculdade Farmácia, Universidade do Porto; 3- Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto; 4- Centro Investigação Ciências Saúde, Universidade Beira Interior, Covilhã; 5- IBILI, Faculdade de Medicina, Universidade de Coimbra; 6- Fresenius Medical Center, Dinefro – Diálises e Nefrologia, SA, Maia.

Toll-like receptors (TLRs), present on the cell surface of innate immune cells, recognize microbial motifs, the pathogen associated molecular patterns and activate downstream signaling pathways [1]. TLR9 is the pattern recognition receptor for microbial DNA. The polymorphism c.1486T>C in *TLR9* gene has been investigated in different diseases and seems to alter the functionality of *TLR9* gene. Inflammation is a common feature in end-stage renal disease (ESRD) patients; however, the mechanisms/factors triggering the inflammatory process are still poorly clarified. It has been suggested that systemic uremic proteins and the dialysis procedure can lead to increased immune cell activation and enhance the inflammatory process [2].

In this work, we aimed to evaluate the impact of the c.1486T>C polymorphism in the inflammatory response of ESRD patients.

One hundred eighty-four ESRD patients from clinical hemodialysis of the north region of Portugal were enrolled in this study. Socio-demographic and hematological data, parameters defining dialysis adequacy, and iron metabolism and inflammatory markers were evaluated; the genotyping of *TLR9* (c.1486T>C) polymorphism was performed by restriction fragments length polymorphisms (PCR-RFLP), using *Afl II* restriction endonuclease.

We found that the prevalence of TC and CC of *TLR9* c.1486T>C polymorphism in our ESRD group of patients was 57.1% and 20.75%, respectively. ESRD patients homozygous for the c.1486T>C polymorphism, when compared with ESRD patients heterozygous or homozygous for the wild-type sequence, showed lower levels of interleukin-6 [1.8 (1.0-2.9 pg/mL) vs 2.5 (1.5-4.4 pg/mL), p=0.022], C-reactive protein [3.1 (1.9-8.4 mg/dL) vs 5.7 (2.5-14.6 mg/dL), p=0.005] and neutrophil counts [3.5 (2.5-4.2 x 10⁹/L) vs 3.8 (3.1-4.9 x10⁹/L), p=0.005]. No significant differences were found in hematological data and in dialysis adequacy markers, neither in iron metabolism.

In conclusion, our data suggest that homozygosity for the c.1486T>C polymorphism is associated with a decreased inflammatory response in ESRD patients under hemodialysis. These results provide new insights in the role of *TLR9* polymorphisms in renal disease, which might have therapeutic impact in a near future.

[1] Kawai T, Akira S. (2010), The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors, *Nat Immunol*, 11,373.

[2] Costa E, Pereira BJG, Rocha-Pereira P, Rocha S, Reis F, Teixeira F, Miranda V, Sameiro-Faria M, Loureiro A, Quintanilha A, Belo L, Santos-Silva A. (2008), Role of prohepcidin, inflammatory markers and iron status in resistance to rhEPO therapy in hemodialysis patients, *Am J Nephrol*, 28, 677-683.

Unraveling post-transcriptional regulation of alternative polyadenylated *MCL1* isoforms by miRNAs in human T Cells

A. Curinha^{1,2}, I. Pereira-Castro¹ and A. Moreira¹

¹ Gene Regulation Group of IBMC – Instituto de Biologia Molecular e Celular, University of Porto, Porto, Portugal;

² Department of Biology, University of Aveiro, Aveiro, Portugal

Polyadenylation is a fundamental processing step of mRNA maturation, essential for its export, stability and translation [1]. As it may occur at the 3' untranslated region (3'UTR), this process can be regulated by microRNAs and RNA binding proteins (RBPS) targeting that region [1]. Bioinformatic analyses have shown that more than 50% of human genes have several polyadenylation sites in the 3'UTR that are used to produce multiple mRNA isoforms by alternative polyadenylation (APA) [1]. This mechanism has a fundamental role in gene expression in a variety of cellular programs [1]. In the immune system, it has been shown that upon T cell activation there is a global switch in polyA (pA) signal selection from a distal to a proximal pA signal, originating mRNAs with shorter 3'UTRs and, consequently, with less microRNA and RBP target sites [2]. The *MCL1* (Myeloid Cell Leukemia Sequence 1) gene encodes an anti-apoptotic protein, which is a member of the Bcl-2 family of apoptosis regulators. *MCL1* is essential for development and maintenance of both B and T lymphocytes in animals [3]. The aim of our study is to map *MCL1* mRNAs 3'ends and characterize the role of miRNAs in the regulation of *MCL1* APA-derived isoforms. By *in silico* analysis and 3' RACE, we mapped the *MCL1* mRNA 3'-ends using a human T cell line (Jurkat E6-1) and primary T cells. Our preliminary results show that *MCL1* produces 3 well-defined mRNA isoforms by APA in human resting and activated T cells, as well as in Jurkat E6.1 cells. To study the miRNAs involved in the regulation of *MCL1* we performed *in silico* analysis and identified several putative miRNA target sites in *MCL1* 3'UTR that are highly conserved in mammals and focused our studies in three of them: miR-92a, miR-29b and miR-17. By RT-qPCR, we show that these miRNAs are expressed in T cells, indicating that they could regulate *MCL1* isoforms. Since *MCL1* plays a major role in suppressing apoptosis, characterization of the *MCL1* mRNA isoforms produced by APA and the miRNAs that regulate its expression may be a useful diagnostic/therapeutic tool in different clinical contexts in the future.

This work was funded by FEDER funds through the Operational Competitiveness Programme – COMPETE and by National Funds through FCT – Fundação para a Ciência e a Tecnologia under the project **FCOMP-01-0124-FEDER-021201 (PTDC/SAU-GMG/116621/2010)**.

References:

- [1] Lutz, C.S. and Moreira, A. (2010), *Alternative mRNA Polyadenylation in eukaryotes: an effective regulator of gene expression*. Wiley Interdisciplinary Reviews: RNA. 2: 23-31.
- [2] Sandberg, R., Neilson, J.R., Sarma, A., Sharp, P.A. and Burge, C.B. (2008) *Proliferating Cells Express mRNAs with Shortened 3' Untranslated Regions and Fewer MicroRNA Target Sites*. Science. 320(5883):1643-7.
- [3] Perciavalle, R.M. and Opferman, J.T. (2012), *Delving deeper: MCL-1's contributions to normal and cancer biology*. Cell Press. 23(1):22-9.

Characterization of Tie1 expression in human erectile tissue and its relation with aging and cardiovascular risk factors

J. Fonseca¹, N. Tomada², A. Magalhães³, A. Gouveia^{1,4} and D. Neves¹

¹ Department of Experimental Biology, Faculty of Medicine of Universidade do Porto & Institute for Molecular and Cell Biology (IBMC) of Universidade do Porto, Portugal

² Department of Urology, Central Hospital of S. João, Porto, Portugal

³ Requimte/Department of Chemistry & Biochemistry of Faculty of Sciences of Universidade do Porto, Portugal

⁴ Faculty of Nutrition and Food Sciences, Universidade do Porto, Portugal

Erectile dysfunction of vasculogenic origin is highly related with aging and other cardiovascular disease risk factors (CVDRF). These conditions cause imbalance in the expression of vascular growth factors in corpus cavernosum (CC) affecting endothelial function. Although the underlying molecular mechanisms are not fully understood, angiopoietins(Ang)-Tie2 system seems to be particularly dependent on aging, considering previous demonstration of upregulation of expression Tie2 and its ligands (Ang1 and Ang2) in the CC of aged individuals. [1] Tie1 is another member of Tie family that has already been identified in the endothelium of human CC. [2] However, its role on angiopoietin regulation remains poorly clarified, thought it seems to downregulate Ang1-Tie2 signaling pathways. In the present study, we characterize the expression of Tie1 in human CC along aging and in aged individuals with CVDRF.

Human CC fragments obtained from programmed surgeries or organ donors were divided in three groups: young (16–35 years), aged (59–74 years) with and without CVDRF (Diabetes Mellitus 2, hypertension and dyslipidemia). Dual-immunolabeling of Tie1 and specific markers of endothelium and smooth muscle (PECAM1 and α -actin, respectively) was performed, and images acquired in an Apotome microscope. Ang1, Ang2, Tie1 and Tie2, protein levels were assessed after Western blotting and labelled bands were evidenced and quantified. The ratio of Ang1/Ang2, Ang1/Tie1, Ang2/Tie1 and Tie1/Tie2 in tissue were also evaluated and statistically compared among groups.

In all analysed CC fragments a 125 kDa band corresponding to Tie1 was detected, presenting an apparent up-regulation in samples obtained from healthy aged but not CVDRF individuals. Bands corresponding to Ang1, Ang2 and Tie2 were also detected. Decrease tendencies in Ang1/Tie1 and Ang1/Tie2 and increase in Tie1/Tie2 in aged individuals with cardiovascular disease were found, although without statistical significance.

The present results suggest that Tie1 interferes in Ang1-Tie2 signaling the Human CC apparently downregulating Tie2-dependent vascular stabilizing mechanisms.

Ongoing RT-PCR study is expected to clarify these findings.

References:

[1] Figueiredo, A., Cordeiro, A. L., Tomada, N., Tomada, I., Rodrigues, A., Gouveia, A. and Neves, D. (2011), *Real-time PCR study of Ang1, Ang2, Tie-2, VEGF, and KDR expression in human erectile tissue during aging*, J Sex Med, 8 (5), pp. 1341-51.

[2] Fonseca, J.A., Tomada, I., Tomada, N., Almeida, H. and Neves, D. (2013), *Immunofluorescent detection of Tie1 in endothelium of the Rat and Human corpus cavernosum during aging*, Microsc Microanal, 19 (4), pp. 39-40.

Lung Cancer – The Role of Trace Elements

M. Cruz¹, P. Ramos¹, M.Moura², D. Lourenço², R. Mendes², A. Santos² and A. Almeida¹

¹ REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

² National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal.

Lung cancer is a major public health problem. According to GLOBOCAN 2012 [1], it has been the most common cancer in the world for several decades (1.8 million new cases, 12.9% of the total, were estimated for 2012), and remains as the most common cancer in men (1.2 million, 16.7% of the total). Late and non-specific symptoms and poor prognosis of the disease contribute for its high fatality. The overall ratio of mortality to incidence is 0.87, making it the most common cause of death from cancer worldwide (ca. one in five, amounting to 1.59 million deaths) [1].

Risk factors most associated with lung cancer are tobacco, occupational exposure, environmental pollution, diet, sex, race, age, genetic susceptibility, family history and previous respiratory diseases [2]. Several trace elements (e.g., Cr, Cu, Mn, Ni, Se, Zn, Fe) have been associated with the disease [3-6], but their actual role is not well defined.

Based on this background, our present work⁷ consists in the determination of trace elements in lung tissue. General objective is to investigate the possible association between them and the development/evolution of lung disease. Specific aims are: 1) to define the reference (“normal”) values for trace elements in lung tissue; 2) to study if there is any tendency for an age-related increase/decrease; 3) to look for eventual differences between smokers and non-smokers individuals; and 4) to compare the trace element levels found in cancerous lung tissue with those “reference” values, in order to make a link with the disease.

Samples (fragments of ca. 1 cm³) from right lung upper lobe are being collected during autopsy exams at the National Institute of Legal Medicine and Forensic Sciences (INMLCF), North Branch. Samples will be solubilized through a closed-vessel microwave digestion procedure and the obtained solutions will be analyzed for a wide range of trace elements by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

References:

[1] http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

[2] van Zandwijk, N. (2009), *A etiology and prevention of lung cancer*, In: Spiro, S.G., Huber, R.M. and Janes, S.M. ed. Chapter 1, Lung cancer: oncogenesis and prevention, European Respiratory Society Monograph, 17(44), 1-14.

[3] Schwartz, M.K. (1975), *Role of trace elements in cancer*, Cancer Research, 35, 3481-3487.

[4] Baumgardt B, Jackwerth E, Otto H, Tölg G. (1986), *Trace analysis to determine heavy metal load in lung tissue*, International Archives of Occupational and Environmental Health, 58 (1), 27-34.

[5] Majewska, U., et al. (2007), *Trace element concentration distributions in breast, lung and colon tissues*, Physics and Medicine in Biology 52(13), 3895-3911.

[6] Fritz, H., et al. (2011), *Selenium and lung cancer: a systematic review and meta analysis*, PLoS One, 6 (11), 1-10.

⁷ Master in Toxicologia Analítica Clínica e Forense, FFUP.

Trace Elements and Cardiovascular Diseases

A. Alves¹, P. Ramos¹, M. Moura², D. Lourenço², R. Mendes², A. Santos² and A. Almeida¹

¹ REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

² National Institute of Legal Medicine and Forensic Sciences, North Branch, Portugal.

Changes in lifestyle and the continued increase in life expectancy that has been witnessing in recent decades, with the consequent increase in the percentage of elderly population [1], have originated a significant increase in the incidence and prevalence of cardiovascular disease (CVD), which have become a major public health worldwide, accounting for nearly half of all deaths in Europe [2].

The major risk factors for CVD are well known: inactivity, obesity/overweight, high cholesterol levels, hypertension, stress, smoking and metabolic syndrome.

However these "traditional" risk factors for CVD do not account for all deaths. Environmental, dietary, and other lifestyle factors also appear to be important in the development of CVD. In particular, disturbances in the homeostasis of trace elements, both the "essential" (e.g. Se, Cu, Zn, Fe, Mn) and the eminently "toxic" (e.g. Pb, Cd, Hg, As) has been identified as potentially involved in the development of CVD. Nevertheless, the importance of this association between trace elements and CVD is less well defined [3] and most existing studies on this topic is simply based on the analysis of body fluids such as blood and urine, since they can be easily obtained.

Based on this background, we are performing a study aimed to find direct evidence, i.e., in the tissues themselves, of any changes in trace elements levels, in order to assess their actual involvement in the pathogenesis of CVD⁸.

In this post-mortem study, samples from 20 "healthy" individuals ("controls") and 50 individuals with evidence of CVD are being collected. Target tissues are kidney (cortex and medulla), myocardium, valves (aortic, mitral and tricuspid), aorta, carotid, and iliac arteries (with and without evidence of atheroma).

Trace elements will be analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after the samples microwave-assisted acid digestion to determine the widest possible panel of elements in the samples.

References:

[1] World Health Organization (1995), *Epidemiology and prevention of cardiovascular diseases in elderly people*, Geneva.

[2] Lloyd, L.J., Langley-Evans, S.C. and McMullen S. (2005), *Childhood obesity and adult cardiovascular disease risk: a systematic review*, International Journal of Obesity, 34(1), 18-28.

[3] Alissa, E.M. and Ferns, G.A. (2011), *Heavy metal poisoning and cardiovascular disease risk: a systematic review*, Journal of Toxicology, 870125.

Acknowledgments: This work is funded by FEDER funds through Programa Operacional Factores de Competitividade - COMPETE and National funds through FCT - Fundação para a Ciência e a Tecnologia under the project PTDC/SAU-ESA/108871/2008.

⁸ Master in Clinical and Forensic Analytical Toxicology, FFUP 2013-2014.

Pleiotropic effects of cardiovascular drugs: evaluation of the ability to prevent myoglobin oxidation mediated by peroxy radicals

A.I.P. Mota, T.R.P. Soares, M.A. Segundo, S.H. Reis, L.M. Magalhães

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

The current therapeutic strategies are based on the design of multifunctional drug candidates that are able to interact with multiple disease related targets. In this context, drugs that have the ability to scavenge reactive oxygen species (ROS) implicated in the development of several human oxidative-stress diseases have beneficial clinical effects. The analytical methods that have been applied to measure the scavenging capacity of drugs are not adequate because the assays are based on the use of synthetic radicals (e.g., DPPH[•] and ABTS^{•+}) and/or target molecules (e.g., fluorescein, luminol and pyrogallol in ORAC assay) that do not represent any reactive species and biotarget, respectively, found in living organisms [1].

In this work, a novel analytical approach is proposed to determine the scavenging capacity of drugs. For this, the peroxy radicals generated from thermo-decomposition of 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH) and an endogenous protein (myoglobin) will be used as models of reactive species and biotarget, respectively. Under reaction conditions (pH, temperature) similar to those found in vivo, the protein peroxidation induced by reactive species was monitored spectrophotometrically by the absorbance decrease at 409 nm. Drugs that have the capacity to prevent protein peroxidation caused a delay of absorbance decrease and their scavenging capacity was assessed by the increase of the area under curve (AUC). The drug concentration that provoked an increase of 50% of AUC (IC₅₀) was determined for several drugs used for cardiovascular pathologies, such as β -blockers (atenolol, labetalol, propranolol) and statins (pravastatin, fluvastatin, lovastatin and simvastatin). Their IC₅₀ values were compared to that obtained for the endogenous antioxidants (glutathione, uric acid and taurine). Among the drugs tested, labetalol (12.5 \pm 1.2 μ M), enalapril (15.2 \pm 1.8 μ M) and fluvastatin (21.0 \pm 2.2 μ M) were those that presented higher ability to prevent protein oxidative damage, even higher than that determined for reduced glutathione (232 \pm 10 μ M). These results indicate that these drugs may have pleiotropic effects by preventing oxidative damage of biomolecules.

Acknowledgements:

L. M. Magalhães thanks FSE and MCTES for the financial support through the POPH-QREN program. Authors acknowledge for the financial support of Pre-Graduate Scientific Research Pluridisciplinary Projects (PP_IJUP_2011_240) and also to FCT for the Strategic Project PEst-C/EQB/LA0006/2011.

References:

[1] Beretta, G., Facino, R.M. (2010), *Recent advances in the assessment of the antioxidant capacity of pharmaceutical drugs: from in vitro to in vivo evidence*, Analytical and Bioanalytical Chemistry, 398, 67-75.

Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson): The effect of processing on the amino acid profile

A.S.G. Costa¹, F.B. Pimentel¹, T.J.R. Fernandes¹, A.C. Ruas¹,
A.F. Vinha^{1,2}, R.C. Alves^{1,3}, M.B.P.P. Oliveira¹

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² Faculty of Health Sciences, University Fernando Pessoa, Portugal.

³ REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

The elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson) is a tropical plant from Southeast Asia, where it is cultivated, but it also grows wild in the Philippines, Malaysia, Indonesia and other countries in the region, including East Timor. Besides being used as food, it is still used in popular medicine to treat some health problems, such as arthralgia, elephantiasis, inflammation, hemorrhoids, vomiting, asthma, dyspepsia, flatulence, colic, constipation, among others [1].

The aim of this study was to characterize the amino acids composition of elephant foot yam from Timor. Fresh and boiled tubers were also compared to evaluate the effect of processing on the amino acids profile.

Samples were prepared as described by Pimentel et al. [2], derivatized with dansyl chloride and analysed by HPLC with fluorescence detection. Analyses were performed in triplicate.

Considering the essential amino acids, for both fresh and boiled samples, the prevailing ones were phenylalanine, threonine, leucine, lysine, followed by valine plus methionine and isoleucine. Similarly, serine was the main amino acid from the non essential fraction, followed by arginine, aspartic plus glutamic acids, alanine and proline. Fresh samples presented higher levels of all amino acids, when compared with the boiled ones. We have concluded that the boiling process has influenced the final concentration of the different amino acids in the samples.

References:

[1] Pullaiah T. (2006), Encyclopaedia of world medicinal plants. Regency Publications. India. Vol 1, pp-145.

[2] Pimentel, F. B., Alves, R. C., Costa, A. S. G., Torres, D., Almeida, M. F., and Oliveira, M. B. P. P. (2014), *Phenylketonuria: Protein content and amino acids profile of dishes for phenylketonuric patients. The relevance of phenylalanine*. Food Chemistry, 149(0), 144-150.

Acknowledgments: R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

BeWo cells differentiation: Syncytialisation induction and anandamide effects in cell viability

A.C. Gomes¹, M. Costa^{1,2}, B. Fonseca^{1,2}, N. Teixeira^{1,2} and G. Correia-da-Silva^{1,2}

¹ Laboratory of Biochemistry, Department of Biological Sciences, Faculty of Pharmacy.

² Institute for Molecular and Cell Biology (IBMC), University of Porto, Portugal.

The placenta is a highly specialized organ that supports the growth of the fetus. Its formation and development requires a highly regulated proliferation, differentiation and apoptosis of trophoblast cells. Cytotrophoblasts proliferate and can differentiate into other type of trophoblasts the syncytiotrophoblasts, multinucleated cells, responsible for the biosynthesis of hormones and proteins. Anomalies in the processes of proliferation and differentiation may have drastic effects in pregnancy such as, miscarriages, preeclampsia and fetal growth restriction. In the last years, endocannabinoids (Ecs) have emerged as a new players in pregnancy events like implantation and decidualization [1,2] but their importance during placental development is still unknown. Our ongoing work shows that the two major Ecs anandamide (AEA) and 2-arachidonoylglycerol (2-AG) induce apoptosis in BeWo cells, a choriocarcinoma cell line used as a model of cytotrophoblasts [3]. In this study we intended to determine AEA actions after BeWo cells- induced differentiation into multinucleated syncytiotrophoblasts.

BeWo cells were treated with forskolin, estradiol or vehicle for 48 and 72 h. Biochemical differentiation was measured by assaying placental ecto-alkaline phosphatase (pALP) activity. BeWo cells fusion was assessed by immunocytochemistry for E-cadherin and nuclei were counterstained with DAPI.

We found that forskolin (10 and 20 μ M) has an inductive effect on syncytialisation after 48 h of exposure, as evidenced by the increase in alkaline phosphatase activity and by the formation of multinuclear cells. Higher concentrations had a dramatic effect on BeWo cell viability. Estradiol was not able to induce syncytialisation in the studied conditions. Treatment with AEA resulted in a decrease in cell viability as it was observed in the non-differentiated cytotrophoblasts. This study demonstrates that AEA actions may affect both trophoblast cell populations suggesting a role for endocannabinoids in trophoblast cells turnover and in placental development.

Acknowledgements: FCT for M.A. Costa PhD grant (SFRH/BD/70721/2010) and B. Fonseca Post-doctoral grant (SFRH/BPD/72958/2010).

References:

- [1] Karasu, T., Marczylo, T.H., Maccarrone, M., Konje, J.C. (2011), *The role of sex steroid hormones, cytokines and the endocannabinoid system in female fertility*. Hum Reprod Update, 17(3), 347-61.
- [2] Fonseca, B.M., Correia-da-Silva, G., Almada, M., Costa, M.A., Teixeira, N.A. (2013), *The Endocannabinoid System in the Postimplantation Period: A Role during Decidualization and Placentation*. Int J Endocrinol, 510-540.
- [3] Costa, M.A., Fonseca, B., Keating, E., Teixeira, N., Correia-da-Silva, G. (2013) 2-arachidonoylglycerol in cytotrophoblasts: Metabolic enzymes expression and apoptosis in BeWo cells. Reproduction. 2013 Dec 9. (in Press).

Differential toxicity in the cerebellum of adolescent and aged rats following a MDMA neurotoxic regimen

A. Teixeira-Gomes¹, V.M. Costa¹, R. Feio-Azevedo¹, F.C. Pereira², M. Duarte-Araújo³, E. Fernandes⁴, M.L. Bastos¹, F. Carvalho¹, and J.P. Capela^{1,5}

¹REQUIMTE (Rede de Química e Tecnologia), Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal ²Farmacologia e Terapêutica Experimental, Instituto Biomédico de Investigação da Luz e Imagem (IBILI), Faculdade de Medicina, Universidade de Coimbra, Portugal ³Biotério do Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, Portugal ⁴REQUIMTE, Laboratório de Química Aplicada, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Portugal ⁵Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Portugal

3,4-Methylenedioxymethamphetamine (MDMA) is a well-known psychoactive drug, commonly used in recreational settings by many adolescents and adults, with acknowledged neurotoxic properties [1]. In humans, MDMA neurotoxic effects were associated to decrease gray matter in the cerebellum [2]. MDMA effects in the cerebellum have been scarcely studied and little is known about the susceptibility of different aging groups. Thus, the aim of this work was to assess the neurotoxicity of MDMA in cerebellum, by evaluating its energetic and oxidative stress status, in adolescent and old rat models after MDMA-binge administration.

Two experiments were conducted. In experiment 1, adolescent male Wistar rats (6 weeks-old) were housed in individual cages, kept in a controlled ambient (temp 22°C; 12h light/dark cycles), and two groups were formed: control (n=5) and MDMA-treated (n=5). The groups received, respectively, an intraperitoneal injection of a saline (NaCl 0.9%) or 5mg/Kg MDMA every 2 hours, to a total of 3 doses [3]. The temperature of each animal was monitored for 7 hours after the first injection. Experiment two was conducted with aged (18 to 21 months-old) male Wistar rats and set as previously described. Aged animals did not receive the 3rd dose of MDMA, given the risk of mortality following the high temperature reached after the 2nd MDMA dose. Seven days later, animals were sacrificed and the cerebellum was collected. Reduced and oxidized glutathione (GSH and GSSG), ATP, and quinoprotein levels were determined.

In adolescent rats, after the 2nd dose of MDMA, the increase in temperature was significantly higher when compared to the control group ($p < 0.0001$), and the temperature persisted high for more than 3 hours after the 3rd dose. In aged rats, a significant increase in temperature occurred after the 2nd dose of MDMA that persisted for the following couple of hours. In particular, in aged animals the temperature reached a peak mean near 41°C after the 2nd MDMA dose, while adolescent animals never surpassed the mean of 39°C after the 3 MDMA doses. In the cerebellum, no differences were found between controls and MDMA-treated animals in the quinoprotein levels or GSH/GSSG ratio in both adolescent and aged rats. Importantly, there were significant decreases in ATP content of the cerebellum in the MDMA group ($p = 0.015$) of aged animals, while in adolescent animals no differences were found in ATP levels.

In conclusion, MDMA binge administration leads to hyperthermia in adolescent and old rats, with higher magnitude in aged animals. One week after the MDMA administration, aged animals presented significant decreases in the cerebellum ATP content, indicating a higher susceptibility of this group to the neurotoxic effects of MDMA.

Acknowledgments:

Supported by the project (PTDC/SAU-FCF/102958/2008), under the frame of “Programa Operacional Temático Fatores de Competitividade (COMPETE)” and “Fundo Comunitário Europeu (FEDER)”. R.F. (BIM) and V.M.C. (SFRH/BPD/63746/2009) acknowledge “Fundação para a Ciência e Tecnologia (FCT)” for their grants.

References:

[1] Capela *et al.* 2009 *Molecular Neurobiology* 39:210-271; [2] Cowan *et al.* 2003 *Drug and Alcohol Dependence* 72:225-235; [3] Goni-Allo *et al.* 2008 *Psychopharmacology* 197:263-278.

Effects of chronic ethanol treatment and withdrawal on the vasoactive intestinal polypeptide content and cholinergic innervation of the rat somatosensory cortex

M. Vilela¹, S. Sousa¹, A. Cardoso¹, M.D. Madeira¹ and P.A. Pereira¹

¹Department of Anatomy, Faculty of Medicine, University of Porto, Portugal

The present study was designed to investigate the effects of chronic ethanol treatment (CET) and Withdrawal (W) on the density of vasoactive intestinal polypeptide-immunoreactive (VIP-ir) neurons and on the cholinergic varicosities in layers II/III, V and VI of the primary somatosensory cortex of the rat. We have also investigated if the administration of nerve growth factor (NGF) would interfere with the content of VIP and with the cholinergic innervation of this cortical area.

It is widely accepted that chronic ethanol intake and W modify the morphology and disturb the function of the central nervous system. In several brain regions, ethanol consumption and W are associated with reductions in neuropeptide expression and cholinergic innervation. VIP is one of the brain neuropeptides that are vulnerable to CET and W. This neuropeptide regulates several physiological functions such as learning and memory, circadian rhythms and cerebral blood flow. It is expressed in various brain regions, including the cerebral cortex. The cerebral cortex receives a dense cholinergic innervation from projection neurons of the basal forebrain and there is evidence suggesting a trophic dependence of VIP neurons on this input. Forebrain cholinergic neurons are dependent on NGF for phenotype maintenance and there is evidence that exposure to ethanol and W alters the NGF neurotrophic support.

Twenty Wistar male rats were assigned to control, ethanol-treated, withdrawn and NGF-treated withdrawn groups. At the end of the experiments, rats were perfused and brain sections were processed for VIP and vesicular acetylcholine transporter immunostaining, and for Nissl staining. Brain sections were analyzed in order to estimate the areal density of VIP-ir neurons and of cholinergic fiber varicosities in the somatosensory cortex.

Our estimates show that the density of VIP-ir neurons and cholinergic varicosities in the somatosensory cortex is altered neither by CET nor by W. Our data also show that NGF treatment of withdrawn rats does not change the expression of VIP in the analyzed layers of somatosensory cortex. Furthermore, we found that the density of cholinergic varicosities is significantly higher in NGF-infused withdrawn rats than in control, ethanol-treated and withdrawn rats.

Our results point toward the existence of region specificity in the effects of CET and W on the VIP expression and on the cholinergic system. This study also reveals that NGF, administered to withdrawn rats, increases to super-normal levels the density of cholinergic varicosities in all analyzed layers of the somatosensory cortex. Our results might be of importance for understanding the role of the cortical VIP and cholinergic system in various functional and behavioral alterations associated with CET and W as well as the potential therapeutic role of NGF in the treatment of these changes.

Effects of chronic alcohol consumption and withdrawal on the density and somatic volume of NPY-containing neurons and on the cholinergic innervation of the hippocampal dentate hilus

S. Sousa¹, M. Vilela¹, P.A. Pereira¹, M.D. Madeira¹ and A. Cardoso¹

¹Department of Anatomy, Faculty of Medicine, University of Porto, Portugal

The present study was designed to investigate the effects of chronic ethanol treatment (CET) and withdrawal (W) on the density and somatic volume of neuropeptide Y-immunoreactive (NPY-ir) neurons and on the density of cholinergic varicosities in the hippocampal dentate hilus. We have also evaluated if the administration of nerve growth factor (NGF) would interfere with the content of NPY and volume of NPY positive neurons as well as with the cholinergic innervation of this brain area.

It is well known that chronic alcohol consumption induces profound morphological alterations in several brain regions, and the hippocampal formation appears to be particularly vulnerable. Furthermore, W from ethanol, instead of stopping these alterations, tends to aggravate the ethanol-induced effects. In the present work, we have analyzed the effects of CET and W on NPY expression and on the cholinergic innervation of the hippocampal dentate hilus. We choose NPY because it has been implicated in the regulation of various functions, including circadian rhythms and cognition that are altered by CET. In addition, there is evidence that the expression of this neuropeptide depends on the trophic support provided by the cholinergic system. The hippocampus receives a strong innervation from basal forebrain cholinergic neurons, which depend on NGF for phenotype maintenance.

Twenty Wistar male rats were assigned to control, ethanol-treated, withdrawn and NGF-treated withdrawn groups. At the end of the experiments, rats were perfused and brain sections were processed for NPY and vesicular acetylcholine transporter (VACHT) immunostaining and for Nissl staining. The densities of NPY-ir neurons and of cholinergic varicosities and the somatic volume of NPY-ir neurons were estimated in the hilus of dentate gyrus. The mean somatic volume of NPY-ir neurons was estimated by applying the optical rotator.

It was found that W, but not CET, increased the number of NPY-ir neurons in the hilus when compared to control rats. Conversely, chronic alcohol consumption markedly reduced the density of VACHT varicosities in the hilus, and W aggravated it. Interestingly, NGF treatment augmented the density of VACHT varicosities to values higher than in controls, but did not change the density of NPY-ir neurons. Furthermore, the mean somatic volume of NPY-containing neurons was unaltered in ethanol treated and withdrawn rats and did not change in response to NGF administration.

These results show that CET reduces the density of cholinergic varicosities but does not affect the density of NPY-containing neurons in the hilus. Furthermore, it was also demonstrated that alcohol W aggravates the ethanol-induced effects on the density of cholinergic varicosities and increases the density of NPY neurons. This study also reveals that NGF administration to withdrawn rats increases the density of cholinergic varicosities to super-normal values and reestablishes NPY expression.

Functional activity of extracts from Goji berries (*Lycium barbarum*)

M. Mendes¹, **A. P. Carvalho**¹, **M. F. Barroso**¹, **J.M.C.S. Magalhães**², **A. M. Gomes**³ and **C. Delerue-Matos**¹

¹ REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Portugal.

² REQUIMTE, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Portugal.

³ CBQF, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Portugal.

Lycium barbarum is a woody perennial plant that produces a berry commonly known as Goji. This fruit has been used for centuries in traditional Chinese medicine to control/prevent several diseases, and is also associated with longevity [1,2]. Currently, and mainly due to their antioxidant properties, Goji berries caught the attention of the Western food industry, since antioxidants can protect cells against damage effects caused by free radicals.

Goji berries can be eaten fresh or dried, alone or in various culinary uses. Most of the commercially available Goji berries in Western countries are dried, because such procedure increases durability and facilitates transportation. Nevertheless, several compounds of interest may suffer numerous modifications during the drying process, e.g. degradation of heat-sensitive chemicals and losses of volatile compounds; on the other hand, other components may increase their concentration.

The present study aims to investigate the differences in biological properties of dried Goji berries commercially available. As the selection of extraction methodologies is of extreme importance especially when it concerns the achieving of compounds of interest with functional properties, a method combining the use of microwaves and solvents was exploited, in order to obtain bioactive compounds available for the pharmaceutical and nutraceutical industries.

A response surface methodology obtained from a multivariate study was used to investigate the performance of the extraction procedure, to study the relevance of the variables required in extraction and to determine the final optimal settings. Results revealed that temperature is of utmost importance during extraction process, whereas time and the polarity of the extracting solvent presented a decreased degree of influence.

References:

[1] Pang, G., Xie, J., Chen, Q. and Hu, Z. (2012) *How functional foods play critical roles in human health*, Food Science and Human Wellness, 1, 26-60.

[2] Jin, M., Huang, Q., Zhao, K. and Shang, P. (2013) *Biological activities and potential health benefit effects of polysaccharides isolated from Lycium barbarum L.* International Journal of Biological Macromolecules, 54, 16-23.

Acknowledgments:

This work is financed by FEDER funds through CCDR-N, in the scope of project Operação NORTE-07-0124-FEDER-000069- Ciência do Alimento, and through FCT – Fundação para a Ciência e a Tecnologia, in the scope of project PEst-C/EQB/LA0006/2013. M. F. Barroso is grateful for the PhD fellowship (SFRH/BPD/78845/2011) financed by POPH -QREN - Tipologia 4.1 - Formação Avançada, subsidized by Fundo Social Europeu and Ministério da Ciência, Tecnologia e Ensino Superior.

Dietary supplements for cognitive function improvement: control of amino acid levels

F.B. Pimentel¹, J. Botelho¹, C.G. Costa^{1,2}, A.S.G. Costa¹,
A.F. Vinha^{1,3}, R.C. Alves^{1,4}, M.B.P.P. Oliveira¹

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² REQUIMTE, Dep. Chemical Engineering, Fac. of Engineering, University of Porto, Portugal.

³ Faculty of Health Sciences, University Fernando Pessoa, Portugal.

⁴ REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

Neurodegenerative diseases affect cognitive functions by progressive and irreversible destruction of brain cells. The medical treatment is often complemented with dietary supplementation in order to suppress the lack of nutrients that is common in these situations. Some amino acids are especially important to prevent or delay the progress of such diseases. For example, tryptophan, tyrosine, and phenylalanine are biosynthetic precursors of important neurotransmitters (serotonin, dopamine, and norepinephrine), and glutamate and aspartate are themselves brain neurotransmitters [1]. The aim of this study was to evaluate the amino acids composition of commercial dietary supplements ($n=8$) for improvement of memory and cognitive function.

Samples were prepared according to Pimentel et al. [2], derivatized with dansyl chloride and analysed by HPLC with fluorescence detection. Arginine, 5-hydroxytryptophan, glutamin, aspartic acid, glutamic acid, phenylalanine, tyrosine, lysine, and methionine were the amino acids considered in this study. Analyses were performed in triplicate.

In general, the results obtained are in accordance with the composition of the dietary supplements analysed. Nevertheless, one sample contained significantly lower amounts of aspartic acid and arginine than those described in the label; other supplement did not describe the amino acid composition. When the amino acids content was higher than that referred by the manufacturer for the individual amino acids, the supplement contained always plant extracts and/or peptides that were additional sources of amino acids.

References:

[1] Fernstrom J. D. (1994), *Dietary amino acids and brain function*. Journal of the American Dietetic Association, 94, 71-77.

[2] Pimentel, F.B., Alves, R.C., Costa, A.S.G., Torres, D., Almeida, M.F. and Oliveira, M.B.P.P. (2014), *Phenylketonuria: Protein content and amino acids profile of dishes for phenylketonuric patients. The relevance of phenylalanine*. Food Chemistry, 149(0), 144-150.

Acknowledgments: R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

Cytotoxicity of gold nanoparticles in the Caco-2 human intestinal epithelial cell line

M. Fernandes¹, C. Pereira¹, E. Pereira², H. Carmo¹, M.L. Bastos¹, S. Fraga¹

¹REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

²REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Gold nanoparticles are a class of nanomaterials with remarkable interest in several areas of research, especially in medicine [1]. Gold nanoparticles have unique optical and magnetic properties that are strongly dependent on the nanoparticle size, shape, surface and agglomeration state [2]. These properties, combined with their chemical stability and lack of evidence of significant toxicity make them a primary choice to various biomedical applications [1].

This work reports the evaluation of cytotoxicity of gold nanoparticles in a human intestinal epithelial cell line (Caco-2). The nanoparticles (20 nm) were coated with three different coatings (citrate, and pentapeptides CALNN and CALNS). To evaluate the cytotoxicity of gold nanoparticles the following tests were performed: (i) the calcein-AM assay (to assess metabolic activity), the propidium iodide assay (to assess plasma membrane integrity), the neutral red uptake assay (to assess lysosomal integrity), and the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay (to measure the reactive oxygen species levels). The cells were incubated with a wide range of concentrations [1, 3, 10, 30, 60 and 100 μ M] for 6 - 72h.

The results of all experiments demonstrated that all tested gold nanoparticles did not cause toxicity regardless of the surface coating. The nanoparticles did not change the morphology of the cells, nevertheless it is possible to visualize the formation of aggregates at the highest concentration tested (100 μ M) (Fig. 1).

Overall, it can be concluded that under our experimental conditions the tested gold nanoparticles are not cytotoxic in this cell line, even at high concentrations. Given the known interference of nanomaterials in several classical *in vitro* cytotoxicity tests, the multiple and complementary assays chosen for the present work give further support to these conclusions.

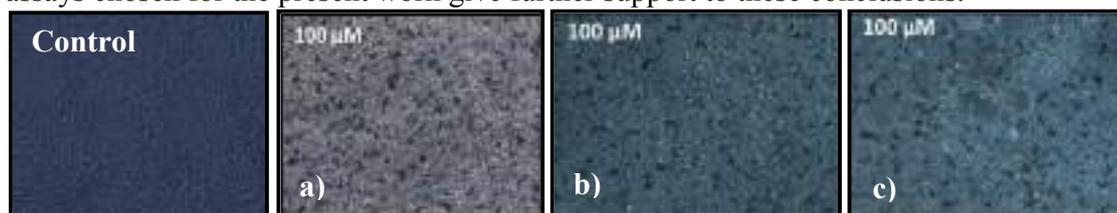


Figure 3 - Representative phase contrast microscopy images of Caco-2 cells at 48 h after incubation with a) citrate, b) CALNN, c) CALNS-coated gold nanoparticles (control and 100 μ M) (100x magnification).

References:

- [1] Khlebtsov, N. and Dykman, L. (2011), *Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies*, Chemical Society reviews, 40 1647-1671.
- [2] Huang, X. and El-Sayed, I. M. (2010), *Applications of Gold Nanorods for Cancer Imaging and Photothermal Therapy*, in: S.R. Grobmyer, B.M. Moudgil (Eds.) Cancer Nanotechnology, Humana Press, pp. 343-357.

Identity and nutrient availability drive the physiological response of intertidal macroalgae to pollution

S. Branco-Neves^{1,3}, M. Rubal^{2,3}, P. Veiga^{2,3}, A. C. Torres^{2,3}, I. Sousa-Pinto^{2,3}, F. Fidalgo^{1,3}

¹ Instituto de Ciências Integrativas & Biosistemas Plant Functional Genomics Group (BioISI)

² Interdisciplinary Centre of Marine and Environmental Research (CIIMAR)

³ Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal (FCUP)

Macroalgae have been used for many years to detect effects of pollution in aquatic environments [1]. However, different species of macroalgae can show different tolerances to pollution and the physiological mechanisms to deal with stress can be modulated by other environmental drivers (e.g. nutrient availability). The objective of this study is to explore the response of two different macroalgae to pollution at two different levels of nutrient availability. For this aim two species of macroalgae *Chondrus crispus* (sensitive to pollution) and *Gelidium pulchellum* (tolerant to pollution) were selected as model species. These species were sampled at two polluted and two reference sites in northwest coast of Portugal. Polluted sites showed higher levels of nutrients and metals than reference ones [2]. To assess the physiological response of the studied species the evaluation of lipid peroxidation as biomarker of oxidative stress and the quantification of free proline as a potent antioxidant were done. The level of malondialdehyde (MDA), a cytotoxic product of lipid peroxidation, is used extensively as an indicator of free-radical production and consequent tissue oxidative stress damage [3]. For a long time proline was only considered as an inert compatible osmolyte. However, several studies showed that proline accumulation can reduce the deleterious effects of many stresses and nowadays it is considered as a potent antioxidant and potential inhibitor of programmed cell death [3]. In fact, proline can now be regarded as nonenzymatic antioxidants that microbes, animals, and plants require to mitigate the adverse effects of reactive oxygen species [3].

The results showed that MDA levels in *C. crispus* (sensitive to pollution) were significantly lower in polluted than in reference sites, while *G. pulchellum* (tolerant to pollution) did not show significant differences for any of the studied biochemical parameters. In *C. crispus* a negative correlation between the levels of proline and MDA accumulation was found. The synthesis of proline and other amino-acids require a source of nitrogen that is a limiting nutrient in marine systems. Therefore, the availability of nutrients at polluted sites seems to modulate the physiological response of *C. crispus* stimulating the production and accumulation of proline. The high level of proline in this species might have been contributed for ROS scavenging, having a protective effect against stress imposed by pollution, that was manifested in a decline of lipid peroxidation. Future experiments will be done to test the importance of nutrients availability driving the physiological response of macroalgae to pollution.

[1] Staples et al. (1995) *Rapid growth of clones of the red alga Chondrus crispus: applications in assays of toxic substances and in physiological studies*, Marine Biology, 122,471-477.

[2] Rubal et al. (2014) *Effects of subtle pollution at different levels of biological organisation on species-rich assemblages*, Environmental Pollution, (submitted).

[3] Gil, S. S. and Tuteja, N. (2010), *Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants*, Plant Physiology and biochemistry, 48, 909-930.

Evaluating the effect of dietary nitrate supplementation on growth, oxygen consumption and reproductive performance of zebrafish (*Danio rerio*)

I. Campos¹, A.P. Carvalho^{1,2}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² CIIMAR/CIMAR, University of Porto, Portugal.

Nitrate and nitrite have been long considered hazardous substances for human and animal health. However, recent research has shown multiple benefits of dietary nitrate, mostly attributed to the reduction of ingested nitrate into nitric oxide, a substance known as a modulator of several physiologic functions. Nonetheless, even though the recently suggested beneficial effects of ingested nitrate have been widely studied in mammals, little is known about this subject on fish.

The present study was conducted in order to assess the effect of dietary nitrate supplementation on zebrafish (*Danio rerio*) growth, reproductive performance and oxygen consumption. Three trials took place successively to analyze each one of those parameters by the mentioned order, using the same set of fish throughout the whole study. Four dietary levels of sodium nitrate (1, 2, 4 and 8%) were tested as a nitrate source, against a control diet with no nitrate supplementation.

Dietary nitrate supplementation showed no improvement in zebrafish growth, but at higher supplementation levels (4 and 8% of sodium nitrate) led to a decreased growth performance, which indicates a toxic effect of nitrate at these levels. Regarding the reproductive performance, nitrate supplementation affected spawning frequency positively at the lowest level (1% sodium nitrate) but showed toxic effects at the highest level (8% of sodium nitrate). Oxygen consumption was significantly reduced in fish fed diets with intermediate nitrate levels (2 and 4% of sodium nitrate) compared to fish fed diets with lower or higher nitrate levels. Results suggest for the first time an oxygen sparing effect of moderate levels of dietary nitrate in fish, as previously found in mammals, which could represent an advantage in aquaculture.

Behavior of osteoblasts and osteoclasts cultured on a nanophased hydroxyapatite/collagen construct

K.C.Piedade^{1,2}, M.H.Fernandes³, S.P.Simões^{4,6}, S. R. Sousa^{2,5} and F.J.Monteiro^{2,7}

¹ Faculty of Pharmacy, University of Coimbra, Portugal

² INEB—Instituto de Engenharia Biomédica, Universidade do Porto, Rua do Campo Alegre 823, 4150-180, Porto, Portugal

³ Laboratório de Farmacologia e Biocompatibilidade Celular, Faculdade de Medicina dentária, Universidade do Porto, Porto, Portugal

⁴ Centro de Neurociências e Biologia Celular de Coimbra, Universidade de Coimbra, 3004-517 Coimbra, Portugal

⁵ REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal

⁶ Departamento de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra, 3000-295 Coimbra, Portugal

⁷ Departamento de Engenharia Metalúrgica e de Materiais, Faculdade de Engenharia (FEUP), Universidade do Porto, Rua Roberto Frias, s/n, 4200- 465 Porto, Portugal

One of the main challenges in developing tissue engineered products for organ repair and regeneration is to adequate them for the environment they will be exposed to. Therefore, the development of functional scaffold systems that perfectly mimic the natural tissue and that can ensure the regeneration of defective tissue to restore normal functions is highly challenging. [1] Bone regeneration has been a matter of discussion for a long time and, as any other mammal tissue, bone is often affected by diseases that cause its destruction. To better mimic the mineral and the main protein components, as well as the microstructure of natural bone, nanohydroxyapatite/collagen composite scaffolds with high and interconnected macro- and microporosity were developed.

Nanohydroxyapatite is a naturally apatite which is synthetically produced and supplied in different forms to create Medical Devices, Personal Care Product formulations, R&D activities and many other applications. When combined to collagen, which is an insoluble fibrous protein found in the extracellular matrix and in connective tissue, nanohydroxyapatite can provide a good stability to the scaffold. [2][5]

By using this compound in tissue engineering, we are stimulating osseointegration at the bone-implant interface. Osseointegration is a key factor for endosseous implants to succeed in achieving and maintaining their long-term stability in direct contact with bone tissue. [3][4] Thus, this project aims at studying the influence of nanostructured HA/collagen biocomposite on the adhesion, viability, proliferation, differentiation and functional activity of osteoblasts and osteoclasts.

References:

[1] X. Wang, J. S. Nyman, X. Dong, H. Leng, M. Reyes. *Fundamental Biomechanics in Bone Tissue Engineering Synthesis Lectures on Tissue Engineering* 01/2010; 2(1):1-225.

[2] <http://www.fluidinova.com/home/hydroxyapatite-supplier/4/>

[3] Luiz Meirelles, Anna Arvidsson, Martin Andersson, Per Kjellin, Tomas Albrektsson, Ann Wennerberg. Nano hydroxyapatite structures influence early bone formation. *Journal of Biomedical Materials Research Part A*, pages 299–307, November 2008.

[4] Xiaolong Zhu, Oliver Eibl, Lutz Scheideler, Jürgen Geis-Gerstorfer. Characterization of nano hydroxyapatite/collagen surfaces and cellular behaviors. *Journal of Biomedical Materials Research Part A*, pages 114–127, October 2006.

[5] Lodish H, Berk A, Zipursky SL, et al. (2000) *Molecular Cell Biology*. 4th edition. Section 22.3 Collagen: The Fibrous Proteins of the Matrix. New York: W. H. Freeman..

Use of Noninvasive sampling in to infer the evolutionary history of wild equids

A.Pereira¹, S.Tavares^{1,2}, R.Monteiro¹

¹ Research Center in Biodiversity and Genetic Resources (CIBIO)

² Department of Biology, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal

The Equidae family was once a very representative group in Africa, Asia and the Americas. Today this family only count with seven species (*Equus africanus*, *E. grevyi*, *E. zebra*, *E. burchellii*, *E. hemionus*, *E. kiang* and *E. przewalski*) and most of them are at risk ^[1].

Noninvasive sampling is a strategy that allows increase the studies in some subjects as conservation biology, ethology and population genetics ^[2].

Microsatellites are repetitive DNA sequences that not only show a highly degree of polymorphism, but also are relatively abundant in genomes and are easy to genotype. They assume a very important role in animal genetics' studies ^[3].

In this study we tested noninvasive biological samples (more specifically feces) collected from the Tibet *E. kiang*. The obtained genetic data will be used to estimate demographic parameters on that population (e.g., N_e) and to detect population substructuring (F-statistics) and diversity estimates (Heterozigosities, Mean allele number).

To obtain the information we need, samples must be processed in two phases: in the first we need to remove the surface of feces (where the DNA of the animal digestive tract is located) into falcon tubes and process them with some reagents that digest the DNA and degrade the rest including potential PCR inhibitors. Then, the tubes are incubated at 56°C, overnight in agitation. In the second stage they will enter in the cycle of many centrifugation, vortex use, add many buffers and incubations. After all these steps, we tested the samples and the negative control on 0.8% agarose gel.

Here, we also use a simple PCR and denaturing gel electrophoresis to allele size determination of some microsatellites.

We expect that these techniques allow a better understanding on population substructuring and diversity estimates on *E. kiang*.

References:

- [1] Moehlman, P.D.R., *Equids: zebras, asses, and horses: status survey and conservation action plan*. 2002: IUCN.
- [2] Taberlet, P., L.P. Waits, and G. Luikart, *Noninvasive genetic sampling: look before you leap*. Trends in Ecology & Evolution, 1999. **14**(8): p. 323-327.
- [3] Vignal, A., et al., *A review on SNP and other types of molecular markers and their use in animal genetics*. Genetics Selection Evolution, 2002. **34**(3): p. 275-306.

Cetacean monitoring between Continental Portugal and Madeira Island

P. Fernandes¹, A. Correia^{1,2} and I. Pinto^{1,2}

¹Department of Biology, Faculty of Sciences, University of Porto, Portugal.

²Interdisciplinary Centre of Marine and Environmental Research – CIIMAR.

Cetacean occurrence data is scarce in Portugal but has been recorded since the XII century, and during this time relationship between Humans and cetaceans evolved from capture to conservation [1]. To meet the requirements of legislative frameworks of European Union [2], cetacean monitoring must be done in order to evaluate the abundance and distribution tendencies of the population over time.

Monitoring studies conducted in the Canary Basin (NE Atlantic) have been focused in coastal regions [e.g.3] and little or no survey effort has been carried out in offshore areas, leading to a lack of data on distribution of many sensitive pelagic species. In this study, platforms of opportunity have been used to conduct 19 sea-surveys along the routes from Continental Portugal (Lisbon and Oporto) to Madeira Island (Caniçal).

A total of 140 sightings were recorded, and 9 species identified: *Balaenoptera acutorostrata*, *Delphinus delphis*, *Globicephala*, *Kogia breviceps*, *Physeter catodon*, *Stenella coeruleoalba*, *Stenella frontalis*, *Tursiops truncatus* and *Ziphius cavirostris*. The dolphins were the most sighted (58%), followed by toothed (24%) and baleen whales (10%). *Delphinus delphis* was the most common species while *Kogia breviceps* and *Stenella coeruleoalba* were the less sighted. 127 sightings were registered on-effort resulting in an overall encounter rate (ER=sightings/100km) of 1,72 on 7402km of survey effort. Distribution maps seem to show that continental coastal areas as well as seamounts have a positive influence in the distribution of cetaceans. Moreover, there is clear segregation among groups along the routes. In general, diversity of species in offshore areas was higher, proving the importance of monitoring this region.

These results show that platforms of opportunity are a cost-effective way to study cetaceans in extensive areas, promoting the knowledge in offshore areas. Moreover, it is proved that the region between Continental Portugal and Madeira Island has a diversity of habitats and, possibly, several hotspots for cetacean species. Thus, it is proposed to maintain these monitoring campaigns in a systematic way, in order to obtain sound data to efficiently support marine management and conservation efforts.

References:

[1] Brito, C. and Sousa, A. (2011), *The Environmental History of Cetaceans in Portugal: Ten Centuries of Whale and Dolphin Records*, PloS one, 6(9), e23951.

[2] Arcangeli, A., Marini, L. and Crosti, R. (2012), *Changes in cetacean presence, relative abundance and distribution over 20 years along a trans-regional fixed line transect in the Central Tyrrhenian Sea*, Marine Ecology, pp. 112-121.

[3] Brito, C., Vieira, N., Sá, E. and Carvalho, I. (2009), *Cetaceans' occurrence off the west central Portugal coast: a compilation of data from whaling, observations of opportunity and boat-based surveys*, Journal of Marine Animals and Their Ecology, 2(1), 10-13.

Interaction between Sparfloxacin and biological membrane models: a multi-technique approach

C. Sousa, M. Ferreira, C. Medforth and P. Gameiro

Requimte, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

Sparfloxacin is a second generation fluoroquinolone antibiotic which acts by penetrating into bacterial cells and inhibiting enzymes related to DNA replication [1]. The misuse of fluoroquinolones has resulted in increased resistance to these antibiotics [2] which constitutes a serious public health issue. One mechanism of bacterial resistance to fluoroquinolones is a decrease in the intracellular concentration of the antibiotic due to mutations in the channel proteins OmpC and OmpF [3]. The uptake of fluoroquinolones through the bacterial membrane can occur by three different pathways: the channel proteins, the lipid bilayer or the lipid-protein interface. Therefore, the study of membrane-antibiotic interactions can be an important tool for improving the understanding of bacterial resistance to fluoroquinolones and an aid in the design of modified fluoroquinolones with improved abilities to cross the bacterial membrane. Our work studies the interaction between Sparfloxacin and *E. Coli* total lipid extract, a model for Gram-negative bacterial membranes. As Sparfloxacin is non-fluorescent, the partition coefficient (K_p) of the drug was determined using multiple techniques (^{19}F NMR spectroscopy, UV-visible absorption spectroscopy, and fluorescence spectroscopy in the presence of a fluorescence probe) and the results compared.

The NMR studies involved determining K_p from variations in ^{19}F chemical shifts upon addition of increasing amounts of lipid to aqueous solutions of Sparfloxacin. Changes in UV-visible absorption spectra of samples with increasing amounts of lipid were also used to determine K_p . Finally, fluorescence studies were carried out using a fluorescence probe ((2-anthroyloxy)stearic acid; 2-AS) incorporated into the lipid membrane.

Similar K_p values are obtained from the three different techniques, confirming that this a useful approach to the determination of partition coefficients for non-fluorescent drugs.

Acknowledgments:

This work was funded by FEDER funds through the Programa Operacional Factores de Competitividade — COMPETE, the Quadro de Referência Estratégico Nacional - QREN and by national funds through Fundação para a Ciência e a Tecnologia (FCT, Portugal) PTDC/SAU-FAR/111414/2009, and PEst - C/EQB/LA0006/2011 projects.

References:

- [1] Klopman, G., Wang, S., Jacobs, M.R., Bajaksouzian, S., Edmonds, K, Ellner, J.J. (1993), Anti-*Mycobacterium avium* activity of quinolones: in vitro activities *Antimicrob. Agents Chemother.* 37, 1799–1806.
- [2] Hardman, J.G. and Limbird, L.E. (2001), Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, New York, McGraw-Hill, 10th ed.
- [3] Ruiz, J. (2003) Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection, *J Antimicrob Chemother.* 51, 1109–1117.

Larval Development of the Barnacle *Balanus perforatus* Reared in Laboratory

T. Azevedo¹, J. Almeida² and I. Cunha²

¹ Faculty of Sciences, University of Porto, Portugal.

² CIIMAR/CIMAR- Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal.

Balanus perforatus is a volcano shaped sessile marine invertebrate belonging to Balanidae family. It can be found on warm and temperate coastlines along the Mediterranean and east Atlantic, from the eastern English Channel to northeast Africa. It is present on shady rock surfaces of the intertidal and subtidal zones, and on man-made marine infrastructures. It is a dominant hard fouler on ship hulls, aquaculture nets and piping, power plants heat exchange coolers, among others. Biofouling represents one of the current issues in the marine environment as antifouling efforts represent not only additional toxic agents inputs but also causing enormous economic losses to maritime industries [1]. In this context, studies on life cycle and development of biofouling species are of great importance, particularly regarding larval development and the transition from planktonic to sessile life stages. Thus, this study describes *Balanus perforatus* larval development, analyzing morphological characteristics of the different larval stages and comparing them with those on other congeneric species of barnacles.

Specimens were collected in Praia da Memória (Portugal) and transported to the laboratory in controlled conditions for immediate examination and isolation of the mature ovigerous lamellae. Eggs were incubated in 1L beaker with aeration and embryos were reared over the six naupliar stages to cypris stage in 2 L glass Erlenmeyers. After hatching nauplii were fed with phytoplankton (*Isochrysis* sp., *Tetraselmis* sp. and *Rhodomonas* sp.) and kept at 20°C under artificial illumination (14h light: 8h dark). Every day a sample was taken and fixed in saline formaldehyde in order to be analysed on an inverted microscope (Nikon Eclipse TS100). Nauplii were measured (total body length, shield width, shield length, length of the fronto-lateral horn and the length till the end of abdominal process, measured from the anterior margin of the shield to the beginning of the bifurcate ramus) and high resolution photographs were taken of the whole nauplii and of particular anatomical features such as naupliar eye, labrum, filaments, thoracico-abdominal process, antennules, antennae and mandibles (Nikon Imaging Software - NIS-Elements). Drawings and measures of the 6 naupliar stages and cypris are presented. *B. perforatus* nauplii are within the normal sizes considering the nauplii of other congeneric species (bigger than *B. amphitrite* var. *amphitrite* but smaller than *B. balanoides* and *B. crenatus*), ranging between $285 \pm 24 \mu\text{m}$ (nauplius I) and $755 \pm 25 \mu\text{m}$ (nauplius VI).

This study presents the first attempt to describe the larval development of *Balanus perforatus* in Northern Portuguese coast.

References:

[1] E. R. Holm (2012), *Integrative and comparative biology*, Barnacles and biofouling, 52 (3), 348–55.

Nutritional evaluation of the invasive bivalve *Corbicula fluminea*

J. Bencatel^{1,2}, J. Campos², R. Sousa² and S. Costa-Dias²

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² CIMAR/CIIMAR Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal.

The Asian clam *Corbicula fluminea* is one of the most relevant invasive species in freshwater ecosystems occurring in several European rivers. It has already a stable population in some Portuguese rivers including Minho, Lima and Guadiana [1]. Their fast growth and sexual maturation, as well as their short life cycle (up to 5 years), high fecundity and dispersal capacity probably account for their success in the colonization of new environments [2].

This small bivalve might alter the ecological conditions of the invaded areas by physically altering the natural habitat of native bivalve species but also creating refuges for some species. Furthermore, *C. fluminea* has a very high filtration rate, feeding on suspended and deposit material and hence, when in high densities, might ingest significant quantities of larvae and juveniles of other bivalve species [3]. However, since *C. fluminea* populations are already established in the system, their biomass represents an important potential food resource for predators of the invaded area. Therefore, in this work, the nutritional value of *C. fluminea* was investigated in two rivers of the north of Portugal, Minho and Lima. For that purpose the lipids and proteins content of individuals from the two populations was analyzed. A discussion is made on the nutritional content of the two populations, taking in account size classes and time of year. Additionally, considerations are made about the influence of the local productivity on the *C. fluminea* populations' nutritional status.

References:

- [1] Sousa, R., Rufino M, Gaspar M et al. (2008), *Abiotic impacts on spatial and temporal distribution of Corbicula fluminea (Müller, 1774) in the River Minho estuary, Portugal*, Aquatic Conserv: Mar. Freshw. Ecosyst, 18, 98–110.
- [2] Sousa, R., Antunes, C. and Guilhermino, L. (2008), *Ecology of the invasive Asian clam Corbicula fluminea (Müller, 1774) in aquatic ecosystems: an overview*, Ann Limnol Int J Lim, 44, 85–94.
- [3] Strayer, D.L. (1999), *Effects of alien species on freshwater molluscs in North America.*, J. N. Am. Benthol. Soc., 18, 74-98.

The Red Fox (*Vulpes vulpes*) in Portugal: Variation in cranial size

A. Almeida & A. Valente

Department of Biology, Faculty of Sciences, University of Porto, Portugal.
Rua do Campo Alegre, Edifício FC4, 4169-007 Porto, Portugal.

The Red fox (*Vulpes vulpes*, Linnaeus, 1758) is a member of the Mammalian order Carnivora, established across the northern hemisphere [1]. Fox can be found in various environments (Arctic to desert). It is an abundant species in Portugal [2], where is not considered threatened [3]. Currently hunting is responsible for most deaths so regulations were created to manage the activity. Skull morphology is a great source of information; reflects the individual's fitness, diet availability, genetic distance and environmental effects. Red fox is a good species to monitor because the large distribution area throughout the world [4]. The present study aim is to analyze fox skull dimensions and use them in order to appraise sex, age and geographic variation effects in the Red fox population in Portugal.

Hunting associations have been contacted since the opening of the hunting season in Portugal to give foxes for our study. A total of 67 skulls were obtained during the 2012/13 hunting season. They were cleaned and a set of twelve craniometrical measures were obtained with a Mitutoyo digimatic caliper (0.02 mm precision for measurements of 0-100 mm, and 0.03 mm precision for measurements of 100-150 mm). Some skulls are damaged and so some dimensions measurements could not be obtained.

Sexual dimorphism is evident as most craniometrical measures are bigger in males, confirming results obtained in previous hunting seasons. Further analysis will be used to test growth/age differences and to verify if the data are in accordance with the Bergman's.

Sampling effort is being continued in the present hunting season because sample size is small but further research should also be soon directed to other approaches such as teeth analysis and to the use of more recent tools such as the morphometric skull analysis.

References:

- [1] Sillero-Zubiri, C., M. Hoffman, et al. (2004). *Canids: Foxes, Wolves, Jackals and Dogs. Status survey and conservation action plan*. Gland, Switzerland and Cambridge UK.
- [2] Santos-Reis, M. and M. L. Mathias (1996). *The historical and recent distribution and status of mammals in Portugal*, *Hystrix* 8(1-2): 75-89.
- [3] Cabral M. J. C., Almeida J., Almeida P. R., Dellinger T., Ferrand de Almeida N., Oliveira M. E., Palmeirim J. M., Queiroz A. I., Rogado L. & Santos Reis M. E. 2006: *Livro Vermelho dos Vertebrados de Portugal*. I. d. C. d. N. A. Alvim (Ed.), Lisboa.
- [4] Nentvichová M. H., Anděra M., Hart V. (2010), *Cranial ontogenetic variability, sex ratio and age structure of the Red fox*. *Central European Journal of Biology*, 5(6): 894-907.

The interplay of erythrocyte cytosolic peroxidases with the membrane under H₂O₂ mediated oxidative stress

D. Gomes¹, **A. Santos-Silva**^{1,2}, **E. Bronze**^{1,2}, **M. Lima**^{3,4}, **C. Catarino**^{1,2} and **S. Rocha**^{1,2}

¹ Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal; ² Institute for Molecular and Cell Biology (IBMC), University of Porto, Portugal; ³ Laboratory of Flow Cytometry, Department of Clinical Hematology, Porto Hospital Center, Santo António Hospital, ⁴ Unit for Multidisciplinary Investigation in Biomedicine, University of Porto, Portugal

Erythrocytes suffer continuous oxidative damage by endogenous and exogenous reactive oxygen species (ROS) and its accumulation appears to damage erythrocytes membranes. The intracellular ROS are mainly due to autoxidation of oxyhemoglobin, generating superoxide that, through dismutation, produces hydrogen peroxide (H₂O₂). The cytoplasmatic enzymes, Peroxiredoxin 2 (Prx2), catalase (Cat) and glutathione peroxidase (GSHPx) scavenge most of the H₂O₂ from the erythrocytes. In a previous work (1), we showed that cytosolic Prx2 binds to the erythrocyte membrane when erythrocytes are under by H₂O₂ mediated oxidative stress. In the present study, our aim was to evaluate the role of Prx2 and of other two erythrocyte cytosolic peroxidases, catalase and glutathione peroxidase, and the crosstalk between them. We incubated erythrocytes for 30 minutes at 37°C, with increasing concentrations of H₂O₂ (0, 5, 10, 20, 40 and 80 µM), in 3 different assay conditions (5 independent experiments): 1) no enzyme inhibition, 2) Cat inhibition and 3) Cat, GSHPx and Prx2 inhibition. Afterwards, the cells were hemolysed and the erythrocyte membranes were isolated and performed immunoblots for Prx2, Cat and GSHPx (1). Cat was not detected in the erythrocyte membrane in any of the studied assay conditions. Prx2 and GSHPx, were not detected in the membrane when no enzymes were inhibited; when Cat was inactive, only GSHPx linked to the membrane at the 2 highest concentrations of H₂O₂; when all enzymes were inhibited, GSHPx was linked to the erythrocyte membrane in all tested H₂O₂ concentrations, while Prx2 was only linked in 10, 20, 40 and 80 µM of H₂O₂. When the enzymes were detected in the erythrocyte membrane, their amount significantly increased with H₂O₂ concentration. These results suggest that Cat does not bind to the erythrocyte membrane under oxidative stress, while Prx2 and GSHPx do and their linkage increases with the oxidative stress within the cell; moreover, GSHPx appears to be more sensitive to oxidative stress than Prx2 because, unlike Prx2, it was linked to the membrane even in lower H₂O₂ concentrations when only Cat was inhibited and when all enzymes were inhibited. The linkage of these cytosolic enzymes to the erythrocyte membrane is still an enigma, as it is still unknown if they link to the membrane to protect it against oxidative damage, or when they are inactive or structurally damaged. Further studies are needed to better understand how these enzymes respond to oxidative stress and their interplay with the erythrocyte membrane.

(1) Rocha S, Costa E, Coimbra S, *et al.*. (2009) *Blood Cells, Molecules, and Diseases*, **43**, 68-73

Acknowledgments: This study was partly supported by a Post-Doc grant (SFRH/BPD/80023/2011) attributed to S. Rocha by FCT and FSE.

Antimicrobial resistant coliform bacteria on beach sands in the North coast of Portugal

S. Lima¹, H. Ferreira^{1,2}

¹ Microbiology, Department of Biological Sciences, Faculty of Pharmacy University of Porto, Portugal.

² REQUIMTE, University of Porto, Portugal

Nowadays we constantly hear about the growing bacterial resistance to antibiotics, turning in a serious threat to human health. Environmental bacterial contamination with antimicrobial resistant isolates can play an important role in dissemination as reservoirs of spread of antimicrobial resistance. We chose beach sands to conduct this study, because beaches are places frequented by a wide range of people that contact with antimicrobial resistant bacteria without knowing.

The aim of this study was the detection of antimicrobial resistant bacteria in sand samples, with special focus on Extended Spectrum Beta Lactamase (ESBL) producing coliforms.

For that purpose, we collected wet sand in three different beaches of the North of Portugal and a portion of 4 gram of these samples was suspended in 40 ml Tryptic Soy Broth (TSB) and incubated overnight at 37°C. Isolates were selected on MacConkey agar and MacConkey agar with ampicilin, ceftazidime, cefotaxime, aztreonam and meropenem. Other approach for experimental work included suspending the samples in sterilized water, followed by supernatant filtration by ramp vacuum. Filters were then placed in the same culture media used for the samples suspended in TSB. After random selection of colonies, we performed a susceptibility test to antimicrobial agents by agar diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI). Screening for ESBL production was also undertaken, by the double disk synergy test and clavulanic acid addition, according to the CLSI guidelines. Finally, selected strains were identified by ID32GN.

ESBL producing *Escherichia coli*, *Citrobacter freundii* and *Klebsiella oxytoca* isolates were detected in the collected samples.

Considering the results the presence of these resistant bacteria might represent a colonization risk in recreational use of these beaches and also a threat in terms of antibiotic resistance environmental dissemination.

Acknowledgements: Susana Rocha, Tânia Vilaça

Study of the microvesicles populations in *Leishmania infantum* exoproteome

Cátia Silva^{1,2}, Nuno Satarém¹ and Anabela Cordeiro-da-Silva^{1,3}

¹ Institute for Molecular and Cell Biology, University of Porto, Portugal

² School of Sciences, University of Minho, Portugal.

³Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal

The secretion pathway to deliver effector molecules by microbial pathogens is a trademark in almost all pathogens. In the beginning of the 21st century it became evident that the release of extracellular microvesicles (MVs) by microorganisms should be taken in consideration. *Leishmania* parasites are responsible for a group of diseases called leishmaniasis. This disease is among the twenty major death causes for children [1]. Therefore it is essential a better knowledge of the parasite biology to enable the discovery of new vaccines or therapeutical approaches. Recently great emphasis has been given to the release of MVs with properties similar to exosomes by several organisms. It is the dawn of a new era in the field of infectious diseases with many possibilities to explore in the relationship between the hosts and the pathogens [2]. Although the capacity of many organisms to release MVs is still a matter of debate, some trypanosomatids, like *Leishmania*, were already shown to be able to secrete MVs [3]. Ultimately, the evaluation of the vesicle populations will enable insights into their origin and function. To evaluate the MVs population present in *Leishmania infantum* promastigote *in vitro* culture we used a strategy developed by the group that enables the recovery of MVs from logarithmic (non-virulent parasites) and stationary (enriched in virulent parasites) [4]. We used a combination of filtration and ultracentrifugation to recover MVs from promastigote cultures and then proceeded with the characterization of the vesicle population concerning the protein profile, size of MVs, stability, presence of DNA and RNA. Significant differences in the evaluated characteristics between the stationary and logarithmic vesicle population hint at stage dependent MVs populations reflecting distinct MVs profiles.

References:

[1] CONTROL OF THE LEISHMANIASIS (2010), *Report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis*, Geneva, 22-26 March 2010.

[2] M. Hosseini, A. A. Fooladi, M. R. Nourani and F. Ghanezad (2013), *The role of exosomes in infectious diseases*. *Inflamm Allergy Drug Targets*, 12(1), 29-37

[3] J. M. Silverman, J. Clos, C. C. de'Oliveira, O. Shirvani, Y. Fang, C. Wang, L. J. Foster and N. E. Reiner (2010), *An exosome-based secretion pathway is responsible for protein export from Leishmania and communication with macrophages*. *J Cell Sci*, 123(6), 842-52

[4] N. Santarem, G. Racine, R. Silvestre, A. Cordeiro-da-Silva and M. Ouellette (2013), *Exoproteome dynamics in Leishmania infantum*. *J Proteomics*, 84, 106-18.

The effect of Arabic gum in microbial growth

Luís Gomes¹, Ana Patrícia Graça^{1,3}, Sílvia Coimbra^{1,2} and Olga Maria Lage^{1,3}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² Biosystems & Integrative Sciences Institute (BioISI) Porto, Portugal

³Centre of Marine and Environmental Research (CIIMAR), Porto, Portugal.

Plants have been used for curative proposes throughout human history. Many of the pharmaceuticals of the actual medicine have active molecules extracted from plants. Arabic gum (AG) is a complex mixture of glycoproteins and polysaccharides produced by the acacia trees. The purpose of this study is to identify the potential synergistic or antagonistic effect of commercial AG (Sigma-Aldrich), in several environmental and clinically relevant microorganisms. The bioactivity tests were performed using liquid microplate assays against *Candida albicans*, *Escherichia coli*, *Bacillus subtilis*, *B. cereus*, *Pseudomonas putida* and *Vibrio anguillarum*. Serial dilutions of AG (30 - 5 mM) were assayed to assess the potential dose-response effect of this extract. The culture assays, performed in duplicate, started with an initial optical density of 0.02 adjusted from an initial culture incubated overnight at 25°C. Preliminary results suggest that 5 mM AG was the concentration providing the most dissimilar results. In stationary growth phase, a slight dose-response was observed for *E.coli* and *P. putida* and no effect was observed in *C. albicans* and *V. anguillarum*.

Acknowledgment: This research was supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology, under the projects PEst-C/MAR/LA0015/2013.

Effects of marine bacteria on *Arabidopsis thaliana* growth

C. Palencia¹, A.P. Graça^{1,3}, S. Coimbra^{1,2} and O.M. Lage^{1,3}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² Biosystems & Integrative Sciences Institute (BioISI) Porto, Portugal.

³ Centre of Marine and Environmental Research (CIIMAR), Porto, Portugal.

Plants' growth, reproduction and development are directly bonded with other organisms like bacteria, fungi and animals in their natural environment. These associations may be beneficial through nutrient exchange or prejudicial due to toxic compounds. With this study we aimed to evaluate the potential synergistic or antagonistic effects of bacteria isolated from marine sponges on *Arabidopsis thaliana* germination. The selection of the bacteria (twenty five) was based on their capacity to also grow on non-saline media. Their growth on different solid culture media: MS (Murashige and Skoog basal medium) under 0%; 50% and 100% seawater; 50 and 100% NA (Nutrient Agar); MS+ 50% NA and MS + 100% NA were evaluated at 25°C for 3 days. The germination of *A. thaliana* was also tested in these media at 25°C for 3 weeks. The best growth media for bacteria were NA and MS + 100% NA followed by MS + 50% NA. Changes in pigmentation were observed in four bacteria due to differences in salinity or nutrient content. *A. thaliana* did not germinate in saline media and growth was reduced in media with NA. Subsequently, bacteria - *A. thaliana* co-cultures were assayed in the best media for each bacterium. Germination and root and leaves development were observed. These preliminary results showed that some bacteria induced changes in plants pigmentation and/or decrease of their growth rate.

Acknowledgment: This research was supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology, under the projects PEst-C/MAR/LA0015/2013. The first author is grateful to the ERAMUS program.

Histopathological lesions associated to canine norovirus

H. Vala^{1,2}, J.R. Mesquita^{1,3}, L. Neves³, R.M.S. Oliveira³, M.S.J. Nascimento³

¹ Escola Superior Agrária de Viseu, Instituto Politécnico de Viseu, Portugal

² Centro de Estudos em Educação, Tecnologias e Saúde (CI&DETS), Instituto Politécnico de Viseu, Portugal.

³ Department of Biological Sciences, Faculty Pharmacy, University of Porto, Portugal.

Noroviruses are nowadays a well-established cause of gastroenteritis in humans, but have been absent from the list of gastrointestinal infectious agents of dogs. In 2010 our group has discovered a novel canine norovirus (CaNoV) and associated with diarrhea in dogs [1]. Since its discovery, CaNoV have also been found in other countries [2], but the pathogenic features, including histopathological characterization, remain to be explored. Hence, the aim of the present study was to study the histopathological lesions of the small intestine of dogs exposed to CaNoV.

Samples of small intestine were collected from 2 adult dogs (2-year-old cross-bred and a 6 year-old cocker spaniel) housed in a kennel where a diarrheic dog had been diagnosed with CaNoV. Samples were fixed (10% neutral buffered formalin), dehydrated (graded ethanol series) and embedded in paraffin wax. Sections (3 µm) were collected on glass slides, submitted to deparaffinization, rehydrated and stained with Haematoxylin and Eosin. Samples were examined by light microscopy (Microscope Zeiss Mod. Axioplan 2) by two independent observers. Histopathological lesions were evaluated in a semi-quantitative score and inflammatory infiltrate was described and classified in a scale from 0 to 3 (absent, mild, moderate and severe).

Macroscopic examination, in both cases, revealed fluid intestinal content with pseudomembranes and petechial lesions. The microscopic findings of case 1 revealed a notorious mononuclear inflammatory infiltrate within lamina propria of small intestine proximal segments, with increasing numbers of lymphocytes and plasma cells, changing from severe to moderate, from the duodenum to jejunum, respectively. The ileum presented only a mild infiltrate. Hypertrophy of the villi, muscularis mucosae, and the inner and outer muscular layers was seen, as well as severe epithelial detachment and mucosal loss, dilated crypts, lined by attenuated epithelium and containing numerous sloughed epithelial cells, cellular and nuclear debris. Also, mitotic figures at crypt basis were seen. In case 2, a more acute pattern was observed with congestion and haemorrhage of some areas of ileum. Follicular lymphoid hyperplasia was seen in proximal intestinal lymphoid tissue, as well as in mesenteric lymph nodes and splenic white pulp. In conclusion, the severe lesions observed can be implied in the mechanism of diarrhoea caused by CaNoV.

Acknowledgement:

FEDER, Programa Operacional Factores de Competividade – COMPETE, FCT (project PTDC/CVT/113218/2009) and CI&DETS (PEst-OE/CED/UI4016). PhD grant of JRM (SFRH/BD/45407/2008) to FCT.

References:

- [1] Mesquita, J.R., Barclay, L., Nascimento MS, Vinjé J (2010), *Novel norovirus in dogs with diarrhea*, Emerg Infect Dis. 16(6):980-2.
- [2] Martella, V., Pinto, P., Buonavoglia, C (2011), *Canine noroviruses*, Vet Clin North Am Small Anim Pract. 41(6):1171-81.

Effect of serial repitching on proteolytic and bioactive properties of brewer's spent yeast extracts: storage stability evaluation

J. Carvalho¹, E. Vieira¹, S. Meireles², T. Brandão² and I.M.P.L.V.O. Ferreira¹

REQUIMTE / Department of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal.

²UNICER, Bebidas de Portugal SGPS, SA, Leça do Balio

Saccharomyces yeast biomass is the second major by-product from brewing industry. Despite its underutilization, mostly used for animal feed, it can be of value as a raw material with different uses. Spent biomass is a good source of nutrients and bioactive compounds with pharmacological applications, namely antioxidant and antihypertensive properties. It also contains numerous enzymes, namely, vacuolar proteases including serine, aspartyl, and metallo proteases, pectinases among others, which can be used for several applications in food industry. Yeast biomass is used in the fermentation process several times (4 to 6 times) until its disposal [1].

This work highlights the influence of serial yeast repitching on nutritional composition, in proteolytic activity and in bioactive properties (antioxidant and ACE inhibitory activities). Additionally, it also evaluates the stability of these properties along storage time at -25°C (0, 1, 4, 8 and 12 weeks).

Extracts from spent biomass with 2, 3 and 4 repitchings (coded as R2; R3 and R4, respectively) were obtained by mechanical disruption using glass beads. Extracts were freeze-dried and solubilized with phosphate buffer pH 7 (ratio 1:4 (w:v)) and different aliquots were storage at -25°C for further analysis (0, 1, 4, 8 and 12 weeks). Proximate composition of biomass was determined according to AOAC Methods. Total protein content of extracts was determined using the Bradford method. The molecular weight distribution of protein extracts was obtained by Size Exclusion HPLC and confirmed by SDS-PAGE. Proteolytic activity was measured according Sigma's Assay, using casein 0.65% as substrate, at pH 7 and 37°C. The antioxidant activity was evaluated through different methods: FRAP; DPPH; Total Phenolics and Reduction Power. ACE inhibitory activity was evaluated using a fluorimetric assay.

No significant differences were obtained between the mean composition (/100g dry biomass) of R2, R3 and R4 biomasses (Protein: 75g±2.05; ash: 0.09g±0.01; lipid 0.65g±0.07; carbohydrates: 9.02g±0.09). The freeze-dry process didn't affect the proteolytic and bioactive properties of the extracts. The molecular weight distribution of the protein extract, obtained by Size Exclusion HPLC and confirmed by SDS-PAGE was between <6.6 KDa and 66 KDa. Protein content, proteolytic activity and antioxidant activities of extracts were increased with the number of yeast repitching. A good correlation was obtained between results from FRAP and Total Phenolics methodologies ($R^2=0.985$). The IC_{50} values for ACE inhibitory activities were also significantly higher for R4 biomass. No significant losses of these properties occurred after 12 weeks of storage ($p<0.05$) for all extracts.

References:

[1] Ferreira I.M.P.L.V.O., Pinho O., Vieira E. and Tavela J.G. (2010), Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications, Trends in Food Science & Technology, 21, 77-84.

Urban stray dog (*Canis familiaris*) packs

V. Casimiro & A. Valente

Department of Biology, Faculty of Science, University of Oporto, Portugal.

Urban stray dog packs are formed by dogs that are abandoned and that reproduce, forming self-perpetuating populations and, as a consequence, stray dog density in urban environments can be very high [1]. Stray dog presence can cause serious management concerns, mostly because they cause impact on biodiversity, they can act as vectors of zoonotic diseases, there are the risk of physical attack to humans, they can cause road collisions, the nuisance of riling trough garbage, the noise disturbance and the deposition of feces [1]. So it is important to understand the reasons that determine the presence of stray dog packs in any specific location within urban areas, namely the importance of human population influence by feeding them (hand out, garbage) or by supplying shelter.

The Oporto City Park (Porto, Portugal) and the surrounding streets (Avenida da Boavista, Estrada da Circunvalação and Avenida do Parque), an area with 1.03 km², was selected and surveyed for stray dogs (September to December 2013). Each dog sighted and each dog sign (footprint, scats, etc.) location was registered (Garmin E-Trex GPS) and all dogs were photographed. During the surveys, environmental information (feeding sites, available shelters, etc.) was also registered.

A large number of dogs was sighted at the City Park during the field work, but only in three occasions dogs could be positively identified as stray dogs. Four dogs were observed (two males and two females) within the City Park limits, near the restaurant area; they seem to belong to the same pack as they were observed together during the first observation. Another stray dog, a female, was sighted outside the City Park limits, in an alley. Dog signs (footprints and scats) are abundant but it is impossible to know if they belong to stray dogs. In several places shelters seem to exist in areas with thick vegetation in the interior of the Park and some abandoned construction might also provide shelter. Besides the dustbins near the restaurant area, a few feeding stations seem to be refilled in alleys near the City Park.

The stray dog density at the City Park seems to be very small, but our results are still preliminary. The high number of both people and pet dogs might be a cause for this small density; the efforts of the City Park administration to avoid wild and stray animal human feeding spots; stray dog density may also be influenced by the intense car traffic in the streets and by the urbanized areas that surround the City Park. In order to better understand the factors that might influence stray dogs, another area was selected and will be included in our study.

References:

[1] Baker, P.J., Soulssbury, C.D. Iossa, G & Harrius, S. (2010). Domestic cat (*Felis catus*) and domestic dog (*Canis familiaris*). In Gehrt, S. D., Riley, S. P. D., Cypher, B. L. (Eds.), *Urban carnivores: ecology, conflicy, and conservation*, The Johns Hopkins University Press, Baltimore, 157-171.

One Health: MAC granulomatous lesions in slaughtered swine as a public health problem

A. Gonçalves¹; B. Enguião¹; C. Ferro²; I. Amorim¹; A. Canadas¹; A. Rema¹; F. Gärtner¹; A. Santos Silva²; J. Correia da Costa^{2,3}, E. Gomes Neves^{1,3}

¹ICBAS, Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto;

²National Reference Laboratory for Mycobacteria, INSA, National Institute of Health, Porto;

³CECA-ICETA, Centro de Estudos de Ciência Animal, Universidade do Porto;

Granulomatous lesions of pig lymph nodes are frequently observed during abattoir meat inspections. The predominant causative agent is *Mycobacterium avium subsp. hominissuis* (Mah), belonging to *Mycobacterium avium complex* (MAC). Members of MAC are ubiquitous and opportunistic pathogens that may infect domestic animals, such as pigs, and humans. In pigs, the infection is usually asymptomatic and the lesions are mainly detected at slaughter. In humans, severe infections have been reported, especially in immunocompromised patients. Furthermore, this pathogen can cause cervical lymphadenitis in children and lung infections in patients with underlying lung disease. In Portugal, there are few reports documenting the prevalence of *Mycobacterium avium*.

From November 2012 until February 2013 at abattoir inspection, 9 281 pigs were examined for the presence of lymph node granulomatous lesions. Among these, lesions were detected in 387 (4,2%) animals from 7 farms. In order to examine if *M. avium* was the causative agent, 47 affected submaxillary lymph nodes were collected. A portion of the lymph node was first homogenized using a mortar, followed by decontamination with detergent and alkali (Kubica method) and the resulting sediments were used for the histological evaluation and culture. *Bacteria* identification was performed by Ziehl-Neelsen (ZN) staining, immunohistochemistry and culture. For primary isolation, we inoculated the sediment in Lowenstein-Jensen medium (a solid egg-base culture media) and in MGIT 960 liquid medium (Bactec MGIT 960 Mycobacterial Detection System, Becton Dickinson). The resulting sediments were used for microscopic examination and culture. Macroscopically, lymph nodes hypertrophy was detected in all the samples and 45 (95,7%) of the total presented gross granulomatous lesions at cross-section. Microscopic examination demonstrated acid-fast bacilli (AFB) in eighteen samples (38%). Culture revealed mycobacteria growth in 24 (51%) out of the 47 affected lymph nodes. All the positive results were identified as *M. avium* spp by a commercial amplification and reverse hybridization method (GenoType Mycobacterium CM, Hain Lifescience). Currently, the identification of the isolates at subspecies level using a multiplex PCR based on the sequences IS 901, IS 1245 and *dnaJ* is being addressed. Additionally, the analysis of 153 new sampled lymph nodes collected from pigs of 9 different farms, slaughtered between September and December 2013, is ongoing.

MAC is a potential zoonotic pathogen, which has to be excluded from the food chain. The control of MAC infection among herds is also very important for epidemiological and public health purposes. The *post mortem inspection* of lymph nodes plays an important role in the detection of mycobacterial infection, but it is essential to identify the etiological agent.

***In vitro* neuroprotective and antioxidant effects of seaweed extracts**

N. Gomes, P. Valentão, P. B. Andrade and C. Grosso

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Seaweeds produce a plethora of secondary metabolites as chemical defense and many of them can also exhibit pharmacological activities useful to the treatment of several human diseases.

Aqueous extract and ethyl acetate fractions of two brown seaweeds, *Cystoseira tamariscifolia* (Hudson) Papenfuss and *Cystoseira usneoides* (L.) M. Roberts, were tested against reactive oxygen and nitrogen species and against enzymes involved in the pathogenesis of Alzheimer's disease and depression, namely, acetyl- and butyrylcholinesterase and monoamine oxidase-A (MAO-A). In addition, their chemical composition was also studied by GC-MS, showing a predominance of fatty acids.

The ethyl acetate fraction of *C. tamariscifolia* was the most potent antioxidant and antidepressant one, displaying IC₅₀ values of 43, 39 and 16 µg/mL against superoxide anion radical (O₂^{•-}), nitric oxide radical (•NO) and MAO-A, respectively (Fig. 1). The anticholinesterases activity was not remarkable.

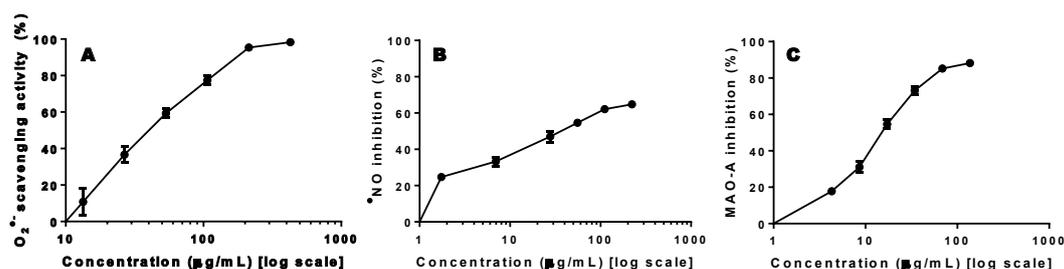


Fig. 1 – Activity of *C. tamariscifolia* ethyl acetate fraction against O₂^{•-} (A), •NO (B) and MAO-A (C). Results show mean ± SEM of three experiments performed in triplicate.

Acknowledgements: The authors are grateful to FCT for grant no. PEst-C/EQB/LA0006/2011 and to “Programa Operacional Regional do Norte ON.2” (P23-NORTE-01-0124-FEDER-000070. Clara Grosso thanks FCT for the Post-Doc fellowship (SFRH/BPD/63922/2009).

Flower extracts of *Filipendula ulmaria* (L.) Maxim inhibit the proliferation of human tumor cell lines

M. João Lima¹, Diana Sousa², Raquel T. Lima^{2,3}, Ana Maria Carvalho⁴, Isabel C.F.R. Ferreira⁴, M. Helena Vasconcelos^{1,2}

¹ Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² Cancer Drug Resistance Group, IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal.

³ CEQUIMED-UP – Centre of Medicinal Chemistry of the University of Porto, Porto, Portugal.

⁴ Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Portugal.

Ethnobotanical surveys document wild plants that have been commonly used worldwide to prepare homemade remedies. *Filipendula ulmaria* (L.) Maxim (meadowsweet) is a good example of a popular medicinal species that can be found throughout most Europe and Asia. The plant is known for its rich antioxidants content, having compounds such as flavonoids and ascorbic acid [1]. Nonetheless, its tumor cell growth inhibitory activity has never been studied. Therefore, the aim of this project was to investigate if the flower extracts of *Filipendula ulmaria* (Fig. 1) have cell growth inhibitory activity in human tumor cell lines.



Fig. 1: Inflorescences of *Filipendula ulmaria* collected in Northeastern Portugal meadows.

The flower extracts were obtained by infusion, methanolic extraction, methanol:water (80:20, v:v) extraction or decoction. Such extracts were screened for tumor cell growth inhibitory activity in three human tumor cell lines: NCI-H460 (non-small cell lung cancer), A375-C5 (melanoma) and MCF-7 (breast adenocarcinoma). One of the most potent extracts from the flowers of *F. ulmaria* (obtained by decoction) was further studied in one of the most sensitive cell lines (NCI-H460), by investigating its effect in cellular proliferation, cell cycle profile and programmed cell death.

Results showed that all the extracts obtained from the flowers of meadowsweet inhibited the growth of the mentioned cell lines. The most potent extract was the one obtained by decoction (GI_{50} of 70.0 ± 8.6 , 96.0 ± 12.4 and 63.3 ± 7.6 $\mu\text{g/mL}$ in the NCI-H460, MCF-7 and A373-C5 cells, respectively). Additionally, further studies of this extract in the NCI-H460 cells (with the GI_{50} and twice the GI_{50} concentration) showed that the reduction in cell growth was due to a strong reduction in cellular proliferation, with a slight increase in the percentage of cells in the G1 phase of the cell cycle, but not to alterations in programmed cell death. Future work will confirm if this extract is non-toxic to human non-tumour cell lines.

References:

- [1] Barros, L., Cabrita, L., Boas, M.V., Carvalho, A.M, Ferreira I. (2011), *Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants*, Food Chemistry, 127, 1600-1608.

Introgression of Iberian maternal lineages onto the Sephardic Jews gene-pool inferred from the mitochondrial DNA

C. Prego^{1,2}, S. Marques², L. Alvarez², M.J. Prata^{1,2}, A. Amorim^{1,2}, A. Goios², L. Gusmão^{2,3}, I. Nogueiro²

¹ FCUP - Faculty of Sciences, University of Porto, Portugal

² IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal

³DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Brazil

The maternal genetic background of Jewish populations has become a hot research topic. The majority of the genetic studies on the subject have been focused on Ashkenazi populations, trying to identify their putative founder lineages [1].

In this study, we focused on a sample of unrelated self-designated Sephardic Jews from Bragança district (NW Portugal), with the main aim of assessing the level of introgression of non-Jews maternal lineages onto the gene-pool of Sephardic Jews. To this end, the mtDNA composition of the Jews from Bragança was compared with the available data for other Iberian populations. In the Jews from Bragança a high diversity of maternal lineages was found, with five haplogroups (HV0b, N1, T2b11, T2e and U2e) putatively identified as Sephardic founder lineages [2]. In the comparative analysis, we focused in two haplogroups: H, which currently it is the most frequent haplogroup across European populations [3], and U5, which was the most frequent in European populations before the Neolithic period [4].

Besides the referred founder lineages in the Sephardic Jews from Bragança the remaining haplogroups represented 60% of their mtDNA diversity, with haplogroup H being by far the most prevalent (37%). U5 was the second most common haplogroup (10.5%). No significant differences were observed in the H and U5 haplogroup frequencies when compared to the Portuguese population (40% and 8%, respectively). Despite that, a shared haplotype analysis has indicated that some lineages might have evolved in the Sephardic Jews after having been introgressed in the population.

References:

- [1] Behar, D.M., Metspalu, E. et al. (2008) *Counting the founders: the matrilineal genetic ancestry of the Jewish Diaspora*. PLoS ONE 3, e2062.
- [2] Teixeira, J.C., Nogueiro, I et al.. (2011) *Mitochondrial DNA-control region sequence variation in the NE Portuguese Jewish community*. FSI genetics supplement series 3, e51-e52.
- [3] Alvarez-Iglesias, V., Mosquera-Miguel, A. et al. (2009), *New population and phylogenetic features of the internal variation within mitochondrial DNA macro-haplogroup R0*. PLoS ONE 4, e5112.
- [4] Brandt, G., Haak, W. et al. (2013), *Ancient DNA reveals key stages in the formation of central European mitochondrial genetic diversity*. Science, 342(6155):257-61.

Biological evaluation of new steroidal compounds: aromatase inhibition and effects in MCF-7aro cells viability

A.Lemos¹, C. Amaral^{1,2}, A. Lopes^{1,2}, C. Varela³, E. Tavares da Silva³, F. Roleira³, S.Costa³, N. Teixeira^{1,2} and G. Correia-da-Silva^{1,2}

¹ Laboratory of Biochemistry, Department of Biological Sciences, Faculty of Pharmacy;

² Institute for Molecular and Cell Biology (IBMC), University of Porto, Portugal.

³ CEF, Center for Pharmaceutical Studies, Pharmaceutical Chemistry Group, Faculty of Pharmacy, University of Coimbra, Portugal.

Breast cancer incidence in women is high at global level. 70-80% of post-menopausal women exhibit estrogen-dependent breast tumors (ER⁺). As these tumors require estrogens for their growth, two main approaches have been applied as endocrine therapy to block estrogen action. One acts directly at the estrogen receptors (SERM's and SERD's), preventing the interaction of estrogens with their receptors. The other is directed to aromatase, the enzyme that catalyzes the last step of estrogens production from androgens, through the use of aromatase inhibitors (AIs) [1]. Based in the interaction of natural substrates and aromatase inhibitors with the enzyme active site, several new compounds have been designed, synthesized and tested for their anti-aromatase activity, by our group, in order to find new and potent AIs [2].

In this study, three steroidal compounds, 4 β ,5 β -epoxyandrost-17-one (**43**), 6 α -methyl-5 α -androst-3-en-17-one (**46**) and 6 α -methyl-3 α ,4 α -epoxy-5 α -androst-17-one (**47**) were obtained by molecular modifications of the androstenedione structure (natural substrate). Their potential anti-aromatase activity was evaluated in human placental microsomes and in an ER⁺ breast cancer cell line, MCF-7aro (aromatase-overexpressing cell line) by the measurement of aromatase activity, using [1β -³H] androstenedione. Their effects in cell viability were also studied in MCF-7aro cells by MTT assay. These new molecules showed to be strong inhibitors of aromatase activity in human placental microsomes and in MCF-7aro cells. Compounds **46** and **47** were the most potent in the inhibition of aromatase activity, inducing a decrease in cell viability in a time- and dose-dependent manner. These evidences were accompanied by morphologic alterations, like membrane blebbing, chromatin condensation and fragmentation, which suggest the involvement of an apoptotic mechanism. These results contribute to the study of the most favorable structural modifications in order to obtain new and potent aromatase inhibitors with an anti-tumoral effectiveness.

Acknowledgements:

FCT for Cristina Amaral and Carla Varela PhD grants (SFRH/BD/48190/2008; SFRH/BD/44872/2008); FEDER Funds through COMPETE and FCT project FCOMP-01-0124-FEDER-020970(PTDC/QUI-BIQ/120319/2010); Dr. Shiu-an Chen (Beckman Research Institute, USA) for MCF-7aro cells.

References:

[1] Fabian, C.J. (2007) *The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer*. Int J Clin Pract 61, 2051–2063

[2] Amaral C, Varela C, Azevedo M, da Silva ET, Roleira FM, Chen S, Correia-da-Silva G, Teixeira N. (2013) [Effects of steroidal aromatase inhibitors on sensitive and resistant breast cancer cells: aromatase inhibition and autophagy](#). J Steroid Biochem Mol Biol., 135,51-9.

Immunohistochemical analysis of phosphorylated mammalian target of rapamycin (p-mTOR) in canine mammary carcinomas

L. Delgado, F. Gärtner, P. Dias Pereira

Department of Veterinary Pathology, ICBAS Faculty, University of Porto, Portugal.

Mammary tumours are the most prevalent neoplasms in not spayed female dogs and more than 50% of cases are classified as malignant neoplasms.

The mammalian target of rapamycin (mTOR) is a 290 kDa serine-threonine protein kinase, constituting an important downstream target of the PI3K/Akt signalling pathway involved in the regulation of overall cellular anabolism, cell growth, proliferation and survival. [3-5] mTOR is activated (p-mTOR) by phosphorylation via the PI3K kinase/Akt signaling pathway.

Activation of the PI3K/Akt/mTOR pathway has been reported in various tumour types in humans. In dogs, activation of this pathway has been reported in canine haemangiomas and haemangiosarcomas tissue sections [5] and in cell lines derived from cases of melanoma and osteosarcoma. [6,7]

To the best of our knowledge, the present study is the first to evaluate the immunohistochemical expression of p-mTOR in canine mammary carcinomas and investigate its relationship with clinicopathological parameters, namely histological type and grade of the lesions and development of lymph node metastasis. For that purpose we collected 45 canine mammary carcinomas and 15 corresponding lymph node metastasis.

Phospho-mTOR cytoplasmic expression was observed in 35/45 cases (78%). We found a relationship between the extent of p-mTOR immunoreactivity and histological grade, with high grade tumours showing decreased expression of this protein, when comparing with intermediate and low grade tumours. No relation was found between p-mTOR expression and histological type of carcinomas.

Of the 15 cases with lymph node metastasis, 12 (80%) presented increased expression of p-mTOR. Analysing the 15 lymph node samples, we observed that 13/15 (86%) showed an equal or inferior expression of p-mTOR when comparing with the respective primary tumor.

References:

- [1] Lana, S.E., Rutteman, G.R. and Withrow, S.J. (2007), *Tumors of the mammary gland*, in Withrow, S.J., "Small Animal Clinical Oncology", Saunders Elsevier, St. Louis, pp. 619–636.
- [2] Sorenmo, K., (2003), *Canine mammary gland tumors*. Veterinary Clinics of North America Small Animal Practice 33(3), 573–96.
- [3] Liao, Y.M., Kim, C. and Yen, Y. (2011), *Mammalian target of rapamycin and head and neck squamous cell carcinoma*. Head & Neck Oncology 3(22), 1-7.
- [4] Monteiro, L.S., Delgado, M.L., Ricardo, S., Garcez, F., Amaral, B., Warnakulasuriya, S., Lopes, C (2013), *Phosphorylated mammalian target of rapamycin is associated with an adverse outcome in oral squamous cell carcinoma*. Oral Surg Oral Med Oral Pathol Oral Radiol. 115(5), 638-45.
- [5] Murai, A., Abou Asa, S., Kodama, A., Sakai, H., Hirata, A. and Yanai, T. (2012), *Immunohistochemical analysis of the Akt/mTOR/4E-BP1 signalling pathway in canine haemangiomas and haemangiosarcomas*. J Comp Pathol. 147(4), 430-40.
- [6] Gordon, I.K., Ye, F. and Kent, M.S. (2008), *Evaluation of the mammalian target of rapamycin pathway and the effect of rapamycin on target expression and cellular proliferation in osteosarcoma cells from dogs*. Am J Vet Res. 69(8), 1079-84.
- [7] Kent, M.S., Collins, C.J. and Ye, F. (2009), *Activation of the AKT and mammalian target of rapamycin pathways and the inhibitory effects of rapamycin on those pathways in canine malignant melanoma cell lines*. Am J Vet Res. 70(2), 263-9.

Effect of *Cordyceps militaris* methanolic extract in NCI-H460 tumor cells

A. Bizarro^{1,7}, I.C.F.R. Ferreira², M. Soković³, L.J.L.D. van Griensven⁴, M. H. Vasconcelos^{5,7*} and R. T. Lima^{5,6*}

¹Department of Biology, School of Sciences, University of Minho, Portugal.

²Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Portugal.

³University of Belgrade, Department of Plant Physiology, Institute for Biological Research “Siniša Stanković”, Serbia.

⁴Plant Research International, Wageningen University and Research, The Netherlands.

⁵Cancer Drug Resistance Group, IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Portugal.

⁶CEQUIMED-UP- Centre of Medicinal Chemistry of the University of Porto, Portugal.

⁷Laboratory of Microbiology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

*corresponding authors

Mushroom extracts have been studied extensively for their potential antitumor effect in tumor cell lines and in animal models [1]. Recently, some of our collaborators have described that the methanolic extract from the medicinal mushroom *Cordyceps militaris* (L.) Link presented tumor cell growth inhibitory activity. In particular, this extract inhibited the growth of the non-small cell lung cancer (NSCLC) cell line NCI-H460, presenting a GI₅₀ of approximately 50 µg/ml [2].

The aim of this work was to further study the effect of *C. militaris* methanolic extract in NCI-H460 cells, regarding its mechanism of action.

NCI-H460 cells were treated with *C. militaris* methanolic extract (at 25 and 50 µg/ml) for 48 h. Viable cell number was then assessed with the Trypan blue exclusion assay. Cellular proliferation was analyzed with the BrdU incorporation assay and cell cycle profile with flow cytometry following propidium iodide (PI) labeling. Levels of apoptotic cell death were determined with flow cytometry following Annexin V-FITC /PI labeling.

Treatment of cells with the *C. militaris* extract caused a dose-dependent decrease in viable cell number. Moreover, a clear and strong decrease in cellular proliferation was observed. In addition, alterations in cell cycle profile were found, with a strong decrease in the S and G2/M phases of the cell cycle together with an increase in G0/G1 phase. Furthermore, treatment with the extract also induced apoptosis in this cell line.

In conclusion, *C. militaris* methanolic extract was shown to interfere with cell proliferation, cell cycle and to induce apoptosis of NCI-H460 cells. Further studies will aim at further understanding the mechanism of action of this extract.

References:

- [1] Ferreira, I.C., J.A. Vaz, M.H. Vasconcelos, and A. Martins. (2010), *Compounds from wild mushrooms with antitumor potential*. *Anti-cancer agents in medicinal chemistry*. 10:424-436
- [2] Reis, F.S., Barros, L., Calheta, R.C., Cirić, A., van Griensven, L.J., Soković, M., Ferreira, I.C. (2013), *The methanolic extract of Cordyceps militaris (L.) Link fruiting body shows antioxidant, antibacterial, antifungal and antihuman tumor cell lines properties*, *Food and chemical toxicology*, 62:91-98.

Genotoxic effects in *Oncorhynchus mykiss* after acute exposure to erythromycin

P. Ferreira^{1,2}, **S. Rodrigues**^{1,2}, **C. Moreira**², **B. Nunes**³, **A.T. Correia**^{1,4}, **S. C. Antunes**^{1,3}

¹ Faculdade de Ciências da Universidade do Porto (FCUP), Departamento de Biologia, Rua do Campo Alegre, 4169-007 Porto, Portugal.

² Centro Interdisciplinar de Investigação Marinha e Ambiental (CIMAR/CIIMAR), Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal.

³ Centro de Estudos do Ambiente e do Mar (CESAM), Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal.

⁴ Faculdade de Ciências da Saúde da Universidade Fernando Pessoa (FCS-UFPP), Rua Carlos da Maia 296, 4200-150, Porto, Portugal.

Pharmaceutical use in aquaculture production systems is nowadays a reality. Several antibiotics used for this purpose end up being discarded within aquaculture effluents, resulting in numerous damages for the aquatic environment. In this way, it is important to evaluate the potential effects of antibiotics in fish health (including cultured and also non target species), once humans may be the final consumers of these organisms. Erythromycin is a macrolide antibiotic frequently used to treat many types of bacterial infections, namely in aquaculture facilities. In the present work, we assessed the genotoxic effects of erythromycin in blood of the freshwater species rainbow trout *Oncorhynchus mykiss*. This species is ecologically important and has a high economic value in Portugal, since it is largely cultured for human consumption. The genotoxic effects of erythromycin were assessed in the blood of *O. mykiss* individuals after an acute exposure (period of 96 h) to a wide range of concentrations (0.001, 0.010, 0.100, 1.000 and 10.000 mg l⁻¹), that are close to the ones in which erythromycin has already been reported in the wild. Additionally, at the end of the exposure period, a subgroup of previously exposed individuals was placed in clean water during 48h, in order to evaluate the recovery process. Blood samples were analyzed by quantifying the erythrocytic nuclear abnormalities (ENAs) occurrence.

References:

S.M. Marques, S.C. Antunes, H. Pissarra, M.L. Pereira, F. Gonçalves, R. Pereira (2009) *Histopathological changes and erythrocytic nuclear abnormalities in Iberian green frogs (Rana perezi Seoane) from a uranium mine pond*. Aquatic Toxicology 91:187–195.

S. Guilherme, M. Valega, M.E. Pereira, M.A. Santos, M. Pacheco (2008) *Erythrocytic nuclear abnormalities in wild and caged fish (Liza aurata) along an environmental mercury contamination gradient*. Ecotoxicology and Environmental Safety 70(3): 411-421

Acknowledgment: This research was supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology, under the projects PEst-C/MAR/LA0015/2013 and PEst-C/MAR/LA0017/2013.

Validation of otolith daily increments in early juveniles of shanny *Lipophrys pholis*

M. G. Carvalho ^{1,2}, A. S. Moreira ^{1,2}, C. Moreira ¹, H. Queiroga ³, P. T. Santos ^{1,2}, A. T. Correira ^{1,4}

¹Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR). Rua dos Bragas 289. 4050-123 Porto. Portugal;

²Faculdade de Ciências da Universidade do Porto (FCUP). Rua Campo Alegre 1021/1055. 4169-007 Porto. Portugal;

³Centro de Estudos do Ambiente e do Mar da Universidade de Aveiro (CESAM). Campus Universitário de Santiago. 3810-193 Aveiro. Portugal;

⁴Faculdade de Ciências da Saúde da Universidade Fernando Pessoa (FCS/UFP). Rua Carlos Maia 296. 4200-150 Porto. Portugal.

The shanny *Lipophrys pholis* is a worldwide intertidal fish usually found in the Portuguese coastal shores. Spawning takes place from October/November to May in rocky nests, after which demersal eggs hatch and larvae disperse to the coastal area. Early juveniles (recruits) return within two to three months, apparently to a particular set of rock tide pools, suggesting a homing behavior. Information available on the ecology of this species is scarce and is mainly from reproductive and behavioral studies, made in some cases from captivity experiments. Otoliths have been successfully used during the last decades to age fishes and by consequence as chronological markers of several life history traits. To assess the periodicity of micro-increment formation on otoliths of *L. pholis*, 90 early juveniles were immersed in alizarin red S or tetracycline hydrochloride for 24 h and sacrificed after 10, 20 and 30 days. Sagittae were observed under ultraviolet and light microscopy. Both dyes appeared as distinct bright rings when viewed under ultraviolet light and micro-increments were clearly visible under light microscopy. The number of micro-increments was significantly related to the duration of the experimental period, exhibiting an overall otolith daily growth rate of $1.24 \mu\text{m day}^{-1}$. The slopes of the linear regressions were not significantly different to 1, indicating that micro-increments in sagittae were deposited on a daily basis. This study provides evidence that daily growth increments are reliable sources of age information for *L. pholis*.

Temperature tolerance limits of *Synechocystis* sp. PCC 6803 and its improvement using compatible solutes

E. Ferreira¹, C. C. Pacheco² and P. Tamagnini^{1,2}

¹ Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Rua do Campo Alegre, Edifício FC4, 4169-007 Porto, Portugal

² IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua do Campo Alegre 823, 4150-180 Porto, Portugal

Cyanobacteria are prokaryotic photosynthetic microorganisms that can be used in many biotechnological applications as cell factories to convert solar energy, by capturing CO₂ from the atmosphere, to produce valuable compounds, such as natural products (sugars and isoprene), biofuels (alcohols, alkanes and hydrogen), and other commodity chemicals. The simple nutritional requirements and transformability of cyanobacteria make these organisms tractable for complex engineering and ideal candidates for synthetic biology approaches [1]. Synthetic biology is the design and construction of new biological parts, devices and systems, as well as the redesign of existing biological systems for useful purposes (<http://syntheticbiology.org>).

Among cyanobacteria, *Synechocystis* sp. PCC 6803 is the best-characterized strain and has a relatively small genome completely sequenced [2] and annotated. Outdoors bioreactors can be used to grow cyanobacteria but these cultures become vulnerable to environmental conditions such as temperature fluctuations, and consequently alter the yield of a given compound. As it was already described, *Synechocystis* sp. PCC 6803 cells seem to suffer from low- and high-temperature stresses <25 °C and >40 °C [3]. Therefore, we evaluated the temperature tolerance limits of this cyanobacterial strain and are currently testing its resilience to daily temperature fluctuations. Simultaneously, we are testing the increase of temperature tolerance by using culture media supplemented with the compatible solutes ectoine and hydroxyectoine. Compatible solutes are known to increase the cells tolerance against various stress factors [4]. In the future, strategies to engineer *Synechocystis* sp. PCC 6803 to produce these compatible solutes will be designed and implemented using a synthetic biology approach.

[1] Ducat, D. C., Way, J. C., & Silver, P. A. (2011). *Trends in biotechnology*, 29(2), 95-103.

[2] Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., & Tabata, S. (1996). *DNA research*, 3(3), 109-136.

[3] Berry, J., & Bjorkman, O. (1980). *Annu Rev Plant Physiol Plant Molec Biol* 31: 491–543.

[4] Pastor, J. M., Salvador, M., Argandoña, M., Bernal, V., Reina-Bueno, M., Csonka, L. N., Iborra, J. L., Vargas, C., Nieto, J. J., & Cánovas, M. (2010). *Biotechnology advances*, 28(6), 782-801.

Mussel news: *Mytilus galloprovincialis* Lamarck

L. R. Silva, A. Miranda, A. Mota, J. Gomes, R. Oliveira, P. Cunha, S. Pinto, T. Soares and T. Sousa

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Marine species are most commonly used as food, because they produce a wide range of biological compounds, which may be also used for the development of products for many fields like medicine. Marine ecosystems led to the discovery of thousands of new compounds in organisms like sponges, soft corals, mollusks and algae. The objective of this work was to improve the knowledge on the chemical composition and bioactivity of the mussel *Mytilus galloprovincialis* Lamarck, by studying its ethanol extract.

Carotenoids, chlorophylls, amino acids and fatty acids were detected, among other compounds (Fig. 1).

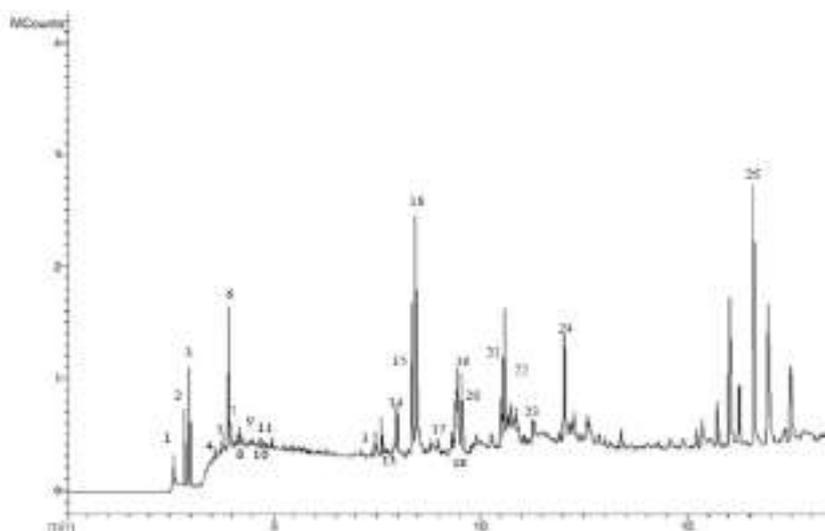


Fig. 1. GC-MS profile of the ethanol extract of *M. galloprovincialis*. Lactic acid (1), alanine (2), glycine (3), valine (4), urea (5), glycerol (6), leucine (7), isoleucine (8), proline (9), serine (10), threonine (11), tetradecanoic acid (12), tyrosine (13), pentanoic acid (14), palmitelaidic acid (15), hexadecanoic acid (16), heptadecanoic acid (17), linoleic and linolenic acids (18), oleic acid (19), stearic acid (20), arachidonic acid (21), eicosapentaenoic acid (22), *cis*-11-eicosenoic acid (23), docosahexaenoic acid (24) and cholesterol (25).

The extract displayed little protective action against reactive oxidant species and showed no antimicrobial activity against *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella typhimurium* and *Escherichia coli*. The use of other solvents may provide more bioactive extracts.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5th year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head) and Patrícia Valentão.

Study of patterns of movement and connectivity in *Emys orbicularis* inside the PAVT

S. Magalhães¹, J. Teixeira²

¹ CIBIO Department of Biology, Faculty of sciences, University of Porto, Portugal.

² CIBIO, University of Porto, Portugal

The need to preserve and conserve is becoming more important day after day. This need becomes even more evident when referring to areas that contain a great wealth and natural values. Conserving species that meet with status of protection becomes a challenge, but more importantly, it becomes a growing need to create plans that can provide means to protect and preserve their habitats. The increasing fragmentation of habitat is a major consequence of human actions in the landscape [1] and one of more relevant threats to biodiversity, and this way maintain the connectivity between habitats shown to us like essential.

The aim of this study is to respond to the need to conserve *Emys orbicularis*, a species of turtle occupying a scarce habitat patches. Turtle movements among ponds have been widely reported and recognized as crucial for population's persistence [2]. In North of Portugal, in extreme North of Boticas, it exist some ponds where we can find these animals. The area of study belongs to PAVT, Arqueological Park of Terva Valley. We use spatial data to create a spatial network structure to help decision-making in landscape management and planning.

At the end of this study we expect to have a network structure that explains the movement between patches and the efforts that these distance represent, as well as giving a possible to to development for these areas without endangering the specie.

[1] Miguel, P., Segurado, P., Neves, N. (2010), *Using spatial network structure in landscape management and planning: A case study with pond turtle*. Landscape and Urban Planning 100 (2011) 67–76

[2] Bowne, D.R., Bowers, M.A., Hines, J.E. (2006), *Connectivity in an agricultural landscape as reflected by interpond movements of a freshwater turtle*. Conserv. Biol. 20 (3), 780–791.

Planctomycetes as a food source for *Daphnia longispina*: preliminary findings

T. Carvalho¹, S. C. Antunes^{1,2}, O. M. Lage^{1,3}

¹ Department of Biology, Faculty of Science, University of Porto, Portugal.

² Centre of Environmental and Marine Studies (CESAM), Campus of Santiago, University of Aveiro, Portugal.

³Centre of Marine and Environmental Research (CIIMAR), Porto, Portugal.

Daphnia sp. is a standard ecotoxicological organism with a well-known life cycle. It plays a key role in the energy transfer in freshwater food webs as a primary consumer (filter feeder), grazing on microalgae, yeast and bacteria [1]. It is commonly reared in the laboratory using microalgal cultures as food source; however alternative or complementary sources of carbon are important to reduce the dependency on a single food source [2]. The potential of planctomycetes as a nutritional source is still unknown. Bearing this in mind, this work aimed to evaluate the potential of the planctomycete *Rhodopirellula rubra* strain LF2 as nutritional source of *Daphnia longispina*. *R. rubra* is a planctomycete isolated from the microbial community of marine macroalgae and is easy to rear in the laboratory in large quantities [3]. Planctomycetes were cultivated according to laboratory procedures to obtain enough food supply for *D. longispina* assay. A life history assay was conducted with *Daphnia longispina* following a bifactorial design. Daphnids were fed with three different concentrations (1/100; 10/1000; 100/1000; v/v) of strain LF2 at exponential growth phase. Additional 3 treatments were included where *D. longispina* were fed with the same concentrations of planctomycetes plus the green microalga *Pseudokirchneriella subcapitata* (1.5×10^5 cells/ml/day). All treatments were compared with the standard microalgae-fed *Daphnia* (control treatment fed with *P. subcapitata*). *D. longispina* was checked daily for mortality and reproduction along 21 days [1]. Results were assessed based on several endpoints measured according to standard protocols: age of first reproduction, offspring, somatic growth rate, r – rate of population increase. Food supply exclusively with *R. rubra* strain LF2 decreased significantly the total offspring and the rate of population increase. However an apparent increase of total offspring was observed when the two different food sources were used. Further studies are needed to confirm these results that are somehow different from the ones obtained previously in a similar assay with *D. magna*.

[1] Antunes SC, Castro BB, Gonçalves F (2003), *Chronic responses of different clones of Daphnia longispina (field and ephippia) to different food levels*. Acta Oecologica 24: S325-S332;

[2] Antunes SC, Castro BB, Gonçalves F (2004), *Effect of food level on the acute and chronic responses of daphnids lindane*. Environmental Pollution 127: 367-375;

[3] Lage, O.M. & Bondoso J. 2011. *Planctomycetes diversity associated with macroalgae*. FEMS Microbial Ecology. 78: 366-375.

Acknowledgment: This research was supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology, under the projects PEst-C/MAR/LA0015/2013 and PEst-C/MAR/LA0017/2013.

Behavior of prostate cancer cells in a 3D bone scaffold of Nanohydroxyapatite/collagen

S. Cruz-Neves^{1,2}, S. R. Sousa^{1,3}, I. Graça^{4,5}, C. Jerónimo^{4,6}, F. J. Monteiro^{1,2}

¹ INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Portugal

² FEUP - Faculdade de Engenharia, Universidade do Porto, Portugal

³ REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Portugal

⁴ Cancer Biology and Epigenetics Group, Research Center, Instituto Português de Oncologia do Porto, Portugal

⁵ ESTSP - Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, Portugal

⁶ Departamento de Patologia e Imunologia Molecular, ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal.

Prostate cancer (PCa) is one of the main healthcare problems of the male population worldwide and it preferentially metastasizes to bone. PCa cell lines, for instance PC3 and LNCaP, are widely used in a variety of two dimensional (2D) cultures, but when adopting three dimensional models (3D), the resemblance of the native microenvironment is higher, as well as the dynamic and interactive exchange between bone components and tumor cells. These 3D models are promising and innovative, since few studies have been done involving these lines cultured in biomaterials. Therefore, the purpose of this work was to study the behavior of PCa cells cultured in nanohydroxyapatite/collagen scaffolds. The porous constructs were produced using the polyurethane sponge impregnation method and the physico-chemical characteristics were analyzed using Scanning Electron Microscope (SEM) and Micro-Computed Tomography (Micro-CT). A porosity of 62.4 ± 1.8 % was obtained and mean pore size around 367 ± 6 μm . Scaffolds viability for biological studies was evaluated using MC3T3-E1 cells. Results from Confocal Laser Scanning Microscopy (CLSM) and SEM shows that MC3T3-E1 cells were able to adhere and colonize the 3D structures. The data obtained indicates that the PC3 cell line adhered, and proliferated inside the 3D scaffolds, while the LNCaP had a different behavior. As a conclusive remark, the use of 3D models can be suitable to provide new insights into the poorly understood mechanisms of bone metastasis and, hopefully, benefit the generation of improved therapies.

The Evolution of the Retinoid Orphan Receptor (ROR) in Lophotrochozoa Protostomes

Emilie Pinheiro^{1,2}, Miguel M. Santos^{1,2}, L. Filipe C. Castro^{1,2} and Raquel Ruivo²

¹ FCUP - Department of Biology, Faculty of Sciences, University of Porto, Portugal

² CIMAR/CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal

Nuclear receptors (NRs) are vital components in endocrine and hormonal systems, regulating various biological processes as development, physiology and reproduction. They belong to a diverse superfamily of ligand-dependent, or independent, transcription factors that regulate gene expression ^[1]. The Retinoic acid receptor-related Orphan Receptors (RORs) are a group of transcription factors suggested to modulate cholesterol metabolism, diurnal rhythm and fasting induction in mammals^[2, 4]. Crystallographic studies have, indeed, put forward cholesterol as RORs natural ligand ^[3]; yet, its ability to bind and activate ROR-dependent gene transcription is still unclear.

Albeit present in all metazoan lineages, the evolutionary history, diversity, and physiological role of NRs are far from fully understood.^[1] In this work we aimed at analysing the ROR repertoire in various lophotrochozoan phyla. Combining genome database mining and PCR-based techniques we obtained the full coding sequence of a ROR orthologue from the annelid *Platynereis dumerilii* and a partial sequence from the mollusk *Osilinus lineatus*. These results will allow us to screen for potential ligands and their ability to activate transcription using *in silico* and *in vitro* techniques: 3D molecular docking and cell-based ligand binding assays, respectively. In combination with phylogenetic analysis, we should contribute for a better understanding of the evolutionary history and molecular function of RORs in the Bilaterian lineage.

[1] Bridgham JT, Eick GN, Larroux C, et al. (2010) PLoS Biol 8(10): e1000497.

[2] Giguère V, Tini M, Flock G, et al. (1994) Genes Dev. 8: 538-553.

[3] Kallen JA, Schlaeppi J-M, Bitsch F, et al. (2002) Structure 10(12):1697-1707.

[4] Pathak P, Li T and Chiang JY. (2013) J. Biol. Chem. 288: 37154-37165.

Development and characterization of a tridimensional intestinal model to study intestinal absorption

C. Pereira^{1,2}, **F. Araújo**^{1,3}, **P. Granja**¹, **B. Sarmiento**^{1,4}

¹ INEB - Instituto de Engenharia Biomédica, University of Porto, Portugal

² FEUP – Faculdade de Engenharia, University of Porto, Portugal

³ ICBAS – Instituto Ciências Biomédicas Abel Salazar, University of Porto, Portugal

⁴ IINFACTS – Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Instituto Superior de Ciências da Saúde-Norte, Department of Pharmaceutical Sciences, CESPU, Gandra, Portugal

*bruno.sarmiento@ineb.up.pt

Gastrointestinal tract, the largest mucosal surface, aims to hinder the entrance of pathogens, toxins and undigested molecules coupled to the digestion and selective absorption of essential nutrients [1]. Particularly in small intestine, intestinal mucosa represents the site of innate and adaptive immune regulation but also of selective absorption by epithelial cells through enzymatic activity, pH inactivation, mucus layer, tight junctions and efflux transporters [2]. As crescent effort to predict the bioavailability of biopharmaceutical drugs, human intestinal in vitro models have been widely employed to assess drug molecules across intestinal mucosa [3]. In the present study is suggested an improved and more physiological tridimensional cell model which incorporate fibroblasts embedded in Matrigel®, Caco-2 cells (enterocytes-like cells), HT29-MTX cells (muco-producing cells) and Raji B cells which convert Caco-2 cells in M-like cells through soluble factors. During the 21 days of culture, membrane integrity as monitored by transepithelial electrical resistance (TEER) measurement. Fibroblasts and epithelial cells in co-culture were visualized by cell staining to vimentin and WGA. To further validate the model, permeability studies were performed using insulin, further analyzed by HPLC.

In intestinal cultures, TEER values showed a desirable integrity ($>400 \Omega \cdot \text{cm}^2$) mainly consequence of Caco-2 cells, which form compact polarized membrane. The introduction of fibroblasts, which intrinsically present lower TEER values, do not compromise the integrity of the model in contrast with the introduction of HT29-MTX cells due to different structure of tight junctions. By imunocytochemistry was confirmed a well-formed network of fibroblasts distinguishable from Caco-2 membrane by their columnar shape. Concerning the permeability studies, the higher the TEER values, the lower the permeability.

A functional intestinal model was achieved as supported by TEER values and apparent permeability. Is expected this model has significant impact in the assessment of the bioavailability of biopharmaceutical drugs facilitating the drug discovery and classification.

References:

1. Antunes, F., Andrade, F., Ferreira, D., Nielsen, H.M., and Sarmiento, B. (2013), Models to predict intestinal absorption of therapeutic peptides and proteins. *Current Drug Metabolism*, 14(1), 4-20.
2. Yun, Y., Cho, Y.W., and Park, K. (2013), Nanoparticles for oral delivery: Targeted nanoparticles with peptidic ligands for oral protein delivery. *Advanced Drug Delivery Reviews*, 65(6), 822-832.
3. Li, N., Wang, D., Sui, Z., Qi, X., Ji, L., Wang, X., and Yang, L. (2013), Development of An Improved 3D in Vitro Intestinal Mucosa Model for Drug Absorption Evaluation. *Tissue engineering. Part C, Methods*.

Investigating the cell growth inhibitory effect of flower extracts of *Sambucus nigra* L. in human tumor cell lines

M. João Lima¹, Joana Pereira¹, Raquel T. Lima^{2,3}, Ana Maria Carvalho⁴, Isabel C.F.R. Ferreira⁴, M. Helena Vasconcelos^{1,2}

¹ Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² Cancer Drug Resistance Group, IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal.

³ CEQUIMED-UP – Centre of Medicinal Chemistry of the University of Porto, Porto, Portugal.

⁴ Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Portugal.

Sambucus nigra L. (elder) is widely used in traditional medicine, especially for its diuretic, anti-inflammatory, diaphoretic and expectorant properties. Its rich flavonoids and ascorbic acid contents makes *S. nigra* flowers a good source of antioxidant compounds [1]. Nonetheless, the tumor cell growth inhibitory activity of such plant material has never been studied. Therefore, the aim of this ongoing project is to investigate if the flower extracts of *S. nigra* have cell growth inhibitory activity in various human tumor cell lines.

The flower extracts were obtained by infusion, methanol extraction, methanol:water (80:20, v:v) extraction or by decoction. These specimens were processed in order to be lyophilized and extracted. Stock solutions of all extracts were prepared in DMSO at a maximum concentration of 100 mg/mL and further stored at -20 °C. Such extracts were screened for tumor cell growth inhibitory activity in three human tumor cell lines: NCI-H460 (non-small cell lung cancer), A375-C5 (melanoma) and MCF-7 (breast adenocarcinoma). The cell growth assay performed was the Sulforhodamine B assay, during which cells were treated for 48 hours with 100 µL of six serial dilutions of each extract, ranging from 25 µg/mL to 400 µg/mL. Doxorubicin was used as a positive control of tumor cell growth inhibition, at a maximum concentration of 150 nM. The effect of the solvent of the extracts (DMSO) on the growth of the cell lines was also evaluated, by treating cells with the maximum concentration of DMSO used (0.20 %).

Some of the extracts showed no tumor cell growth inhibitory activity up to the highest concentration tested, whereas other extracts have some tumor cell growth inhibitory potential. However, those extracts showed different activity over time, indicating possible lack of stability. The results will be confirmed with fresh aliquots of those extracts. The most potent extract will then be selected to be further studied in the most sensitive cell line. Such studies will consist in investigating its antiproliferative potential, the effect on cell cycle profile and on programmed cell death.

References:

[1] Barros, L., Cabrita, L., Boas, M.V., Carvalho, A.M, Ferreira I. (2011), *Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants*, Food Chemistry, 127, 1600-1608.

Carbapenem-resistant *Pseudomonas aeruginosa* in imported fruit

M. Pinto^{1,2} and H. Ferreira^{1,2}

¹Microbiology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal

² REQUIMTE, University of Porto, Portugal

Pseudomonas aeruginosa is a widespread pathogen in the hospital environment that is frequently found on medical equipment, and colonizes tissues of long-stay patients. It is considered an opportunistic pathogen that induces pneumonia, bloodstream, and urinary tract infections associated with high mortality rates and high treatment failure because of its resistance against many antibiotics.^[1]

Molecular studies indicate that pseudomonal resistance to carbapenems is generally due to chromosomally encoded efflux pumps or other mechanisms that prevent uptake of the drug molecule and also carbapenemase production.^[1]

Fruits and vegetables are increasingly recognized as important vehicles for transmission of human pathogens that were traditionally associated with food of animal origin. With the increased globalization of food products, microorganisms are expanding to reach areas where they had never been detected before generating what has been called "trade-related infections." The foodborne illness affects up to 30 percent of the population in industrialized countries. International efforts to control the transmission of trans-frontiers diseases are becoming increasingly important.^[2]

The aim of our work was the detection of antimicrobial resistant bacteria in fruits, produced by organic farming or conventional farming, in our country and imported. Approximately 10g of different fruits were suspended separately in 40 ml of Tryptic Soy Broth (TSB) and incubated at 37° overnight. Isolates were selected by spreading 200 µl of broth on MacConkey agar and isolated in Mac Conkey agar with ampicillin. Colonies grown on ampicillin agar were randomly selected and susceptibility to antimicrobial agents was determined by the agar diffusion method, according to the Clinical Laboratory Standards Institute (CLSI) and screened for beta-lactam resistance. Identification of the selected strains was achieved by ID 32 GN.

One sample of a citrine imported from China, showed a *Pseudomonas aeruginosa* expressively resistance to imipenem, meropenem and ertapenem.

Detection of this multi-resistant non fermenting Gram negative, in fruit to be eaten uncooked is a worrying question in terms of food safety and population colonization and alerts for the hypothesis of dissemination of bacteria and resistance mechanisms, provided by imported fruits.

References:

[1] Fuste, E. *et al.* (2013) Carbapenem-resistance mechanisms of multidrug-resistant *Pseudomonas aeruginosa*. *J. Med. Microbiol.* 62, 1317–1325.

[2] Hodges, J. R. & Kimball, A. M. (2005) The global diet: trade and novel infections. *Glob. Health* 1, 4.

Acknowledgements: Pedro Cecílio, Bárbara Loureiro

Efficiency of cAMP, forskolin or prostaglandin E2 in the decidualization of human endometrial stromal cells

M. Diniz da Costa^{1,2}, B.M. Fonseca^{2,3}, G. Correia-da-Silva^{2,3} and N.A. Teixeira^{2,3}

¹ Faculdade de Ciências da Universidade do Porto

² Instituto de Biologia Molecular e Celular da Universidade do Porto (IBMC)

³ Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto

Decidualization of the endometrium is an essential step for pregnancy outcome since it is responsible for creating a favourable environment for blastocyst implantation and placenta development. This process involves important morphological and biochemical changes being characterized by the differentiation of endometrial stromal cells (ESC) into decidual cells [1]. The molecular mechanism of decidual transformation is induced by the coordination between progesterone and cAMP second messenger signalling pathways [2]. Although some cellular events that support ESC differentiation are well recognized, the molecular pathways that drive the initiation and progression of this process remain elusive. By using a recently immortalized human ESC cell line [3], we investigated the optimum conditions for *in vitro* differentiation of human endometrial stromal cells. The St-T1b cells were incubated with cAMP, forskolin or prostaglandin E2 (PGE2), always supplemented with medroxyprogesterone acetate (MPA). The decidual cell reaction was confirmed by morphological examination and by the expression of insulin-like growth factor binding protein-1 (IGFBP-1), a specific protein that are not expressed in undifferentiated stromal cells, analyzed by qRT-PCR. Results showed that in the experienced conditions, differences in the morphological and biochemical markers were observed. The second messenger cAMP was the most efficient inducer as revealed by morphological and biochemical markers followed by forskolin and PGE2. Although, PGE2 is known to induce differentiation of primary cultures, the St-T1b cells revealed a weak differentiation. Forskolin, which is an agent able to enhance cAMP levels, showed marked morphological changes accompanied by an increase in the expression of biochemical markers. The results confirmed that St-T1b could response to different molecules as revealed by morphological and biochemical markers, though with different intensities.

Acknowledgements: The authors thank Fundação para a Ciência e Tecnologia (FCT) for the post-doctoral grant attributed to Bruno Fonseca (SFRH/BPD/72958/2010).

[1] Gellersen, B., Brosens, I.A. and Brosens, J.J. (2007) *Decidualization of the Human Endometrium: Mechanisms, Functions, and Clinical Perspectives*. Seminars in Reproductive Medicine 1: 445–453.

[2] Gellersen, B., Brosens, J. (2003) *Cyclic AMP and progesterone receptor cross-talk in human endometrium: a decidualizing affair*. The Journal of endocrinology 178: 357–372.

[3] Samalecos, A., Reimann, K., Wittmann, S., Schulte, H.M., Brosens, J.J., Bamberger, A.M., Gellersen, B. (2009) *Characterization of a novel telomerase-immortalized human endometrial stromal cell line, St-T1b*. Reproductive biology and endocrinology : RB&E 7:76.

Fluoxetine effects in zebrafish embryos, insights to psychopharmaceuticals toxicity

P. Rodrigues^{1,2}, V. Cunha^{2,3} and M.Ferreira²

¹ FCUP - Dept of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

² CIMAR/CIIMAR- Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal

³ ICBAS/UP- Institute of Biomedical Sciences Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal

Pharmaceutical drugs in the aquatic environment are a growing problem, in particular psychopharmaceutical (PP), associated with economic crisis, among other social problems. Fluoxetine (FLX) is a serotonin selective reuptake inhibitor (SSRI) with the commercial name of Prozac and one of the most used antidepressant in the world. This PP, as well as other pharmaceuticals, are not completely eliminated at water treatment facilities, so ultimately they can reach the aquatic environment. Therefore it is essential to understand their impact and toxicity to non-target species and ultimately to aquatic environments. Zebrafish embryos were chosen for this study due its transparency allowing the monitoring of embryonic development. Moreover zebrafish brain formation begins at 9hpf [1], and the fact that FLX acts in neural receptors makes this species a possible target at sensitive early stages of development.

The aim of this study is to identify the effects of FLX in zebrafish embryos development by assessing anomalies in the embryonic development and mortality.

Embryos were obtained after reproduction of adult zebrafish in a ratio of 1:2 (male:female) in tanks (30L) with a “breeding cage” with a net bottom covered with marbles to mimic rocky substratum and stimulate reproduction.

Embryos at 1hpf (hours post fertilization) were exposed, in a 24-well plate, to FLX at different concentrations (0.0015, 0.05, 0.1, 0.5 and 0.8µM). Embryos were observed at three different time stages (8, 32 and 80 hpf) and several parameters were observed (mortality rate, 75% epiboly, delay/arrest of the division, abnormal cell masses, development delay, pericardial edema, head, eyes, tail and vitelline sac anomalies).

Preliminary results show an increase mortality in the presence of FLX between 8 and 32hpf, being the lowest concentration the most lethal, between 32 and 80 hpf the mortality stayed the same. In accordance to mortality results, embryos exposed to the lowest concentrations (0.0015 and 0.05µM) showed higher percentage of anomalies such as pericardium edema swelling, tail and eyes anomalies with the exception of development delay that showed higher percentage in the highest concentration (0.8µM). In conclusion, FLX seems to have lethal and toxic (abnormal development) effects in zebrafish embryos that can ultimately have a negative effects to fish populations exposed to this compound.

References:

[1] Kimmel C.B, Ballard W.W, Kimmel S.R, Ulmann B, and Schilling T.F (1995) Stages of Embryonic Development of the Zebrafish. *Developmental dynamics* 203:255-310

Activity of *Thymus caespititius* against *Candida* species

P. Oliveira¹, J. Coelho¹, C. Cavaleiro², L. Salgueiro², E. Pinto^{1,3}

¹ CEQUIMED, laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal.

² CEF/Faculdade de Farmácia, Universidade de Coimbra, Portugal.

³ Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Portugal.

Fungal infections have been gaining importance in recent years, largely due to the increasing number of immunocompromised patients, who require effective and potent fungicidal drugs. Vulvovaginal candidosis, often recurrent and recalcitrant, represents an important cause of morbidity. Although new therapies have emerged, the reduced number of available antifungals, most of them fungistatic, taken together with emerging resistance, urged the search for therapeutic alternatives. Previous research demonstrated the antifungal activity and the mechanism of action of some essential oils (EO). In the present work we report the antifungal activity of the EO *Thymus caespititius* and its main compound α -terpineol against *Candida* species, in order to support their use as antifungal agents.

The EO of *T. caespititius* obtained by hydrodistillation was analysed by gas-chromatography and gas-chromatography/mass spectroscopy. The antifungal activity was evaluated for the EO and α -terpineol against eight strains of six *Candida* species (*C. albicans* ATCC, M1 and D5; *C. glabrata*; *C. parapsilosis*; *C. krusei*; *C. tropicalis* and *C. dubliniensis*) using the reference CLSI broth macrodilution protocol M27-A3. Minimum inhibitory and minimum lethal concentrations (MICs and MLCs, respectively) were determined. Afterwards, the inhibition of germ tube formation was evaluated in *C. albicans* ATCC and D5.

The EO of *T. caespititius* is characterized by high amounts of α -terpineol (35.7%). The EO demonstrated activity against all *Candida* species, with MIC values ranging from 0.64 to 2.5 μ L/mL and MLC values equal to, or just one log₂ dilution above the MICs. Regarding the major component of the EO, α -terpineol also displayed activity against all tested strains, with the MICs and MLCs ranging from 0.64 to 1.25 μ L/mL. In addition, the EO revealed an important inhibitory effect on germ tube formation, a virulence factor in *C. albicans* pathogenesis. At the sub-inhibitory concentrations of 1/16 MIC, germination was completely inhibited in the clinical *C. albicans* strain D5. For the strains ATCC the inhibition was around 80%, being almost complete at 1/8 MIC. α -Terpineol seems not be the only responsible for this effect, considering that only MIC/2 was able to inhibit almost completely the germ tube formation.

In addition to the fungicidal activity of the EO of *T. caespititius* against *Candida* spp., the important inhibition of filamentation in *C. albicans* strains is interesting, since filamentation has long been shown to be essential for virulence in *C. albicans* and its inhibition alone appears to be sufficient to treat candidosis. Our results support the concept that EO might be useful in the clinical management of candidosis, particularly of its mucocutaneous presentations such as vulvovaginal candidosis. The association with other commercial antifungal compounds could bring benefits, by the effect on germ tube formation.

Acknowledgments: The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT) through grants: PEst-OE/SAU/UI0177/2011; CEQUIMED-PEst-OE/SAU/UI4040/2011.



P

POSTERS

**FRIDAY
14TH**

**POSTER
SESSIONS**

Current situation of Architecture in Portugal- Economic crisis and the future business model

Carlos Manuel Oliveira Borges¹

Masters thesis, Faculty of Architecture University of Porto, Portugal.

With this investigation I want extrapolate the causes for the current situation in which the architecture and all stakeholders are living nowadays in Portugal. The crisis in Portugal caused many architects to have less work. Today we live not only na economic crisis but alto other problems, aggravated by the crisis and affecting Portuguese architecture.

This investigation will try understand what these problems are and how we can overcome them, will be the main purpose of this investigation. During the investigation, it will be necessary study the profession evolution in Portugal, in order to understand the citizen as client so that we can identify the barriers between clients and architects.

Means of an inquiry understand what these barriers can unable in a greater acceptance of architecture. Understand in Portugal there are architecture without architects, citizens without any academic resources. Investigate if the citizens see architects as someone who will solve their problems.

It is necessary to face reality and to work for the future understanding what is wrong. My investigation attemps to uncover gaps in the profession and in professionals who can devalue the career and statute of architects. After I understand the problems, I will try to anticipate the future business model for architects.

Between the Digital and the Analogic project

M. Rodrigues¹

¹ Faculty of Architecture, University of Porto, Portugal.

This paper aims to reflect on the tools used in an architecture project, categorizing them as *analogic* or *digital* and comparing the processes that are associated with its use.

For this analysis, it is essential to understand the meaning of some key words such as "technology", "analogic project" and "digital project" (based on the ideas of Heidegger¹, Leach² and Lisboa³). Afterwards, an historical overview of the computer's use in the architecture project will be presented, focusing in the past 50 years and, particularly, in the understanding of a more analogic or digital application (proposed by Kolarevic⁵). After this introduction, the work will focus on the analysis of projects/ works that can fit in the proposed theme.

The housing project of Malagueira, in Portugal, designed by the architect Álvaro Siza, and the work developed by the architect José Pinto Duarte, in his PhD thesis (entitled *Customizing Mass Housing: a discursive grammar for Siza's Malagueira Houses*), will be presented. In this work, carried out at MIT, Duarte sought to create a digital lexicon, with the Malagueira's Housing Unit, achieved through the identification of the essential elements of the project, and its implementation in a digital environment. On the international scene, the work of the architect Mark Burry in the continuation of the "Sagrada Familia" in Barcelona, is a major example that includes the procedural and methodological duality that underlies this work. The other international case study is the Guggenheim Museum in Bilbao, designed by the architect Frank Gehry, which presents itself as a work where the analogic design was materialized through digital technology.

The paper also aims to understand which implications a tool based on the digital logic, brings to a discipline that relies on analogic media like drawings or models. The division between analogic or digital is proposed according with the relation established with the architect, divisible among those tools that can hold a corporeal connection (defended by Vieira⁷ or Pallasmaa⁸), or those that are immaterial. The fact that all the instruments used by the architect are virtual must be clear, since they all promote an abstraction of the reality, verifiable on aspects such as scale, materiality and detail.

References:

- [1] Heidegger, M. (1982), *The question Concerning Technology, and Other Essays*, London, Harper Torchbooks.
- [2] Leach, N. (2006), *Esqueçamos Heideger!*, in Afonso, R. & Furtado, G. (Eds.), "Arquitetura, máquina e corpo : notas sobre as novas tecnologias na arquitetura", Porto, FAUP, pp. 67-81.
- [3] Lisboa, F. (1997), *Desenho de Arquitectura Assistido por Computador*, Porto, Faup.
- [4] Kolarevic, B. (2005), *Architecture in the Digital Age*, London, Taylor and Francis.
- [5] Vieira, J. (1995), *O Desenho e o projecto são o mesmo? : outros textos de desenho*, Porto, Faup.
- [6] Pallasmaa, J. (1982), *The thinking hand : existential and embodied wisdom in architecture*, Chichester, John Wiley & Sons.

Architectural textured surfaces panels for optical purposes

**Eduardo Veiga¹, André Chaves ¹, Inês Castanheira³, Pedro Flores¹, Pedro Joel Costa¹,
Gonçalo Furtado¹, Jorge Lino²**

¹ Faculty of Architecture, University of Porto, Portugal.

² Faculty of Engineering, University of Porto, Portugal.

³ Faculty of Fine Arts, University of Porto, Portugal.

The “Multidisciplinary Project No. 62” is being developed at the University of Porto, using a multidisciplinary research, with input from interaction design, architecture and engineering.

The object of research is the study of structures and textured panels for modular architectural surfaces, especially for interiors, modelled and manufactured with computer support and the subsequent assembly of its various components. Each panel will have a size of 20x20cm, and their combination will result in a wall panel similar to traditional Portuguese wall tiles. The aim of this research is to create a multimedia interface actuated by various human senses.

The research emphasizes a methodological approach that is both scientific and artistic, which later results are both academic and business. The modular components are produced in composite materials, such as paper, using a binder of high performance epoxy resin (transparent bicomponent). Technological elements used include electronic devices (sensors and actuators, fiber optics, processor type as “arduino”, circuit boards with electronic application of optical fibers) that enables the reception and emission of sensory stimuli. We are currently conducting experiments on materials and technologically advanced devices, such as optical fibers and sensory technology, by performing small prototypes designed to test solutions conceived within the aim of project.

The expected result includes a prototype versatile and innovative (aesthetic and functional), capable of applying to different spatial contexts interiors. Its performance allows, given the visual and tactile aspects, the playfulness of such spaces.

A microcosm of light and beauty: the Chapel of Nossa Senhora da Conceição

Celso Ramos

Master Degree in Architecture in Faculty of Architecture, University of Porto, Portugal.

In the board I will present some of the main aspects and findings of the investigation I carried out for my dissertation on the Chapel of Nossa Senhora da Conceição[1].

To conduct this study I made a new plan of the Chapel, which lead to new findings in the geometry and the conception of the building. The observation's focus was conducted from an architectural point of view.

Despite the aspect of a small jewel, in close range the Chapel fails to meet the high expectations generated from afar. However, in the inside the building recovers the precious characteristics causing several historians to consider it in similar terms to Walter Watson's description as “one of the most beautiful interiors to be found anywhere”[2]. This is due in part to the Chapel's extraordinary use of the elements to make the light part of the ornament. This display will illustrate this aspect through photography and a description of how this is done. In addition, attention will be paid to the ornaments' symbolic functions.

Another fact discovered was the existence of some optical corrections related to the Chapel's main axis. This subject will also be briefly presented.

The presentation will conclude with consideration of some proportional relations between the Chapel and the Vitruvian's man as drew by Cesare Cesariano.

References:

[1] Ramos, Celso (2013), *A Capela da Nossa Senhora da Conceição em Tomar*, FAUP, Porto.

[2] Watson, Walter C. (1908), *Portuguese Architecture*, Archibald Constable and Company, London, pp. 232.

Identification of photographic processes: photography lab FBAUP

Bastos, Ana Rita Simões

Faculty of Fine Arts, University of Porto, Portugal.

Is possible to create images without the use of cameras, but these, as we know, play an important role, and judging by the existing formats, the multiplication of results is highly pronounced. The add to registry there is the factor of sensitization of the photographic paper: an equally extensive procedure. Was this multiplicity of photographic processes has gained prominence over the internship in the lab. Thus, we became interested in doing a survey of possible photographic processes to achieve in the lab, since it implied explore all the possibilities.

The relevance of the theme chosen is due to the fact that we observed a certain unawareness of students on the possibilities and potentialities of the photography lab. Moreover, we believe in the importance of existing a document that identifies all processes.

Thus, the main objective of this research was to bridge the flaw identified during the internship in the lab, admitting that this publication will serve to identify potential work processes to develop the institution.

In order to achieve our goal, we did a survey of all photographic processes and a brief description about each of them. To facilitate the understanding of speech in question we produce at least an image, matching to each process. This study resulted in a small publication with a reflection on each of the developed photographic techniques, using the illustration through photographic images.

The type of methodology presented here aims at developing a original publication on the integrated application of knowledge and skills acquired over the four years of the Degree in Communication Design FBAUP — these skills that have been developed and enhanced over the internship lab — and theoretical information gained during the readings.

Acknowledgements:

Designer Joana Bravo;

Master João Lima;

Professor PhD José Carneiro.

Assessment of the antioxidant properties of blueberry pomace as potential food preservative

A.F. Caldas¹, H.H. Tournour^{1,2}, L.M. Cunha¹, M.A. Segundo³, L.M. Magalhães³

¹ REQUIMTE, DGAOT, Faculty of Sciences, University of Porto, Portugal.

² Faculty of Food Science and Nutrition, University of Porto, Portugal.

³ REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Blueberry juice processing by-products (pomace) are a rich source of polyphenolic compounds, which have demonstrated enhanced antioxidant properties preventing the oxidative damage of food components and/or living organisms [1]. The extraction of these bioactive compounds from blueberry residues could represent a profitable source of food antioxidants replacing the conventional synthetic compounds (e.g. butylated hydroxytoluene and butylated hydroxyanisole). Therefore, the antioxidant compounds obtained from blueberry pomace could be an important component for food and nutraceutical industries.

In the present work, blueberry pomace obtained from *Vaccinium corymbosum* species harvested in Monção (north of Portugal) in July 2013 was used as model sample. The blueberry pomace was dried in oven at 55 °C during one week, grounded and sieved (0.7 to 1.4 mm) before storage at -18 °C. The extraction conditions namely, the solvent (water and ethanol/water mixtures (80/20, 50/50 and 20/80, v/v)), pH (2.0, 5.3, 9.0) and extraction time were studied aiming to attain the maximum extraction yield of phenolic compounds. For this, the microplate Folin-Ciocalteu procedure was used to determine the total amount of phenolic compounds attained in each extraction condition [2]. The maximum value for total phenolic extraction was obtained with ethanol/water (50/50, v/v) at pH 2.0 as solvent extractor and an extraction time of 90 min, providing 20.3 ± 0.3 mg gallic acid /g of dried blueberry pomace. These extraction conditions were further applied to extract larger quantities of blueberry pomace in order to obtain the residue rich in phenolic compounds for application as food preservative. Current work is dedicated to the assessment of the antioxidant properties of this residue by several analytical methods (ABTS^{•+}, DPPH[•] and ORAC assays) and to determine its ability to prevent oxidation of a protein food model (peptone).

Acknowledgements:

The authors thank financial support Erasmus Mundus Action 2 Strand 1 Lot 16 Argentina Program - Project 2 Eurotango (PhD Scholarship Hernán H. Tournour EUROTANGO 2). L.M. Magalhães thanks FSE and MCTES for the financial support through the POPH-QREN program. We also acknowledge FCT for the Strategic Project PEst-C/EQB/LA0006/2011.

References:

- [1] Li, C., Feng, J., Huang, W-Y., An, X-T. (2012), *Composition of Polyphenols and Antioxidant Activity of Rabbiteye Blueberry (Vaccinium ashei) in Nanjing*, Journal of Agricultural and Food Chemistry, 61 (3), 523-531.
- [2] Magalhães, L.M., Santos, F., Segundo, M.A., Reis, S. and Lima, J.L.F.C. (2010), *Rapid microplate high-throughput methodology for assessment of Folin-Ciocalteu reducing capacity*, Talanta, 83 (2), 441-447.

Assessment of children exposure to PM₁₀ and PM_{2.5} levels at an Oporto school

M. Oliveira^{1,2}, V. Barbosa¹, C. Delerue-Matos¹, M. C. Pereira², S. Morais^{1*}

¹ REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal*. sbm@isep.ipp.pt

² LEPAE, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Portugal

World Health Organization indicated that more than two million premature deaths each year can be attributed to the effects of urban outdoor and indoor air pollution [1]. Particulate matter (PM) consists in a mixture of solid or solid/liquid particles suspended in air varying in size, shape, origin and chemical composition [2]. PM with aerodynamic diameter smaller than 10 µm (PM₁₀) are able to reach the respiratory tract below the larynx while particles with less than 2.5 µm (PM_{2.5}) can reach the lower parts of respiratory tract where they deposit in the smaller conducting air ways and gas exchange regions of lungs [3]. PM air pollution has been linked to health effects such as asthma, cardiac diseases and impaired lung function [4]. Children spend the most part of their days at school and represent a vulnerable group because their lung structure and immune system are not fully developed [4].

The present work reports the children exposure to PM₁₀ and PM_{2.5} levels at an Oporto pre-school influenced by intense traffic emissions. PM samples were daily collected inside a common playroom and at the school-yard with 25 to 90 children aging 3 to 5 years old attending at the school.

PM₁₀ and PM_{2.5} concentrations in the school outdoor air ranged from 27.3 to 36.9 µg/m³ and from 11.3 to 36.2 µg/m³, respectively. The pre-school indoor environment presented PM₁₀ and PM_{2.5} levels ranging respectively, from 16.0 to 46.7 µg/m³ and from 4.4 to 26.6 µg/m³. The assessed PM concentrations will be discussed according to the available legislation as well as the possible health impact on schoolchildren.

Acknowledgements:

This work was supported by the *Fundação para a Ciência e a Tecnologia* by the IJUP project PP_IJUP2011 121. M. Oliveira is grateful to FCT for her fellowship SFRH/BD/80113/2011.

References:

- [1] WHO (2006), *Air Quality Guidelines, Global Update 2005*, WHO Regional Office for Europe, Copenhagen, Denmark.
- [2] Breyse, P.N., Diette, G.B., Matsui, E.C., Butz, A.M., Hansel, N.N. and McCormack, M.C. (2010), *Indoor air pollution and asthma in children*, Proc Am Thorac Soc, 7, 102-106.
- [3] Oliveira, M., Slezakova, K., Delerue-Matos, C., Pereira, M.C. and Morais, S. (2013), *Exposure to Polycyclic Aromatic Hydrocarbons and the Associated Health Risks in Schoolchildren: a Review*, in: Bandeira, GC and Meneses, HE (eds) "Handbook of Polycyclic Aromatic Hydrocarbons: Chemistry, Occurrence and Health Issues", Nova Science Publishers, New York, pp. 289-307.
- [4] Salvi, S. (2007), *Health effects of ambient air pollution in children*, Paediatric Respiratory Reviews, 8, 275-280.

Biomechanical study of cervical spine

T. Teixeira¹, L. C. Sousa², R. Natal², M. Parente², J. M. Gonçalves³, R. Freitas³

¹ Faculty of Engineering, University of Porto, Portugal

² Department of Mechanical Engineering and IDMEC - Faculty of Engineering, University of Porto, Portugal

³ Department of spine surgery, Orthopaedics Service - Centro Hospitalar de Vila Nova de Gaia, Vila Nova de Gaia, Portugal

The cervical spine is one of the more complex structures of the human skeleton. The knowledge of the spinal kinematics is important in order to understand the load transfer through the intervertebral disc, the ligaments and the joints. This knowledge facilitates diagnosis and treatment of instability, development and evaluation of spinal implants and surgery planning. The finite element method is an excellent tool for studying the biomechanical behavior of cervical spine as it allows spine motion analysis in order to plan surgical interventions and predict its outcomes[1].

The goal of this study was to provide a 3D finite element mechanical model of the human C5-C6 cervical segment. The choice of this functional unit was due to the fact that these vertebrae are still little studied and because they are the most vulnerable to injury [2].

A finite element model of human C5-C6 cervical segment was established based on CT scanning data. The finite element mesh was created using the commercial finite element package ABAQUS. After building the vertebrae model, the intervertebral disc was designed with its components, such as nucleus pulposus, annulus fibrosus and fibers which were assumed to sustain tensile force only.

This study was performed to investigate responses of the C5-C6 segment to static loads, such as axial compression, flexion, extension, lateral bending and torsion. Although the finite element model developed for this study is not fully complete, proved to be a very useful tool for understanding the biomechanical behaviour of the cervical spine.

Acknowledgments

The authors gratefully acknowledge the collaborative work of the medical team from Centro Hospitalar de Vila Nova de Gaia.

References:

- [1] Wheeldon, J.A., Pintar, F.A., Knowles, S. and Yoganandan, N. (2006), *Experimental flexion/extension data corridors for validation of finite element models of the young, normal cervical spine*, Journal of biomechanics, 39 (2), 375-380.
- [2] Galbusera, F., Fantigrossi, A., Raimondi, M.T., Sassi, M., Fornari, M. and Assietti, R. (2006), *Biomechanics of the C5-C6 spinal unit before and after placement of a disc prosthesis*, 253-261.

Quality control of geotextiles in waste landfills

F.D. Carvalho, A.J. Simão, D.M. Oliveira, J.R. Carneiro and M.L. Lopes

Department of Civil Engineering, Faculty of Engineering, University of Porto, Portugal.

Geosynthetics are polymeric materials used in the construction of many infrastructures, such as waste landfills, roads, railways, tunnels, dikes or reservoirs. These relatively new materials offer many advantages when compared to more traditional ones: easy of use and application, low cost, environmental-friendly and good integration with the landscape. Geotextiles (one of the main groups of geosynthetics) are permeable fabrics, typically made from polypropylene or polyester, which have the ability to filter, drain, separate, protect or reinforce.

The geotextiles play a number of important roles in landfill applications: protection of the geomembranes (fluid barrier) from getting damaged, filtration layer to prevent clay or soil intrusion or permeable separation layer. For performing correctly their functions, the materials must have minimum values of certain properties (values present in the construction project).

In this work, we describe the quality control process for the selection of a geotextile for application in a waste landfill. In our case study, we evaluated if the geotextile could, or not, be used in a specific waste landfill. For that, laboratorial tests were carried out in order to determine some relevant properties of the material, such as: thickness, mass per unit area, tensile strength, tearing strength or static puncture resistance. At the end, we compared the values obtained in laboratory with the minimum values required for the landfill.

Results showed that almost all properties of the geotextile complied with those required in the construction project. However, thickness was slightly below. Based on this, our final recommendation would be to reject the material.

Quality control of geomembranes in waste landfills

J. Barbosa, J. Loureiro, M. Sousa, J.R. Carneiro and M.L. Lopes

Department of Civil Engineering, Faculty of Engineering, University of Porto, Portugal.

Geosynthetics are polymeric materials used in many civil engineering applications, such as waste landfills. The use of geosynthetics, instead of more traditional materials, has technical, economical and environmental benefits. In a simple way, these materials can be divided into geotextiles, geomembranes and related products (like geogrids, geonets or geocomposites). The functions of geosynthetics are many and include: reinforcement, protection, separation, fluid barrier, filtration or drainage. The fluid barrier function is exclusive for geomembranes and geosynthetic clay liners.

The geomembranes (very low permeability barriers) have a key role in waste landfills, being responsible for the containment of high pollutant products. These materials must have high quality to perform correctly, and during a long period of time, their functions.

This work presents the quality control needed during the selection and application of a geomembrane in a waste landfill. In our case study, we evaluated (1) if a geomembrane fulfilled, or not, the requirements for being applied and (2) if the geomembrane seams (made by thermo-fusion) were performed correctly.

Laboratorial tests were carried out to determine if the proposed geomembrane fulfilled the construction project requirements. These tests included determination of thickness, tensile strength, deformation, tearing strength and puncture resistance. The efficiency of the seams was also evaluated in laboratory (by destructive tests).

The geomembrane properties were in accordance with those required by the project and, based on that, the material was accepted. The quality of the geomembrane seams was also good.

Parenting and substance abuse: a comparative investigation in parents with alcohol, heroin and cocaine abuse.

J. Silva¹, O. Cruz²

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

² Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

The goal of this exploratory investigation is to analyze the disciplinary behaviors, the parental attitudes and the risk factors associated with drug consumption in substance-abusing parents. Assuming that each substance produces different effects in the subject that consumes it, we intend to analyze the differences in parenting practices, according with the substance of election.

Participants were 59 fathers and 27 mothers, 31 being heroin users, 31 cocaine users and 24 alcohol abusers. Participants' age ranges from 30 to 60 years. The disciplinary behaviors were collected with the Escala de Comportamentos Disciplinares Parentais – ECDP (Parenting Disciplinary Behaviors Scale [1]), the child-rearing attitudes were assessed with the Portuguese version of the Adult-Adolescent Parenting Inventory – AAPI-2 A Form [2], and the risk factors associated with substance abuse were assessed with the Portuguese version of the Addiction Severity Index – ASI-X [3], the short version of this instrument being used for research purposes.

Concerning the disciplinary behaviors, the results show that alcoholic parents rely more often in physical punishment, while substance abuse parents rely more often in inductive behavior. In what concerns child-rearing attitudes, alcoholic parents exhibit more inappropriate expectations, role reversal and oppression of child's autonomy than parents with substance abuse. Regarding the risk factors associated with substance abuse, alcoholic parents report more family problems, while heroin users exhibit more medical and psychological issues and cocaine users show a higher risk of involvement in criminal and illegal activities.

The results of this study point out to differences in parenting according to different kinds of substance use. Alcoholic parents appear to be more punitive in their disciplinary performance, while parents with substance abuse seem to incur in higher risk factors associated with negligence.

Keywords: parenting, substance abuse, disciplinary behavior, child-rearing attitudes, risk factors associated with substance abuse.

References:

- [1] Cruz, O., Oliveira, A., & Ribeiro, C. (2011). Questionário de comportamentos disciplinares parentais.
- [2] Bavolek, S., & Keene, R. (1999). The Adult-Adolescent Parenting Inventory (AAPI-2). Family Development Resources.
- [3] Brown, E., Friedman, A., & Frank, A. (1997). Supplementary administration manual for the Addiction Severity Index (ASI) instrument. US: Center for substance abuse prevention.

Impact of Sexual Orientation on Parental Justification and Social Support

A. Mesquita¹, A. Santos¹, C. Casimiro¹, L. Gonçalves¹, P. Lourosa¹ and V. Ramos¹

¹ Faculty of Psychology and Educational Sciences, University of Porto, Portugal.

Lesbian and gay parenting is a topic that has just recently merited the attention of Portuguese researchers. No major significant differences between non-heterosexual parents and heterosexual parents with respect to their educational skills and the psychosocial development of their children were found ^[1]. Nevertheless, can prejudice towards lesbian and gay parent families influence the way non-heterosexuals parent their children? ^[2]. This study aims to investigate the degree to which non-heterosexual parents feel pressured to justify the quality of their parenting (*parental justification*). Furthermore, it explores the frequency of *social support* (from family and friends) that non-heterosexual parents receive, comparatively to their heterosexual counterparts. The sample was composed by 67 participants, with ages ranging from 24 to 52 years old; 65.7% were partnered; the heterosexual sample comes from blended families. Data was collected through an online questionnaire (non-heterosexual sample) and by administering questionnaires face to face (heterosexual sample). The *Parental Justification Scale* ^[3] was used to measure the construct of parental justification ($\alpha = .88$), and the *Social Support Scale* (Gato, 2012) to measure the construct of social support ($\alpha = .84$).

Non-heterosexual (NH) parents ($M = 2.67$, $SD = 1.42$) didn't differ significantly from heterosexual (H) ones ($M = 3.07$, $SD = 1.26$) in the need for parental justification ($t_{59} = 1.11$, $p = .274$). Taking into consideration that heterosexual parents integrate blended families, that can be considered a possible indicator of their need to ensure themselves and others that they are good parents, after the rupture in the familiar structure. Both groups (H: $M = 3.01$, $SD = 0.76$; NH: $M = 3.25$, $SD = 0.98$) also didn't differ in the perception of social support ($t_{59} = 1.07$, $p = .288$), probably due to the economic capacity of both samples, which can evocate a lesser demand for support. Furthermore, non-heterosexual ($M = 3.22$; $SD = 1.22$) didn't differ significantly from heterosexual ones ($M = 3.30$, $SD = 0.82$) in the support received from their families ($t_{25.68} = .25$, $p = .807$), which is a possible indicator that families may feel more open and available, even in the presence of non-normative sexual orientations of their relatives. Although this study has some limitations (convenience sample and selectivity of the educational level), it contributes to a growing awareness of the need to recognize that the various facets of parenting are more distinguished by established relationships with their children than by supposed characteristics inherent to the sexual orientation of parents.

References:

- [1] Gato, J. and Fontaine, A. (2011), *Impacto da orientação sexual e do género na parentalidade: Revisão dos estudos empíricos com famílias homoparentais*, *Ex aequo*, 23, 83-96.
- [2] Bos, H.M.W, van Balen, F., and van den Boom, D.C. (2007), *Child Adjustment and Parenting in Planned Lesbian-Parent Families*, *American Journal of Orthopsychiatry*, 77 (1), 38-48.

Clinical Training: Effect on Empathic Communication Skills Acquired in the Second Year. Relationship with Emotion Recognition Ability and Alexithymia

G. Vasconcelos¹, B. Melo¹ and I. Castro-Vale¹

¹ Medical Psychology Unit, Department of Clinical Neurosciences and Mental Health, Faculty of Medicine, University of Porto, Portugal

Empathy is a key element in doctor-patient relationship, improving clinical outcomes. Furthermore, there is evidence of a positive relationship between empathy and ability to recognize facial expression of emotions, important in nonverbal communication.[1] Given its importance, improving empathy should be a goal, which is possible through many strategies.[2] This subject is vital because most studies show a decline of empathy along medical education.[3] Alexithymia has been reported as another factor negatively associated with empathic capacity.[4] The main objective of this study is to analyze the influence of clinical training in empathic abilities of medical students, by comparing differences between students of the second and sixth year. It is intended to characterize the students in their levels of empathy, ability to recognize facial expression of basic emotions and alexithymia, as well as the associations between these variables.

A cross-sectional study was designed, with data collection being taken at the end of second and sixth year, at academic year 2012/2013. Medical students were invited to complete self-administered questionnaires: socio-demographic data; Emotion Recognition Ability test, based on JACFEE (ERA–JACFEE); the empathy scale Interpersonal Reactivity Index (IRI); and the Toronto Alexithymia Scale (TAS- 20).

A total of 189 students completed the questionnaires: 32% males, 56% 2nd year. The mean age (SD) of 2nd and 6th year students was 20 (1) yo and 24 (1) yo, respectively. In our sample, 6th year students scored higher in all IRI subscales than 2nd year students, with significant differences for empathic concern IRI subscale ($p=0.049$). As for ERA–JACFEE test, no significant differences were found. Empathic concern scores were also significantly higher in women than men ($p=0.036$). Concerning the perspective taking IRI subscale, significantly higher scores were found for non-alexithymic students, when comparing to the ones with alexithymia ($p=0.004$).

The results suggest that empathy does not decrease along clinical training, but a longitudinal study is needed. These findings are opposite to most studies, but consistent with studies carried out in Portugal. Facial expression of emotions recognition and alexithymia also deserve further exploration.

References:

- [1] Ekman, P. (1992), *An argument for basic emotions*, Cognition & Emotion, 6(3-4), 169-200.
- [2] Batt-Rawden, S. A., Chisolm, M. S., Anton, B. and Flickinger, T. E. (2013), *Teaching empathy to medical students: an updated, systematic review*, Acad Med, 88(8), 1171-7.
- [3] Neumann, M., Edelhauser, F., Tauschel, D., Fischer, M. R., Wirtz, M., Woopen, C., Haramati, A. and Scheffer, C. (2011), *Empathy decline and its reasons: a systematic review of studies with medical students and residents*, Acad Med, 86(8), 996-1009.
- [4] Swart, M., Kortekaas, R. and Aleman, A. (2009), *Dealing with feelings: characterization of trait alexithymia on emotion regulation strategies and cognitive-emotional processing*, PLoS One, 4(6), e5751.

Teaching Communication Skills to medical students: association between Empathy, Emotion Recognition Ability and Alexithymia.

B. Melo¹, G. Vasconcelos¹, I. Castro-Vale¹

¹ Medical Psychology Unit, Department of Clinical Neurosciences and Mental Health, Faculty of Medicine, University of Porto, Portugal.

Empathy is, in the clinical context, the appreciation of patient's feelings and the communication of this understanding.[1] It is known that physician's empathy promotes the quality of health care.[2] Several studies have demonstrated that empathic skills can be taught to medical students.[1] Furthermore, the ability to recognise facial expression of emotions facilitates empathy.[3] Alexithymia is expected to influence negatively empathy levels.[4] This work aims to study the evolution of empathy by teaching communication skills, and its association with the ability to recognise facial expression of basic emotions and alexithymia.

The subjects of this study were 2nd year medical students randomly selected from the 17 classes of Faculty of Medicine, University of Porto, attending 2nd semester's Medical Psychology course, where they learn special communication skills. They filled self-administered questionnaires: socio demographic data and Toronto Alexythimia Scale (TAS-20) at the beginning of the 2nd semester, and Interpersonal Reactivity Index (IRI), and the Emotion Recognition Ability test, (ERA-JACFEE) at the beginning of the 2nd semester and, once again, at the end.

65 students [mean age (SD): 20 (3)] were included, 35% male. All 4 dimensions of IRI were higher in women at baseline, being empathic concern the only presenting significant differences ($p=0.017$). Students with mean classifications below 13.5/20 had significantly lower personal distress scores at the end of the course compared to baseline ($p=0.008$). Concerning the ERA-JACFEE test, we found an increase in the ability to recognise all emotions but surprise and happiness, being disgust and sadness the only achieving significant differences ($p=0.028$ and 0.049 respectively). A post-HOC Bonferroni correction showed a significant lower score for the fantasy domain of IRI in males with alexithymia when comparing to those without alexithymia ($p=0.032$).

The preliminary results of our study suggest that training can improve the ability to recognize facial expressions of basic emotions as well as the emotive empathy dimension (personal distress) in a subset of students.

[1] Stepien, K.A. and A. Baernstein (2006) Educating for empathy. A review. *J Gen Intern Med*,21(5): p.524-30.

[2] Kim, S.S., S. Kaplowitz, and M.V. Johnston (2004)The effects of physician empathy on patient satisfaction and compliance. *Eval Health Prof*, 27(3): p. 237-51.

[3]Regenbogen, C., et al.(2012), The differential contribution of facial expressions, prosody, and speech content to empathy. *Cogn Emot*. 26(6): p. 995-1014.

[4]Swart, M., R. Kortekaas, and A. Aleman (2009) Dealing with feelings: characterization of trait alexithymia on emotion regulation strategies and cognitive-emotional processing. *PLoS One*,4(6): p. e5751.

The life experience of parenting in adoption

A. Costa¹ and M. Barbosa-Ducharne².

¹ Department of Psychology, Faculty of Psychology and Education Sciences, University of Porto, Portugal.

² Department of Psychology, Faculty of Psychology and Education Sciences, University of Porto, Portugal.

Families start a new beginning in their life cycle when they become parents. Parenthood might be similar in different types of families. However, different current family structures imply specifics of parenthood related, in particular, with the way that the parental system emerges in the family. Adoption provides families who could not have biological children the possibility of realizing their wish of parenting and constituting a family. Therefore, it is essential to understand how the transition to parenting occurs in families who adopted a child and how they exercise their adoptive parenting.

The present dissertation presents an empirical study whose object is to explore the relationship between a specific process of adoptive families - the life experience of adoption - and the exercise of parenting which, in its basic components, is common to all types of families.

125 adoptive parents with 125 adopted children participated in the research. Two assessment instruments were used; an interview - EPA Portuguesa - which resulted from the process variables and the life experience of adoption and an enquiry - QPP - to evaluate the ideas, behaviors and parental affection.

The results show that the participant families present a greater positive than negative emotionality and used more inductive discipline strategies than punitive ones. In families who say they are happy, the result of adoption was very positive and they are very pleased with the family that was formed by adoption. Differences at the level of life experiences of adoption and parenthood in function of characteristics of the child and parents were observed.

This study shows how the adoption process is experienced by families and how the way that parents exercise parenthood is impacted, as well as, how the exercised parenthood influences the life experience of adoption. The results of this study have implications for the adoption practice.

Adoption of older children: Waiting period, transition, adaptation and integration. Exploratory Study

M. Cruz¹, and M. Barbosa-Ducharne².

¹ Department of Psychology, Faculty of Psychology and Education Sciences, University of Porto, Portugal.

² Department of Psychology, Faculty of Psychology and Education Sciences, University of Porto, Portugal.

Adoptive families have specific characteristics and tasks involving both the adoptive parents and the adopted children. These characteristics and tasks related to the period after adoption (decision, waiting and transition to parenthood/affiliation) and also with the pos-adoption period (integration, adaptation, communication about adoption). The adoption of older children has many different and particular characteristics and requires additional challenges. The aim of this study is to explore, with families who have adopted a six or more year-old child, how the adoption process took place and how was the integration of the child in the family both the parent's perspective and the child's perspective (and the comparison between them) in order to come up with new clues to the adoption practice in Portugal.

This study involved 125 parents and 58 adopted children, aged 5 to 15 years old. Data were collected through the Adoption Process Interview – Portuguese version and the Strengths and Difficulties Questionnaire, among parents and through the ECAA - Interview for Children and Adolescents on Adoption, conducted with the children.

In this study, the parents who adopted aged 0 to 6 children had percept more challenges/difficulties along the adoption process such as greater rejection by other family members, a longer integration period for the child and a lower satisfaction with the adoption. However, at the present moment, they consider that the child is well adapted and they express satisfaction concerning the adoption process, the child and his/her family. Also children, despite having some doubts and fears along the way, seem to find themselves integrated and accepted by the adoptive family.

These data raise important clues for intervention in adoption. On one hand, they reveal that the challenges of adopting an older child can be overcome and that child's preparation to adoption can be an asset. Also prospective adoptive parents should be aware of the implications of adopting an older child, but also they should be given access to empirical data that prove the integration and adjustment ability of older children, as well as the satisfaction that the adoption of these children can bring to the family.

OLFACTORY MEMORY: PERFORMANCE IN OLFACTORY IDENTIFICATION, SERIAL EVOCATION AND OLFACTORY RECOGNITION TASKS

J. Sousa & A. Pinto

Faculty of Psychology and Educational Sciences, University of Porto, Portugal

In our everyday life, we are surrounded by a wide range of odours, on which we input different attributes, emotions and levels of significance, being, some of them, more familiar to our olfaction and that makes us remember, in this way, ancient memories related to them. According to investigations accomplished [1-2] olfactory memories are more ancient and completely different from visual and verbal memories, as the feeling of going back in time is more intense when associated to olfactory memories to a determined happening which makes us remember something from the past. Olfactory tasks such as discrimination and identification of odours comprise a more complex olfactory information process, so there is a significant influence of the cognition in these tasks, which is not verified in sensitivity tasks [3]. Through odours we can evaluate the performance in olfactory tasks, simple or complex, being possible, through these same tasks, to evaluate the memory for odours.

Considering the results found in this research area, one study was designed, whose main goal was to verify what was the age influence over the performance of tasks such as olfactory identification, serial evocation and the olfactory episodic memory. It was carried out with two groups of different ages, with 20 individuals each, one of youths with ages between 18 and 35, and the other of middle-aged adults with ages between 40 and 57 years old. The results obtained indicated a tendency to a decrease in the performance with the age increase, although no statistical significance was found in the olfactory identification task. In the serial evocation task, was verified a significant difference in the participant's performance with the age increasing and with the difficult increase of the task too. In relation to the olfactory episodic memory, was demonstrated a difference connected with the aroma, the participant's age and the life time the aroma was associated.

The results obtained were discussed and analysed within this research area, concerning memory and olfactory tasks.

References:

- [1] Willander, J., & Larsson, M. (2006). Smell your way back to childhood: Autobiographical odor memory. *Psychonomic Bulletin & Review*, 13, 240-244.
- [2] Willander, J., & Larsson, M. (2007). Olfaction and emotion: The case of autobiographical memory. *Memory & Cognition*, 35, 1659-1663.
- [3] Hedner, M., Larsson, M., Arnold, N., Zucco, G., & Hummel, T. (2010). Cognitive factors in odor detection, odor discrimination and odor identification tasks. *Journal of Clinical and Experimental Neuropsychology*, 32, 1062-1067.

The Associative Movement of Parents Educational Dimensions of Participation in Associations (O Movimento Associativo de Pais Dimensões Educativas da Participação nas Associações)

I. Oliveira¹, T. Medina²

¹ Master in Educational Sciences

² Associate professor of Faculty of Psychology and Educational Sciences of University of Porto, Portugal.

This work is based on the fulfillment of nine semi-structured interviews to directors and former directors of Parents' Associations in the district of Porto, Portugal. This research work aimed to contribute to the reflection about the Parents' Associations and what is their role in the relationship between other parents and the school as well as for the understanding of educational aspects of Parents' Associations, as places of formal/non-formal educative contexts and informal enhancers of multiple learnings.

Parents' associations mobilize, today, thousands of parents and education authorities, by having a regular and daily intervention in the life of a significant number of schools in the Portugal country, constituting a movement whose social importance is rising and can't be concealed.

Accordingly, considering education as a process that occurs throughout all life, in all contexts and spaces, it is also important to understand how the parents' association leaders recognize the formative/educative sense of their participation and intervention.

The association leaders, interviewed as part of this research, when talking about their life experiences in the parents' association, acknowledged the importance of their role in the school, in building of a more democratic society and of the importance of parents' associations as contexts of participation, political, and social intervention, which shows an active citizenship that enhances his members in learning and improving ways to influence the society. Recognizing the parents' associations as educative/formative spaces, these leaders identify different types of learning, such as learn how to speak in public and discuss the problems of education, to connect with other parents, to drive meetings, writing documents, and so on. At the same time, they consider the parents' association movements as a school them self, different from traditional school, a "life" school, so understood by the diversity and multiplicity of situations experienced and by the knowledge acquired, a school of "human relations" and solidarity in the right path for individual values development and for the individual exercise of participatory citizenship.

Valorisation of Saffron by-products

M. Rebelo¹, A.S.G. Costa¹, M.B.P.P Oliveira¹

¹ REQUIMTE, Faculty of Pharmacy, University of Porto, Portugal.

Saffron is the dried stigmas of *Crocus sativus L.* and it is the most expensive spice in the world (1g of spice requires 165 flowers by manual work). Nowadays, the main producers are Iran, Spain, Italy and France but Portugal may be also a potential producer due to its climatic conditions [1].

Saffron has several properties such as antioxidant capacity, tumor inhibition, antiseptic activity and immunomodulatory potential [2] but few studies were conducted in by-products of *Crocus sativus L.* like petals of its flowers.

The main objective of this work is to study the by-product (without value) of the saffron production with special attention to the flowers.

Several extraction procedures were developed: different solvents were used, at two different temperatures, during 1 hour to 3 hours in order to obtain the same extraction rate in the different conditions [3]. The described conditions were tested with different amounts of sample and solvent.

The extracts were tested for total phenolics as a comparison model between all extracts, revealing that the best extraction method were: water, 1 hour at room temperature, with only 0.25 g of sample.

Therefore, it can be concluded that the extracts have more affinity with water and the saffron petals showed a significant antioxidant power. This preliminary study allows to obtain a promising sustainable extract with different applications. The study is on-going.

References:

- [1] Srivastava, R., Ahmed, H., Dixit, R.K., Dharamveer, Saraf, S. (2010), *Crocus sativus L.: A comprehensive review*, Pharmacognosy reviews; 4(8):200–8.
- [2] Hasan, I., Ansari, A. H., Sherwani, A.M.K., Zulkifle, M. (2011) *The incredible health benefits of saffron : A review*, Journal of Pharmacy Research; 4(7), 2156–2158
- [3] Costa, A.S.G., Alves, R.C., Vinha, A., Barreira, S.V.P., Nunes, M.A., Cunha, L.M., Oliveira, M.B.P.P. (2014), *Optimization of antioxidants extraction from coffee silverskin, a roasting by-product, having in view a sustainable process*. Industrial Crops and Products. *In Press*

Acknowledgments: This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

Carotenoids content of cherry tomato fruits: A comparative study

A.B. Aires¹, M. Arantes¹, A.S.G. Costa¹, R.C. Alves^{1,2}, A.F. Vinha^{1,3}, M.B.P.P.Oliveira¹

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

³ Faculty of Health Sciences, University Fernando Pessoa, Portugal.

Consumption of cherry tomato fruits has been increasing due to the rising public demand for convenience and awareness of fruit's health benefits. Carotenoids, natural pigments synthesized by plants and microorganisms but not by animals, contribute to the different colours of tomato fruits and are recognized as playing an important role in several biological functions [1].

In this work, we studied some carotenoid contents (chlorophyll a, chlorophyll b, lycopene and β -carotene) of five cherry tomato cultivars (*red cherry chucha*, *red cherry*, *striped cherry*, *yellow cherry*, and *red cherry cacho*). Measurements were performed spectrophotometrically according to Vinha *et al.* [2].

Quantitative chemical differences were observed among all cherry tomato cultivars ($p < 0.05$). Higher lycopene amounts were found in *red cherry* tomatoes, whereas β -carotene presented the highest value in *yellow cherry* tomatoes. Chlorophyll a and chlorophyll b contents were inversely correlated with lycopene and β -carotene levels. *Striped cherry* cultivar was the only one that presented similar values of chlorophylls, lycopene and β -carotene contents.

In conclusion, these preliminary results showed that tomato carotenoids levels depends mostly on the cultivars.

References:

[1] Rao, A.V. and Rao, L.G. (2007), *Carotenoids and human health*, Pharmacological Research, 55, 207-216.

[2] Vinha, A.F., Alves, R.C., Barreira, S.V.P., Castro, A., Costa, A.S.G., Oliveira, M.B.P.P. (2014), *Effect of peel and seed removal on the nutritional value and antioxidant activity of tomato (Lycopersicon esculentum L.) fruits*. LWT – Food Science and Technology, 55(1), 197-202.

Acknowledgments: R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

Antimicrobial activity of synthetic and natural compounds

C. Soares¹, M. Maia¹, M.J.R.P. Queiroz², J.C. Quintela¹, A.P. Almeida¹, E. Pinto^{1,3}

¹ CEQUIMED, Faculdade de Farmácia, Universidade do Porto, Portugal.

² Centro de Química, Universidade do Minho, Braga, Portugal.

³ Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Portugal.

Opportunistic bacterial and fungal infections remain major complications in immunocompromised hosts. Concerning bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, as well as species of *Enterococcus* and *Klebsiella* are the most commonly involved [1]. In recent years, serious fungal infections have increased significantly, and they are responsible for high mortality and morbidity in hospitals. The epidemiology of fungal infections has changed and among the pathogenic species to humans, *Candida*, *Aspergillus* and dermatophyte species deserve our attention. The continued emergence of resistant bacteria is a public health concern. On the other hand, antifungal drugs often exhibit limitations with fungistatic action, high toxicity, many drug interactions, insufficient bioavailability and development of resistance or innate resistance by emerging species [2]. Thus, it is imperative to continue searching for new antimicrobial drugs.

The antimicrobial activity of seven compounds obtained by chemical synthesis and six products from *Cecropia catarinensis* (three plant extracts and three isolated compounds) were evaluated against clinically relevant bacteria (*S. aureus*, MRSA, *E. coli* and *P. aeruginosa*) and fungi (*C. albicans*, *A. fumigatus* and dermatophyte species), according to the reference method approved by CLSI (M7-A7, M27-A3 and M38-A2).

Antifungal activity was observed in four synthetic tested compounds. However, no activity was observed against *C. albicans* and *A. fumigatus* for the higher concentration tested. Dermatophytes showed a variable susceptibility according to the species, being *Epidermophyton floccosum* the most susceptible. Furthermore, the active compounds were fungicidal. Concerning bacteria, none of compounds were active against Gram negative bacteria (*E. coli* and *P. aeruginosa*). Only one compound showed a bacteriostatic activity against Gram positive *S. aureus* (ATCC and MRSA strain).

Concerning natural products, crude methanolic extract and the two semi-purified fractions did not revealed antimicrobial activity. However, one of the isolated compounds, ursolic acid, showed antibacterial activity against Gram positive bacteria, including *S. aureus* MRSA. The selectivity against Gram positive bacteria were confirmed against *Enterococcus faecalis* and *Bacillus cereus*.

References:

[1] Majumdar, S. S., Padiglione, A. A. (2012), *Nosocomial Infections in the Intensive Care Unit*, *Anaesthesia and Intensive Care Medicine*, 13, 204-208.

[2] Pfaller, M. A. (2012), *Antifungal Drug Resistance: Mechanism, Epidemiology and Consequences for Treatment*, *The American Journal of Medicine*, 125, 3-13.

Acknowledgments: This work is funded through national funds from FCT-Fundação para a Ciência e Tecnologia under the project CEQUIMED-Pest-OE/SAU/UI4040/2011

Anti-angiogenic properties of a palladium derivative compound, the Pd2Spm

F. Costa¹, A.L.M. Batista-de-Carvalho², M.P.M. Marques^{2,3}, and C. Diniz¹

¹REQUIMTE/FARMA, Faculty of Pharmacy, Department of Drug Science, Laboratory of Pharmacology, University of Porto;

²Molecular Physical-Chemistry R&D Unit, Faculty of Sciences and Technology of University of Coimbra;

³Department of Sciences of Life, Faculty of Sciences and Technology of University of Coimbra.

Tumor growth is largely dependent on angiogenesis. The emergence and growth of blood capillaries (from pre-existing vasculature) that will allow adequate blood supply to the tumor and facilitate their growth involves a complex series of interplays between different cell types and reciprocal interactions of vessels with the extracellular matrix and tissue that needs additional vasculature. In healthy tissues, it is associated to homeostatic pro- and anti-angiogenic factors, while in cancer an imbalance of these factors occurs with a prevalence of the former. Inhibitors of angiogenesis can be used to control tumor growth, rendering the development of anti-angiogenic compounds a promising area for cancer treatment [1]. This work aimed at studying the effect of an anti-angiogenic compound (Pd2Spm) developed the University of Coimbra [2,3].

Fertilized chicken embryos allow an easy access to a very vascularized tissue (the chorioallantoic membrane: CAM). Inoculation of the drugs under study (Pd2Spm and doxorubicin: DOX; PBS as a control) was carried out in the CAM of thirteen-fertilized embryos. After 48 h, CAM samples were taken and viewed under a microscope, the respective digital images being acquired for later analysis (using a dedicated software). Results (mean±s.e.m) are expressed as a percentage of the control (PBS); p<0.05 was taken to reflect statistically significant differences (ANOVA followed by Holm-Sidak).

The gathered results (for n=3) evidenced that Pd2Spm (40 µM) was effective in reducing both the length and branches of the CAM vessels, an effect comparable to that obtained with DOX (100 µM): the total master segments length was: 35±4 and 32±5% respectively for Pd2Spm and DOX; the number of branches was equal to 73±14 and 64±9% for Pd2Spm and DOX; the total length was 46±4 and 43±1% for Pd2Spm and DOX; total branches length was 45±5 and 41±1% for Pd2Spm and DOX.

In conclusion, Pd2Spm, a newly palladium derivative, revealed good properties as an anti-angiogenic agent which further envisage its putative use as an anticancer agent.

Work supported by FCT (FEDER funding and COMPETE, projects Pest-OE/SAU/UI215/2011 and Pest-OE/QUI/UI0070/2011, PhD grant SFRH/BD/72851/2010)

[1] Kubota, Y. (2012), *Tumor Angiogenesis and Anti-angiogenic Therapy*, Keio J Med; 61 (2): 47–56

[2] Fiuza, S. M. et al. (2011), *Biologic Activity of a Dinuclear Pd(II)–Spermin Complex Toward Human Breast Cancer*, Chem Biol Drug Des, 77: 477–488

[3] Tumala, R. et al. (2010), *Characterization of Pt-, Pd-spermine complexes for their effect on polyamine pathway and cisplatin resistance in A2780 ovarian carcinoma cells*, Oncol Rep, 24(1): 15–24

Development of sustained release matrix tablets of vitamin B1 containing hydrogenated castor oil (Cutina® HR)

B. Ribeiro, J. Couto, M. Sampaio, M. Pereira, P. Cardoso, S. Silva, M. Estanqueiro, J. Conceição, J. M. Sousa Lobo

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Sustained release oral delivery systems are designed to achieve therapeutically effective concentration of drug in the systemic circulation over an extended period of time, thus achieving better patient compliance and allowing a reduction of both the total dose of drug administered and the incidence of adverse effects [1]. Among the different approaches intended for this aim, matrix systems still appear as one of the most employed. These systems can be defined as a system that controls the release of a drug molecularly dispersed in a support resistant to desegregation [2].

The aims of this work were the development of a sustained release tablets formula using hydrogenated castor oil as lipophilic matrix and vitamin B1 (2%, w/w) as a model drug, production of tablets and their technological characterization. Vitamin B1 was used as a model drug because it is easy water soluble and presents a short half-life.

The developed formula was composed by vitamin B1 (2%, w/w), Cutina® HR (36%, w/w) as lipophilic matrix, starch (20%, w/w), cellulose microcristalline (15%, w/w) and dibasic calcium phosphate (15%, w/w) as diluents, magnesium stearate (1.2, w/w) and talc (10.8%, w/w) as lubricants. The formula components were mixed on a Turbula Wab mixer for 15 minutes and directly compressed using a single-punch compression machine Korsch 9048-71 (Germany). Technological characterization, such as weight variation, hardness determination and friability, were carried out [3]. Additionally, *in-vitro* drug release was evaluated in two units in a Sotax model AT 7 dissolution apparatus. The dissolution medium consisted of 500 mL water, maintained at 37°C ± 0.5°C and stirred at 100 rpm. Samples (10 mL) were withdrawn at predetermined time intervals (15, 30, 45, 60 and 90 minutes), without volume reposition. Samples were filtered and assayed by spectrophotometry at 273 nm (isosbestic point of vitamin B1).

The obtained tablets presented an average weight of 527.8 mg and none of the units differed ± 5% of the average weight. The studied tablets presented a hardness average of 85.8 N and, in the friability test, the obtained loss of mass was 0.88% (< 1.0%). After the dissolution test, the maximum cumulative drug released obtained was 64.93%.

The results showed that the developed tablets complied with the requirements stated in the Portuguese Pharmacopoeia and the sustained release rate of vitamin B1 was obtained [3].

References:

- [1] Sánchez-Lafuente, C., Faucci, M.T., Fernández-Arévalo, M., Álvarez-Fuentes, J., Rabasco, A.M. and Mura, P. (2002), Development of sustained release matrix tablets of didanosine containing methacrylic and ethylcellulose polymers. *International Journal of Pharmaceutics*, 234, 213-221.
- [2] Lopes, C.M., Sousa Lobo, J.M. and Costa, P. (2005), Formas farmacêuticas de liberação modificada: polímeros hidrofílicos. *Revista Brasileira de Ciências Farmacêuticas*, 41 (2), 143-154.
- [3] *Farmacopeia Portuguesa 9*: Ministério da Saúde, INFARMED, I.P., 2008.

Immunocytochemical and functional characterization of interstitial cells of Cajal in the rat ileum

A. Alves-Lação¹, M. Duarte-Araújo¹, S. Gonçalves-Monteiro¹, F. Ferreirinha¹, M.A. Costa¹, P. Correia-de-Sá¹

¹Laboratório de Farmacologia e Neurobiologia, UMIB, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, Portugal.

Gastrointestinal motility disorders (GI) can affect nearly 1/3 of the population and are responsible for high morbidity in the long term. The primary players involved in the control of GI motility are enteric neurons, interstitial cells (ICs) and smooth muscle cells [1]. Unpublished data from our group indicate that ICs are endowed with muscarinic M₃ and tachykininergic NK₁ receptors and might regulate transmission between activated neurons and smooth muscle fibers by releasing intermediate messengers, such as purines and NO among others (see e.g. [2]). In addition, these mesenchymal-originated cells are essential for the generation and propagation of rhythmic slow waves in the small intestine through gap junction communication, which may be impaired in pathological conditions (e.g. diabetes) [3]. Unfortunately, unlike well-characterized ICs from mice and guinea-pigs [4], major caveats exist in the investigation of these cells in the rat intestine because these are functionally heterogeneous, ultrastructurally different and exhibit distinct c-Kit (tyrosine kinase receptor Kit) dependency along phenotype development and maintenance [5]. Since histological categorization bears on understanding cell function and IC networks in the rat show inconsistent c-Kit immunolabeling, less specific mesenchymal cells markers have been attempted, like the intermediate filament protein vimentin [6]. Recently, Ca²⁺-activated ionic channels, like Ano1 (a chloride channel) [7] and SK3 (a potassium channel)[8], were indicated as potential IC of Cajal (ICC) markers, whereas platelet-derived growth factor receptor α (PDGFR α) immunolabeling was considered specific for fibroblast-like cells (FLC) [9]. This study was designed to unequivocally identify ICCs in cell cultures from the rat ileum by immunocytochemistry in order to study their role on purinergic neurotransmission in this species.

Cells were obtained after brief digestion of small pieces of the ileum of 5-11 days old Wistar rats with collagenase [10]. Immunofluorescence confocal microscopy evaluation of the cells cultured for 5-15 days, showed that isolated cells consisted in a mixed population; some cells exhibited positive labeling for vimentin (1:150), whereas others were positive to α -smooth muscle actin (SMA-FITC, 1:300). Although both groups of cells exhibited positive immunoreactivity against the SK3 antibody (1:300), labeling was much more intense in SMA-FITC-negative cells. FLC exhibited positive labeling to both SMA-FITC and PDGFR α (1:500). Cultured cells loaded with the Ca²⁺ sensitive fluorescence dye, Fluo-4 NW, responded to ATP (50 μ M) by increasing intracellular Ca²⁺ levels. In contrast to the inconsistent c-Kit immunolabeling, preliminary tests in whole-mount preparations of the longitudinal muscle-myenteric plexus of the rat ileum indicate that the Ano1 (1:100) antibody may be a good marker for rat ICCs, since it clearly identified ganglionic and intramuscular ICC networks.

Although further studies are required to confirm our data (e.g. Ano-1 testing in cultured cells, differentiation of the Ca²⁺ signature among distinct cell types in culture), the results so far obtained demonstrate that immunocytochemical characterization of ICs unable to respond to c-Kit labeling is possible using an array of alternative cell markers, which include specific antibodies and response to known signaling mediators

[1] Zhu et al., 2013, Pflugers Arch., Oct 8. [Epub ahead of print]; [2] Vieira et al., 2009, Neurogastroenterol Motility, 21: 1118-e95; [3] Lammers et al., 2001, Exp Physiol. 96:1039-48; [4] Ward et al., 1994, J Physiol. (London), 480: 91-97; [5] Horiguchi & Komuro, 1998, Cell Tissue Res., 293: 277-84; [6] Mendez et al., 2010, FASEB J., 24: 1838-51; [7] Hwang et al., 2009, J Physiology. (London), 15: 4887-4904; [8] Fujita et al., 2001, Am J Physiol, 281: C1727-33; [9] Grover et al., 2012, Neurogastroenterol Motility, 24: 844-52; [10] Kim et al., 2013, Korean J Physiol Pharmacol., 17: 149-156. *Work supported by FCT (FEDER funding, PEst-OE/SAU/UI0215/2011).*

Expanding the chemical space of antitumor xanthenes: a new route paved by a chromene moiety

D. Marques^{1,2}, J. Neves^{1,2}, R. Vieira^{1,2}, J. Soares^{1,2}, S. Reis³, M. Pinto^{1,2,4}, C. Azevedo¹, C. Afonso^{1,2,4}.

¹ Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP).

² Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. ³ REQUIMTE ⁴ Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal.

Xanthenes hold a dibenzo- γ -pyrone scaffold and have been isolated from a variety of natural sources (higher plants, fungi and lichens) and also obtained by synthesis. Xanthone derivatives are important due to the variety of biological activities they present, particularly antitumor. ^[1] The chromene moiety is a privileged motif present in several antitumor compounds ^[2]. Bearing this in mind, our group has been synthesizing xanthenes with this moiety and a hit compound (XC36) has emerged as a promising antitumor compound ^[3]. In this work, we present a synthetic pathway to obtain a key chromene intermediate (CJ1), which will be used in the synthesis of derivatives of XC36 with potential antitumor activity (Fig. 1). The synthesis of CJ1 involves the protection of a salicylic acid derivative followed by its *O*-dimethylpropargylation and respective cyclization to give the chromene.

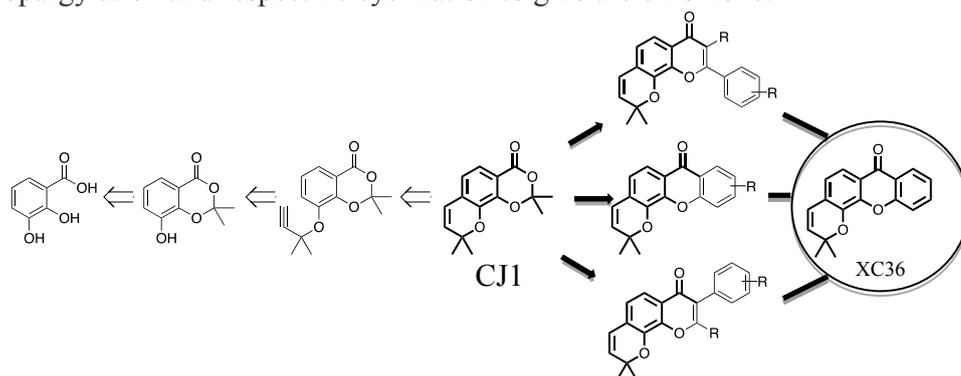


Fig. 1 - The synthetic pathway to CJ1 and the XC36's derivatives

This synthetic process represents a practical route to a versatile building block (CJ1), which will enable the synthesis of diverse molecules in a fast and reliable way, reaching a chemical space that would be otherwise hardly accomplished.

References:

- [1] Pinto, M.M.M., Sousa, M.E. and Nascimento, M.S.J. (2005), *Xanthone derivatives: New insights in biological activities*, Current Medicinal Chemistry, 12 (21), 2517-2538.
- [2] Patil, S. *et al* (2013), *Chromenes: Potential new chemotherapeutic agents for cancer*, Future Medicinal Chemistry, 5 (14), 1647-1660
- [3] Azevedo, C. *et al* (2013), *Pyranoxanthenes: Synthesis, growth inhibitory activity on human tumor cell lines and determination of their lipophilicity in two membrane models*, European Journal of Medicinal Chemistry, 69, 798-816.

Acknowledgments:

FCT – Fundação para a Ciência e a Tecnologia under the project CEQUIMED – PEst-OE/SAU/UI4040/2011, FEDER funds and COMPETE program under the project FCOMP-01-0124-FEDER-011057 and U.Porto/Banco Santander Totta (PP_IJUP2011-58)

New inhibitors of p53-MDM2 interaction with chalcone scaffold

D. Pereira¹, S. Carvalho¹, J. Soares³, S. Cravo^{1,2}, L. Saraiva³, M. Pinto^{1,2}, H. Cidade^{1,2}

¹Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP) e Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

²Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal

³REQUIMTE, Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

Chalcones represent an outstanding class of naturally occurring compounds with interesting biological activities, being the antitumor activity one of the most reported in the literature [1,2]. It is thought that this biological activity can be explained by the interference with different molecular targets, namely by the activation of the tumour suppressor protein p53 [3]. Considering these, and with the objective of obtaining chalcones with potential antitumor activity by interfering with the p53-MDM2 interaction, several chalcones were synthesized by aldol condensation of a 2'-hydroxyacetophenone with properly substituted benzaldehydes *via* microwave assisted organic synthesis (MAOS). Some of these chalcones were used as building blocks to obtain the prenylated derivatives by the substitution reaction in the presence of prenyl bromide. The structures of all synthesized chalcones were determined by NMR techniques (¹H NMR, ¹³C NMR, HSQC and HMBC). The effect of the synthesized chalcones on the p53-MDM2 interaction was evaluated using a yeast-based assay, consisting of *Saccharomyces cerevisiae* cells co-expressing human p53 and MDM2, as described previously[4].

References:

- [1] Go, M. L., Wu, X., Liu, X. L. (2005) *Chalcones: An update on cytotoxic and chemoprotective Properties*, Curr. Med. Chem., 12, 483-499.
- [2] Boumendjek, A., Ronot, X., Boutonnat, J. (2009) *Chalcones Derivatives Acting as Cell Cycle Blockers: Potential Anti-Cancer Drugs*, Curr. Drug Targets, 10, 363-371.
- [3] Zhang, Y., Srinivasan, B., Xing, C., Lü, J. (2012) *A new chalcone derivative (E)-3-(4-methoxyphenyl)-2-methyl-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one suppresses prostate cancer involving p53-mediated cell cycle arrests and apoptosis*, Anticancer Res., 32(9), 3689-98.
- [4] Leão, M., Pereira, C., Bisio, A., Ciribilli, Y., Paiva, A.M., Machado, N., Palmeira, A., Fernandes, M.X., Sousa, E., Pinto, M., Inga, A., Saraiva, L. (2013) *Discovery of a new small-molecule inhibitor of p53-MDM2 interaction using a yeast-based approach*, Biochem. Pharmacol., 85(9),1234-45.

Acknowledgments:

This work is funded through national funds from FCT – Fundação para a Ciência e a Tecnologia under the project CEQUIMED – PEst-OE/SAU/UI4040/2011 and REQUIMTE (PEst-C/EQB/LA0006/2011) FEDER funds and COMPETE program under the projects FCOMP-01-0124-FEDER-011057 and FCOMP-01-0124-FEDER-015752.

Facilitation of acetylcholine release via the activation of adenosine A_{2A} receptors in rats with Experimental Autoimmune *Myasthenia Gravis*

C. Costa¹, R. Roque-Bravo¹, P. Correia-de-Sá¹ & L. Oliveira¹

¹Laboratório de Farmacologia e Neurobiologia, UMIB, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS) – Universidade do Porto, Portugal.

Myasthenia gravis (MG) is an autoimmune disease mainly (yet not only) motivated by auto-antibodies directed towards muscle-type nicotinic acetylcholine receptors (nAChRs) leading to neuromuscular transmission failure during repetitive movements. Our group presented compelling evidence that activation of pre-synaptic facilitatory A_{2A} receptors by endogenously formed adenosine during high-frequency nerve firing may contribute to overcome tetanic depression in healthy rats [1]. Thus, we hypothesized that benefits could be drawn for the treatment of MG by shifting the equilibrium from a prevailing inhibitory A₁ receptor tone towards the activation of co-localized A_{2A} receptors by increasing the frequency of nerve stimulation. This can be accomplished either directly, by stimulating the A_{2A} receptor with selective agonists, or indirectly, by enhancing the amount of adenosine released as such or formed from the catabolism of adenine nucleotides at the motor endplate [2]. This is particularly relevant since we recently showed that the expression of A_{2A} receptors is conserved on motor nerve terminals of rats with Experimental Autoimmune *Myasthenia gravis* (EAMG) [3]. As a proof of concept, this study was designed to evaluate the effects of the adenosine precursor, AMP, and of the selective A_{2A} adenosine receptor agonist, CGS21680C, on [³H]-ACh release from indirectly stimulated (5 Hz, 750 pulses) phrenic-nerve hemidiaphragm preparations isolated from EAMG rats as compared to healthy littermates. The EAMG model was generated by immunizing Wistar rats with the R97-116 peptide, a synthetic peptide corresponding to a specific region of the α subunit of the rat nicotinic AChR, made up in a solution containing the Complete Freund's Adjuvant (CFA) [4]. Thirty days after the first inoculation, the animals were boosted with the R97-116 peptide made up in the Inactive Freund's Adjuvant (IFA). Control animals received the CFA emulsion without the peptide; animals from the naive group were not submitted to any kind of treatment. Clinical scoring was based on the presence of tremor, hunched posture and fatigue; muscle strength was assessed by the grip strength test (BIOSEB, France) (see e.g. [4]). Pre-treatment of the preparations with CGS21680C (2 and 3 nM) facilitated the evoked release of [³H]-ACh in all groups of animals, EAMG (24±8, n=4 and 31±6%, n=3), control (23±8, n=3 and 42±8%, n=3) and naive (36±12, n=3 and 63±2, n=3), respectively. Likewise, AMP (100 μ M) also significantly ($P<0.05$) increased neurotransmitter release in EAMG, control and naive animals by 15±8% (n=5), 29±8% (n=4) and 31±5% (n=4), respectively. Thus, despite both CGS21680C and AMP increased transmitter release from stimulated phrenic motor nerve terminals in all animal groups, activation of facilitatory A_{2A} receptors is somehow relatively impaired in myasthenic animals as compared with healthy littermates.

These preliminary results suggest that neuromuscular transmission failure in MG may also reside in deficits on the physiological mechanisms underlying transmitter release adaptation to repetitive nerve activity. Despite this constrain, observation that activation of the pre-synaptic A_{2A} receptor could still promote ACh release from myasthenic motor nerve endings confirms our prediction that this receptor may be considered as a putative pharmacological target for the treatment of MG. This is particularly relevant since the nucleoside, via A_{2A} receptors activation, also decreases immune cell responses [5,6] and, therefore, may exert a two-in-one role to restore use-dependent neuromuscular facilitation and immune suppression in patients with MG.

[1] Oliveira et al., 2004, *J. Physiol. (Lond.)*, **560**, 157-58 ; [2] Correia-de-Sá et al., 1996, *J. Neurophysiol.*, **76**, 3910-19; [3] Guerra-Gomes et al., 2013, IJUP2013, personal communication; [4] Baggi et al., 2004, *J. Immunol.*, **172**, 2697-2703; [5] Csoka et al., 2008, *FASEB J* **22**: 3491-3499; [6] Ohta et al., 2012, *Front Immunol* **3**: 190..

Work supported by FCT (PTDC/SAU-FCF/108462/2008 and PEst-OE/SAU/UI0215/2011) and by U.Porto/Santander Totta (PP-IJUP2011-232)

Distribution of adenosine A_{2A} receptors in the kidney of hypertensive diabetic rats

O. Cunha², C. Abreu², C. Carvalho², M. Mota², D. Patinha¹, C. Diniz², M. Morato^{1,2} and A. Albino-Teixeira¹

¹ Department of Pharmacology and Therapeutics, Faculty of Medicine of Porto, University of Porto, Portugal.

² REQUIMTE - Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Diabetes mellitus is a chronic disease that progressively damages several organs, including the kidney, where it causes diabetic nephropathy, a prime cause of end-stage renal disease with associated high morbidity and mortality [1]. Adenosine is produced in the kidney and regulates multiple physiological functions in this organ [2]. Studies regarding the role of adenosine in diabetic nephropathy rarely take into account that most patients also simultaneously have hypertension, aggravating the prognosis. The aim of this work was to study the distribution of adenosine A_{2A} receptors in the kidney of spontaneously hypertensive rats (SHR) with streptozotocin (STZ)-induced diabetes.

In this study, eight male 12 weeks-old SHR rats were used. On day 0, animals were i.p. injected with STZ (65 mg/kg) or saline (controls). On day 21, animals were anesthetized with pentobarbital sodium (65 mg/kg; i.p.), the left kidney was removed, fixed in formalin and included in paraffin. Tissue sections (4 µm) were incubated with primary antibodies directed against adenosine A_{2A} receptors and the resulting immuno-complexes detected with a biotinylated secondary antibody. The chromogenic reaction was enhanced with ABC using DAB as substrate, imaged using bright field optics on a microscope and acquired with a CDD camera connected to a computer.

In control animals, adenosine A_{2A} receptor immunoreactivity was markedly observed in the collecting duct and in the loop of Henle, with a less marked immunoreactivity in the distal tubule and the glomeruli (both in mesangial cells and podocytes) and almost no immunoreactivity in the proximal tubule. In all the structures, the immunoreactivity was mostly found in the cell nuclei, although it was also observed in the basal membrane of distal tubules and in the luminal membrane of collecting ducts. In STZ animals, the distribution of the immunoreactivity against adenosine A_{2A} receptors was similar to that of controls, although it seems to be less marked.

While we wait for the results of the quantitative study, these qualitative data suggest that STZ alters the distribution of adenosine A_{2A} receptors in the kidney of SHR animals, which might be relevant for the pathophysiology of diabetic nephropathy.

Acknowledgments: Supported by Abbot Diabetes Care and FEDER/QREN/COMPETE and Strategic Programs (FCT project grants PTDC/SAU-FCF/67764/2006, Pest-C/SAU/LA0002/2011, Pest-C/EQB/LA0006/2011) and D. Patinha grant QREN/POPH (FCT-SFRH/BD/43187/2008).

References:

- [1] Arora, M.K. and Singh, U.K. (2013), *Molecular mechanisms in the pathogenesis of diabetic nephropathy: An update*, *Vascular Pharmacology*, 58, 259-271.
- [2] Vallon, V., Mühlbauer, B. and Osswald, H. (2006), *Adenosine and Kidney Function*, *Physiology Reviews*, 86, 901-940.

Cardiac P2X4 purinoceptors predominantly decrease atrial rate with only minor effects on ventricular active tension in the rat

S. Nogueira-Marques¹, T. Rodrigues¹, B. Bragança¹, N. Oliveira-Monteiro¹, A.C. Pereira¹,
A.P. Fontes-Sousa¹, P. Correia-de-Sá¹

¹ Laboratório de Farmacologia e Neurobiologia / UMI B, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Portugal.

Increasing evidences indicate that ATP and other nucleotides play important roles in cardiovascular pathophysiology through the activation of subtype-specific P2X and P2Y purinoceptors (reviewed in [1]). It has been shown that overexpression of the ionotropic P2X4 receptor (P2X4R) increases cardiac contractile force and survival in a model of myocardial infarction-induced cardiac failure [2]. However, no reports have been published so far of the role of P2X4R on the control of heart rate.

Experiments were performed on spontaneously beating atria and electrically-paced right ventricular strips of Wistar rats superfused with gassed (95% O₂ + 5% CO₂) Tyrode's solution, at 37°C. Isometric muscle tension was continuously monitored on a computer screen via a PowerLab data acquisition system. Values are expressed as mean±SEM of *n* number of preparations; statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Bonferroni's post-test.

ATP (100 μM) exerted a transient reduction in atria active tension (Phase I), which was followed by a gradual recovery (Phase II) until washout of drug. Sustained and more robust decreases in the rate of atrial contractions (negative chronotropism) were obtained less than 1 min after nucleotide application. Inhibition of extracellular ATP catabolism by ecto-NTPDases with POM-1 (100 μM) sensitized atria to negative chrono- and inotropic effects of the nucleotide, indicating that ATP-induced cardiodepression does not necessarily require its metabolism into adenosine. Ivermectin (10 μM), a P2X4R allosteric enhancer, significantly potentiated the ATP-induced negative chronotropism. The P2X4R antagonist, 5'-BDBD (10 μM), attenuated the negative chronotropic effect of ATP, but it favored the negative inotropic action of the nucleotide on spontaneously beating rat atria. ATP (0.1 and 1 mM) concentration-dependently decreased the active tension of right ventricular strips paced electrically. This scenario was not affected by 5'-BDBD (10 μM), unless the extracellular concentration of KCl is increased from 2.7 to 5 mM; under the latter experimental conditions, blockade of P2X4R with 5'-BDBD (10 μM) enhanced (*P*<0.05) the negative inotropic effect of ATP (1 mM) from 12±2% (*n*=7) to 24±6% (*n*=3), respectively.

In conclusion, we showed that P2X4R plays an important role to decrease atrial rate in response to extracellular ATP accumulation without significantly changing ventricular active tension. Preliminary data from our laboratory led us to propose that cooperation between P2X4R and adenosine A₁ receptors might be beneficial to convert supraventricular tachycardia to sinus rhythm given that both receptors lack major effects on ventricular contractile activity. In contrast to normal physiological conditions, activation of P2X4R may be advantageous in ischemic, traumatic or stressful circumstances leading to increased extracellular potassium levels as it might additionally contribute to increase ventricular contractility and prevent heart failure [2].

[1] Erlinge & Burnstock (2008). *Purinergic Signal*; **4**: 1-20; [2] Sonin *et al.* (2008). *Am J Physiol Heart Circ Physiol*; **295**: 1191-97.

Work supported by FCT through projects FCOMP-01-0124-FEDER-028726 (FEDER, COMPETE, FCT PTDC/DTP-FTO/0802/2012) and PEst-OE/SAU/UI0215/2011.

Antioxidant activity and bioactive compounds of honey

**L. P. M. Ribeiro^{1,2}, D. M. D. Nascimento^{1,2}, S. E. F. Soares², A. S. G. Costa²,
R. C. Alves^{1,3}, L. M. Cunha¹, M. B. P. P. Oliveira²**

¹ REQUIMTE, DGAOT, Faculty of Sciences, University of Porto, Portugal.

² REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

³ REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

Consumers are, nowadays, more concerned with natural products and their effect on health and well-being. Honey, as a natural product, is increasingly consumed due to its association with a reduced risk of certain diseases/disorders, as coronary heart disease, cataracts, inflammation, cancer, and others [1]. Honey is a good source of antioxidants, such as flavonoids and other phenolic compounds [2], characteristic influenced by multiple factors, as climatic conditions, soil composition, flora diversity and botanical origin.

The present work aimed to evaluate the total phenolic and flavonoid contents and the antioxidant activity (DPPH[•] scavenging activity and ferric reduction antioxidant power (FRAP)) of 10 honey samples with different floral and geographical origins.

The total phenolic contents ranged from 101.62 to 628.50 mg gallic acid/kg, being the highest values found in the heather honey. A similar variation was observed in total flavonoid content (from 10.18 to 58.42 mg epicatechin/kg). The results also showed that total phenolic compounds are more important contributors for antioxidant behavior of honey than flavonoid compounds. Chestnut honey was the sample with the highest total antioxidant activity.

References:

[1] Arráez-Román, D., Gómez-Caravaca, A. M., Gómez-Romero, M., Segura-Carretero, A. e Fernández-Gutiérrez, A. (2006), *Identification of phenolic compounds in rosemary honey using solid-phase extraction by capillary electrophoresis–electrospray ionization–mass spectrometry*, Journal of Pharmaceutical and Biomedical Analysis, 41, 1648-1656.

[2] Estevinho, L., Pereira, A.P., Moreira, L., Dias, L.G. & Pereira, E. (2008), *Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey*, Food and Chemical Toxicology, 46, 3774-3779.

Acknowledgments: S. Soares and R. Alves are grateful to FCT for a PhD grant (SFRH/BD/75091/2010) and a post-doc grant (SFRH/BPD/68883/2010), respectively. This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069-CIÊNCIA DO ALIMENTO).

Heavy metals levels are increased in samples of kidney tumoral tissue: a microscopy technique, SEM-XRM, analysis

SEM. Cunha¹, A. Silva¹, R. Pinto¹, P. Pereira¹, F. Cruz¹ and C. Martins da Silva¹

¹ Department of Urology, Faculty of Medicine, University of Porto, Portugal.

There are numerous studies that have examined the association between heavy metal contamination and carcinogenesis, although the precise mechanisms for the carcinogenesis of heavy metals are not completely understood. In this work, we investigated the affinity of circulating heavy metals particles in samples of renal tumor and surrounding renal tissue [1].

Samples from renal tumor and adjacent renal tissue from 7 patients submitted to radical or partial nephrectomy were fixed in a 3% glutaraldehyde solution. A scanning electron microscopy coupled with X-ray microanalysis (SEM-XRM) was used to detect and quantify heavy metals *in situ*, after the samples were dehydrated in ethanol and critical point-dried in a Balzer's apparatus. The preparations were mounted on metal stubs and coated by carbon under vacuum and examined in a JEOL JSM-6301F.

The heavy metals studied were, arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni) and zinc (Zn), tungsten (W), iron (Fe) and nickel (Ni). This study was approved by local Ethics Committee. All the patients signed the Informed Consent. SEM-XRM revealed the presence of Cd, and other heavy metals such as Ni, Cr, Fe, W and Ni particles in the tumor tissue samples. Interestingly, in renal adjacent tissue, no heavy metal particles were detected. There is an anomalous sequestration of heavy metals in the kidney tumor tissue.

This finding opens a new venue for future studies on the detailed kinetics of microscopic particles of heavy metals and could be fundamental to understand the impact of heavy metals/ carcinogenesis, namely in renal carcinoma.

[1] Müller, I. Helmers, E. Barchet, R. Schweinsberg, F. (1994) *Cadmium concentration in the renal cortex of kidney tumor patients and controls*. J Trace Elem Electrolytes Health Dis,8 (3-4), 173-176

Potential antitumour xanthenes: synthesis and pharmacokinetics

J. Neves^{1,2}, D. Marques^{1,2}, R. Vieira^{1,2}, J. Soares^{1,2}, S. Reis^{2,3}, M. Pinto^{1,2,4}, C. Azevedo¹, C. Afonso^{1,2,4}.

¹ Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP).

² Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. ³ REQUIMTE ⁴ Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal.

Xanthenes are natural compounds with a dibenzo- γ -pyrone scaffold, which show a great variety of biological activities. Therefore, they are considered privileged structures in medicinal chemistry and a source of inspiration for new drug candidates [1]. Our group has been focusing on synthesizing xanthonic derivatives both more potent and with better pharmacokinetic properties [2].

In this work the synthesis of 4-hydroxyxanthone, an important precursor of several bioactive xanthenes, is presented (Fig.1). Classic and green chemical synthesis approaches were used.

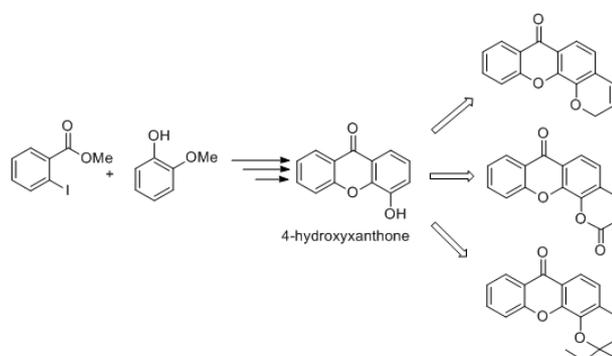


Fig.1 – Synthesis of 4-hydroxyxanthone and derivatives

Keeping in mind druglikeness [3], a small library of 4-hydroxyxanthone derivatives was planned (Fig.1) in order to search for new promising antitumor compounds. Pharmacokinetic behavior was predicted using a different set of rules e.g. Lipinski and Veber. These rules use several molecular and structural descriptors such as log P, hydrogen donors/acceptors, polar surface area and rotatable bonds.

This work will allow planning new molecules, more potent and with better ADMET (absorption, distribution, metabolism, excretion and toxicity) properties.

References:

[1] Pinto, M.M.M., Sousa, M.E. and Nascimento, M.S.J. (2005), *Xanthone derivatives: New insights in biological activities*, Current Medicinal Chemistry, 12 (21), 2517-2538.

[2] Azevedo, C. *et al* (2013), *Pyranoxanthenes: Synthesis, growth inhibitory activity on human tumor cell lines and determination of their lipophilicity in two membrane models*, European Journal of Medicinal Chemistry, 69, 798-816.

[3] Gleeson, M. *et al* (2011), *Probing the links between in vitro potency, ADMET and physicochemical parameters*, Nature Reviews Drug Discovery, 10, 197-208

Acknowledgments:

FCT – Fundação para a Ciência e a Tecnologia under the project CEQUIMED – PEst-OE/SAU/UI4040/2011, FEDER funds and COMPETE program under the project FCOMP-01-0124-FEDER-011057 and U.Porto/Banco Santander Totta (PP_IJUP2011-58)

A computational study to investigate the binding of new potential antitumor xanthone derivatives to human serum albumin

R. Vieira^{1,2}, D. Marques^{1,2}, J. Neves^{1,2}, J. Soares^{1,2}, A. Palmeira^{1,2}, S. Reis^{2,3}, M. Pinto^{1,2,4}, C. Azevedo¹, C. Afonso^{1,2,4}.

¹ Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP).

² Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. ³ REQUIMTE ⁴ Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal.

Xanthenes are O-heterocyclic compounds with a variety of interesting biological activities [1]. Our group has been focusing on the synthesis of xanthone derivatives with antitumor activity. From our in-house library, a hit compound (XC36) showed promising antitumoral activity [2]. Several analogues were designed aiming for an increased potency and a better pharmacokinetic profile.

Regarding the pharmacokinetic profile, the study of binding to human serum albumin (HSA) is an important issue. The degree of affinity between drugs and HSA can dictate its distribution into target tissue, affects metabolism, elimination, toxicity and therefore the therapeutic activity [3].

Herein, we present a computational chemistry study of the binding of XC36 analogues to HSA. HSA structure was obtained from Protein Data Bank (pdb code: 2VUE). The ligands were drawn (ChemBioOffice 2013) and subjected to energy minimization (ArgusLab) by molecular mechanics using the force field method. Docking simulations were conducted (PyRx/AutoDock Vina) considering the HSA as a rigid unit, while the ligands were allowed to be flexible. The best docking scores were selected and the putative binding sites were defined. Furthermore, the type of interactions and the HSA involved residues were investigated.

The obtained results allowed us to enhance our knowledge about the binding of the studied XC36's analogues to HSA, suggesting and orienting the design of new drug candidates with better pharmacokinetic profile.

References:

[1] Pinto, M.M.M., Sousa, M.E. and Nascimento, M.S.J. (2005), *Xanthone derivatives: New insights in biological activities*, Current Medicinal Chemistry, 12 (21), 2517-2538.

[2] Azevedo, C. et al (2013), *Pyranoxanthenes: Synthesis, growth inhibitory activity on human tumor cell lines and determination of their lipophilicity in two membrane models*, European Journal of Medicinal Chemistry, 69, 798-816.

[3] Howard, M.L., Hill, J.J., Galluppi, G. R. and McLean, M.A. (2010), *Plasma protein binding in drug discovery and development*, Combinatorial Chemistry and High Throughput Screening, 13 (2), 170-187.

Acknowledgments:

This work is funded through national funds from FCT – Fundação para a Ciência e a Tecnologia under the project CEQUIMED – PEst-OE/SAU/UI4040/2011, FEDER funds and COMPETE program under the project FCOMP-01-0124-FEDER-011057 and U.Porto/Banco Santander Totta (PP_IJUP2011-58)

Development of a toothpaste containing roots of *Euclea natalensis*

D. Santos², J. B. P. Silva¹, J.C. Meneses², Á. Bastos², E. F. Silva¹, P. Lobão², M. H. Amaral², J. M. Sousa Lobo², C. Gomes¹

¹ GeoBioTec Research Unit, University of Aveiro, University Campus of Santiago, Aveiro, Portugal

² Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Teeth cleaning and disinfection of the oral cavity are habits that date back to the Greek and Roman civilizations. The people of that time used for this purpose the stems and roots of plants, because these materials possess abrasive properties.

The roots of *Euclea natalensis* are used by various African peoples in oral hygiene (used as natural dentifrice), and it has been considered as the main plant used for this purpose in Mozambique (popularly known by Mulala). Traditionally the root bark is removed and the inner part is chewed until it gets crumbed, after being rubbed against the teeth and gums. This process temporarily leaves the mouth and teeth with an orange coloration, disappearing after a few hours [1].

In literature, many authors consider other applications particularly in the treatment of dental caries, gingival bleeding and treatment of oral diseases because of its antifungal and antibacterial activity (against microorganisms such as *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus kristinae*) [2,3].

The roots were obtained at typical market in Maputo (Mozambique) and then treated in the laboratory, proceeding to wash with purified water and then drying in a ventilated oven, at approximately 40°C. After drying, the roots were pulverized and passed through a sieve with a mesh size of 90µm. Then we proceeded to develop a basis for incorporation of the powder prepared.

After the geochemical characterization of the root [4], the toothpaste base was prepared in which was added 2.5% of its component. The final pH obtained was 6.9, which complies with the recommended requirements for dentifrices [5]. The toothpaste obtained showed appreciable physical stability and good viscosity and texture characteristics. This work must be continued in order to verify the effectiveness of the powder with respect to antiseptic, toning and cicatrizing properties of the root of Mulala.

References:

- [1] Filipe, M., Gomes, E.T., Serrano, R. and Silva, O. (2008), Caracterização farmacognóstica da raiz de *Euclea natalensis*. Workshop plantas medicinais e fitoterapêuticas.
- [2] Lall, N. and Meyer, J. J. (2000), Antibacterial activity of water and acetone extracts of the roots of *Euclea natalensis*, *J Ethnopharmacol*, 72 (1-2): 313-6.
- [3] Lall, N. et al. (2006), Antifungal activity of naphthoquinones and triterpenes isolated from the root bark of *Euclea natalensis*. *South African Journal of Botany*, 72 (4): 579-83.
- [4] Silva, J.B., Silva, E.F., Santos, D., Meneses, J.C., Lobão, P., Amaral, M.H., Lobo, J.M., and Gomes, C. (2013), Caracterização geoquímica da raiz de *Euclea natalensis* (Mulala). III CIBAP-Açores.
- [5] Priste, L.N., Bahia, M.F. and Vilar, E. (1995), *Dermofarmácia e Cosmética*. II Volume. Ed da Associação Nacional de Farmácia.

Development of formulations containing mud from saline of Santiago da Fonte, Aveiro, for dermocosmetics and dermo therapy applications

J. H. Gomes¹, D. Santos², F. E. Almeida¹, J. C. Meneses², H. Amaral², J. B. Silva¹, J. Conceição², C. F. Gomes¹, J. M. Sousa Lobo²

¹ GeoBioTec Research Unit, University of Aveiro, University Campus of Santiago, 3810-193 Aveiro, Portugal

² Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal

Since 19th century, Cuba uses, empirically, mud salt in balneotherapy for treating various pathologies [1, 2]. From 1989 began the scientific characterization, in physicochemical and bacteriological terms, of Peloids / mud saline of Cuba [1, 2]. Therefore, and according to several researchers, including Romero Sánchez [1], investigations have been made papers about the use of saline muds, with the aim of its use in thermal therapy and for the development of dermocosmetics products.

Given this very purpose the authors of this communication proceeded initially the study of mud from saline of Santiago in Aveiro, thoroughly investigating their mineralogical, physical and chemical properties most relevant. In a second step involved the refining and upgrading of mud characterized before.

To refine and benefit the mud collected in the crystallizer, an equipment was developed to concentrate and desalt the fraction required. The determination of size particle distribution was determined using sieves with the mesh sizes of: 500µm, 250µm, 125µm and 63µm. Analysis of the distribution of grain size in the mud showed that it is very thin (90% of grain particles are lesser than 63µm and 41% of the particles have grain lesser than 4µm).

In the third stage, mud benefits formulations were developed and were applied to standard methods evaluation of relevant properties of the mud / pelloid in view of the possible use of mud / Peloids in dermocosmetic and dermo therapy. Were detected and quantified in the mud pathogenic microorganisms and, by this, the samples were sterilized. To the base formulation increasing concentrations of mud were added (4%, 5%, 6% and 10%), proceeding the mixing of samples.

After 30 days, it was found that the physical stability remained unchanged for formulations containing 4%, 5% and 6% of mud. For higher concentrations, a slight physical instability was observed. The base formulation used for the incorporation of mud proved to be very satisfactory, taking into account the results obtained in rheological tests.

References:

[1] Romero Sánchez, J. *Características de las aguas minerales y fangos, uso y control de la calidad de estos recursos*; [01/10/2013]. Disponível em: <http://www.sld.cu/galerias/pdf/sitios/mednat/peloides.pdf>.

[2] Pita, M.T.S. (2006), *Consideraciones sobre el control sanitario de los fangos medicinales o peloides*, Rev Cubana Hig Epidemiol, 44 (3).

Marine macroalgae: a source of excellence of natural compounds with beneficial effects for health - A review

C.G. Costa^{1,2}, F.B. Pimentel¹, R.C. Alves^{1,3}, A.S.G. Costa¹, M.B.P.P. Oliveira¹

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² REQUIMTE, Dep. Chemical Engineering, Fac. of Engineering, University of Porto, Portugal.

³ REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

Marine biodiversity represents an exceptional source of natural resources. Using those in a sustainable basis may ensure alternative food sources for human consumption, which scarcity is anticipated. Macroalgae are an outstanding example of this biodiversity and are considered to be an excellent source of a wide number of compounds with beneficial effects for health [1].

According to their pigmentation macroalgae can be distinguished in red (Rhodophytaea), green (Chlorophytaea) and brown (Phaeophytaea). Some of them are widely used as food ingredients in oriental countries and, because they are a good source of fibre and some technologically important compounds (phycocolloids), they are also considered a very attractive raw material for the food, pharmaceutical and cosmetic industries [2].

In this communication, we reviewed a number of common types of algae used for human consumption, its nutritional composition and overview the main compounds associated with health beneficial effects, namely those with interesting biological activities such as antidiabetic, anti-allergic, anti-inflammatory, antioxidant, neuroprotective, immunomodulatory, prebiotic, that have been described in various species with economic potential for several applications [1-3].

References:

[1] Holdt, S.L. and S. Kraan. (2011), *Bioactive compounds in seaweed: functional food applications and legislation*. Journal of Applied Phycology, 23(3), 543-597.

[2] Wijesekara, I., R. Pangestuti, and S.K. Kim. (2011). *Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae*. Carbohydrate Polymers, 84(1), 14-21.

[3] Pangestuti, R. and S.K. Kim. (2011). *Biological activities and health benefit effects of natural pigments derived from marine algae*. Journal of Functional Foods, 3(4), 255-266.

Acknowledgments: R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

Development and characterization of an oil-in-water cream containing honey

A. Araújo¹, J. Fonseca¹, F. Rodrigues², M.H.Amaral¹, M.B.P.P. Oliveira², D. Santos¹, J. M. Sousa Lobo¹

¹ Department of Drug Sciences, Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto

² REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto

Nowadays, there is a great tendency in the cosmetic area in the development of multifunctional cosmetics that use natural compounds with highly moisturizing content. Not only the aesthetic but also the natural properties of compounds are taken in consideration by consumers. Since ancient times, honey has been used for its medicinal properties [1].

The aim of this work was the development of an oil-in-water cream containing honey from the *Barroso* region and to evaluate its properties and benefits for the skin.

Oil-in-water creams containing different percentages of honey (1 or 2 %, w/w) were prepared and, during one month, these formulations and the respective dermatological base (cream without honey) were submitted to the following analyzes: viscosity (performed with a shear rate of 5, 10, 20, 30 and 50 rpm, and at a temperature of 20°C), texture (evaluation of firmness and adhesiveness), pH and biometric tests (skin hydration, pH, transepidermal water loss, and skin relief) in human volunteers. In order to study the antioxidant activity, this was evaluated by the DPPH [2] assay and total phenolic content (TPC) by the Folin-Ciocalteu method [3].

The incorporation of honey in the oil-in-water cream caused an increase in viscosity and firmness, but decreased the pH. However, these parameters remained stable over 30 days of storage at 20°C. We noticed an increase in skin moisturizing 2 hours after the application of creams containing honey. However, the differences between the creams containing 1 % and 2% of honey were not significant. Regarding the antioxidant capacity, the differences were also not significant, presenting the cream containing 2% of honey results slightly lower.

To improve efficacy studies, formulations should be applied in more volunteers and analyzes carried out for longer time.

Acknowledgments:

F. Rodrigues is grateful to FCT for a PhD grant (SFRH/BDE/51385/2011). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069-CIÊNCIA DO ALIMENTO).

References:

- [1] Ediriweera, E. R. H. S. S., et al. (2012), *Medicinal and cosmetic uses of BEE's Honey – A review*, An International Quarterly Journal of Research in Ayurveda, 33(2): 178-182.
- [2] Guimarães, R., et al. (2010), *Targeting excessive free radicals with peels and juices of citrus fruits: Grapefruit, lemon, lime and orange*, Food and Chemical Toxicology, 48(1): 99-106.
- [3] Alves, R.C., et al. (2010), *Antiradical Activity, Phenolics Profile, and Hydroxymethylfurfural in Espresso Coffee: Influence of Technological Factors*, Journal of Agricultural and Food Chemistry, 58(23): 12221-12229.

How to assess combination effects between ecstasy and often co-consumed licit substances?

I. Durán¹, H. Carmo², and D. Dias da Silva².

¹ Faculdade de Medicina, Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200-319. Porto, Portugal. ² REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Ecstasy (MDMA or 3,4-methylenedioxymethamphetamine) is a phenylethylamine structurally similar to amphetamine and mescaline that has turned into a major drug of abuse worldwide over the last three decades. It has become particularly popular among rave participants because it enhances energy, endurance, sociability and sexual arousal.

Ecstasy misusers repeatedly abuse several drugs simultaneously, combining legal and illegal substances to modulate the expected effects of MDMA (either by alleviating the unpleasant ‘come-down’ effects or extending the duration of the ‘high’). Studies on the progression of drug abuse revealed that the recreational use of MDMA is usually preceded by legal drugs, such as caffeine, alcohol and nicotine, and followed by illicit drugs. Ethanol is the most frequent substance co-consumed with MDMA. There is also a high prevalence of smoking among MDMA users. Caffeine exposure results from pill contamination or from the ingestion of caffeine-rich to reduce drowsiness and fatigue.

This polydrug abuse pattern is one the most serious confounding factors when evaluating MDMA toxicity, as it hampers the correlation of toxicological events with one specific drug. Nevertheless, the understanding of the toxicity and the investigation of potential pharmacodynamic and/or pharmacokinetic interactions between MDMA and other recreational agents has received rather little attention, and most of the information on the topic is inferred from intoxication cases. For toxicological risk assessment the mathematical prediction of mixture effects, using knowledge on single substances, is therefore desirable.

In an effort to elucidate whether MDMA combinations were able to yield significant toxicity several studies were conducted to evaluate the joint effects of MDMA and three other amphetamines. The combination effects of different mixtures were assessed by using two mathematical models broadly employed in pharmacology: concentration addition (CA) and independent action (IA) [1]. A clear additive effect between these drugs was revealed [1]. A particularly notorious finding was that substantial mixture effects occurred even when each drug was present at levels that were individually ineffective [1]. While potential interactions between amphetamines could be relatively easy to anticipate based on their overlapping mechanistic, metabolic and detoxifying pathways, a much more complex task is to evaluate relevant interactions with substances that are neither structurally nor pharmacologically related but are almost always associated with MDMA abuse.

In this review, we discuss the current state of the art in toxicology of mixtures of MDMA with licit drugs (ethanol, tobacco and caffeine) in relation to the investigation of the mechanisms underlying pharmacokinetic and pharmacodynamic interactions and the tools that can be used to evaluate mixture toxicity.

References:

[1] Dias da Silva D, Carmo H, Silva E (2013) The risky cocktail: what combination effects can we expect between ecstasy and other amphetamines? *Arch Toxicol* 87(1):111–122.

Valorization of *Arbutus unedo* L. berries: a study on their nutritional and phytochemical composition

C. Albuquerque¹, A. F. Vinha^{1,2}, A. S. G. Costa¹,
R. C. Alves^{1,3}, M. B. P. P. Oliveira¹

¹ REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal.

² Faculty of Health Sciences, University Fernando Pessoa, Portugal.

³ REQUIMTE, School of Engineering, Polytechnic Institute of Porto, Portugal.

In the Mediterranean region, fruits and leaves of *Arbutus unedo* L. (Ericaceae) are traditionally used due to their therapeutic benefits (antiseptic, diuretic, laxative, hypotensive). The fruits are also used in the production of jams, or fermented and distilled into liquors [1], common practices in rural areas of Portugal. As they are still considered an underutilized fruit-tree species [1], the aim of this study was to study the nutritional and phytochemical composition of fresh *A. unedo* L. berries harvested in the North of Portugal (Minho), to promote their consumption and valorization.

Nutritional composition was evaluated according to AOAC methodologies [2]. Total phenolics, flavonoids, anthocyanins, and carotenoids contents were evaluated by spectrophotometric methods [3-5].

Berries moisture was approximately 56 %. Carbohydrates were the most representative components of the nutritional profile of *Arbutus unedo* L. fruits (~42%), followed by protein (~1.1%), ash (0.3%) and lipids (0.3%). Total phenolics, flavonoid and anthocyanin contents per gram of fresh fruit were, respectively, 1.50 mg gallic acid eq., 1.29 mg catechin eq., and 16.5 µg cyanidine-3-glucoside eq. Chlorophyll a (1.9 µg/g), chlorophyll b (2.7 µg/g), lycopene (0.7 µg/g) and β-carotene (2.4 µg/g) were also found in the samples. These results suggest that *Arbutus unedo* L. fruits may exert biological activity and health promoting effects, and that their consumption should be valorized.

References:

- [1] Ruiz-Rodríguez, B.-M. *et al.* (2011), *Valorization of wild strawberry-tree fruits (Arbutus unedo L.) through nutritional assessment and natural production data*, Food Research International, 44 (5), 1244-1253.
- [2] AOAC International (2000). Official methods of analysis of AOAC International. 17th edition. Association of Analytical Communities, Gaithersburg, MD, USA.
- [3] Soares, M. *et al.* (2013). *Angolan Cymbopogon citratus used for therapeutic benefits: Nutritional composition and influence of solvents in phytochemicals content and antioxidant activity of leaf extracts*, Food and Chemical Toxicology, 60, 413-418.
- [4] Burdulis, D. *et al.* (2008). *Method development for determination of anthocyanidin content in bilberry (Vaccinium myrtillus L.) fruits*. Journal of Liquid Chromatography & Related Technologies, 31 (6), 850-864.
- [5] Vinha, A. F. *et al.* (2014). *Effect of peel and seed removal on the nutritional value and antioxidant activity of tomato (L. esculentum L.) fruits*, LWT - Food Science and Technology, 55, 197-202.

Acknowledgments: R. Alves is grateful to FCT for a post-doctoral research grant (SFRH/BPD/68883/2010). This work has been supported by FCT (PEst-C/EQB/LA0006/2013) and QREN (NORTE-07-0124-FEDER-000069- CIÊNCIA DO ALIMENTO).

Therapeutic efficacy and safety of BNIPDaoct-loaded PLGA nanoparticles for the treatment of visceral leishmaniasis

S.C. Lima^{1,*}, M. T. Baltazar^{2,3}, R. J. Dinis-Oliveira^{2,3,4,5}, A. Cordeiro-da-Silva^{1,6}

¹BMC-INEB, Infection and Immunology Unit, Parasite Disease Group, University of Porto

²IINFACTS - Institute of Research and Advanced Training in Health Sciences and Technologies, Department of Sciences, Advanced Institute of Health Sciences – North (ISCS-N), CESPU, CRL, Gandra

³REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto

⁴Department of Legal Medicine and Forensic Sciences, Faculty of Medicine, University of Porto

⁵Forensic Sciences Center – CENCIFOR, Portugal

⁶Department of Biological Sciences, Faculty of Pharmacy, University of Porto

Bisnaphthalimidopropyldaoctane (BNIPDaoct) was the recently identified as anti-*Leishmania* therapeutic agent. The development of a nanoparticle-based drug delivery system for BNIPDaoct should overcome its low aqueous solubility and probably improve its anti-parasitic activity. This project intended to characterize the *in vivo* efficacy of BNIPDaoct-loaded PLGA nanoparticles (BNIPDaoct-PLGA NPs) as well as their safety in terms of drug-induced tissue toxicity.

The biodegradable and biocompatible PLGA was chosen for the production of the nanoparticles by a nanoprecipitation method. The characterization of the unloaded and BNIPDaoct-PLGA NPs was achieved by dynamic light scattering, HPLC for BNIPDaoct quantification and transmission electron microscopy. The BNIPDaoct-PLGA NPs exhibited a narrow size distribution (156.0 nm) identical to unloaded PLGA NPs (151.7 nm), with low polydispersity values indicating that the NPs were of relatively uniform size. The mean zeta potential of the BNIPDaoct-PLGA NPs was -8.4 mV and the encapsulation efficiency of BNIPDaoct was 88%, as a consequence of the drug hydrophobic nature. The BNIPDaoct-PLGA NPs were evaluated using the acute visceral leishmaniasis model, by treating the mice at week 2 post infection, with free BNIPDaoct, BNIPDaoct-loaded PLGA NPs and AmBisome[®]. Treatments with the BNIPDaoct and with AmBisome[®] were comparable and lead to significant reduction in the parasite load in the spleen ($P < 0.05$) and in the liver ($P < 0.01$) when compared with the untreated controls.

The safety of the nanoformulation was assessed using several oxidative stress biomarkers: reduced and oxidized glutathione, lipid peroxidation and myeloperoxidase in different organs. Identical levels of drug-induced tissue toxicity by BNIPDaoct-NPs and AmBisome[®] were observed.

In conclusion, an efficient and safe BNIPDaoct-nanoparticle delivery system for visceral leishmaniasis therapy, was developed.

Acknowledgments: Funding from U.Porto/Santander Totta – project 227/2011 and from FCT through project PTDC/SAU-ENB/113151/2009 are acknowledged. S.C. Lima thank FCT and FSE (III Quadro Comunitário) for the grant SFRH/BPD/37880/2007.

*Present address: REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto

Mitoxantrone causes time- and concentration-dependent toxicity in H9c2 differentiated cells at pharmacological relevant concentrations

A. Reis-Mendes¹, E. Sousa², F. Remião¹, F. Carvalho¹, M.L. Bastos¹, and V.M. Costa¹

¹ REQUIMTE (Rede de Química e Tecnologia), Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal

² CEQUIMED-UP, Dep. Química, Lab. Química Orgânica e Farmacêutica, Faculdade de Farmácia, U.Porto and CIIMAR – Interdisciplinary Centre of Marine and Environmental Research

Background: In clinical practice, the activity of mitoxantrone (MTX) has been demonstrated in patients with advanced breast cancer, prostate cancer, acute leukemia and lymphoma [1]. Furthermore, MTX has been used on the treatment of multiple sclerosis [1]. Currently available therapeutic strategies for treating various types of cancer have many limitations and adverse effects. One of the systems most affected by cancer chemotherapy is the cardiovascular system [2]. Cardiotoxicity leading to heart failure is a common side effect that can affect up to 18% of MTX-treated patients [2]. **Aim:** This work aims to highlight the mechanisms involved in the MTX-induced cardiotoxicity in differentiated H9c2 myocytes, namely the effects toward mitochondria, nuclei and lysosomes, using therapeutic relevant concentrations. **Methods:** H9c2 cells were differentiated using Dulbecco's Modified Eagle Medium supplemented with 10 nM retinoic acid (RA) and 1% fetal bovine serum (FBS) for 7 days (medium changed every two-days) [3]. The differentiation was assessed by contrast phase microscopy and the fluorescent nuclear dye Hoechst 33258 [4]. After cell differentiation protocol, cells were incubated with MTX in concentrations that ranged from 0.01 μ M to 5 μ M and two time-points were selected (24 and 48h) for cytotoxicity tests. The cytotoxicity tests used were: lactate dehydrogenase leakage (LDH) assay, the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay and the neutral red (NR) uptake assay [5]. **Results:** The differentiation by 10 nM RA and 1% FBS led to morphological changes making cells more resembled to the cardiac phenotype. The differentiation also resulted in a lower proliferation rate as verified by Hoechst 33258 staining. The lower MTX concentrations (0.01 and 0.1 μ M) did not cause cytotoxicity in H9c2 cells. However, for the higher concentrations (1, 2, and 5 μ M), cytotoxicity was time- and concentration-dependent for all the assays, being the NR assay more sensitive for the MTX toxic effects. **Conclusions:** MTX is a cardiotoxic drug that alters several pathways in H9c2 cells. Those pathways need to be further addressed as to reveal putative targets for cardioprotection.

Acknowledgments: This work was supported by FCT [EXPL/DTP-FTO/0290/2012] -QREN initiative with EU/FEDER financing through COMPETE. VMC acknowledges FCT for her Post-doc grant SFRH/BPD/63746/2009.

References:

- [1] Seiter, K., 2005. Toxicity of the topoisomerase II inhibitors. *Expert Opin Drug Saf* 4, 219-234.
- [2] Costa, V.M., Carvalho F., Duarte J., Bastos M.L., Remião F., 2013. The heart as a target for xenobiotic toxicity: the cardiac susceptibility to oxidative stress. *Chem Res Toxicol* 26:1285-1311.
- [3] Ruiz, M., Courilleau, D., Jullian, J.C., Fortin, D., Ventura-Clapier, R., Blondeau, J.P., Garnier, A., 2012. A cardiac-specific robotized cellular assay identified families of human ligands as inducers of PGC-1 α expression and mitochondrial biogenesis. *PLoS One* 7 (10), e46753.
- [4] Soares, A.S., Costa, V.M., Diniz, C., Fresco, P. 2013. Potentiation of cytotoxicity of paclitaxel in combination with Cl-IB-MECA in human C32 metastatic melanoma cells: A new possible therapeutic strategy for melanoma. *Biomed Pharmacother* 67:777-789.

Development, characterization and stability evaluation of silicone emulsions containing dithranol

M. Estanqueiro, G. Moreira, S. Sousa, S. Albino, M.H. Amaral, P. Lobão, J. Conceição, J.M. Sousa Lobo

Research Centre for Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Psoriasis is a disease with an accelerated cellular turnover, having an accumulation of dead cells in the skin surface. The clinical manifestations and the co-morbidities of psoriasis have a major impact on the quality of life as well as on the average life expectancy of these patients [1, 2]. Psoriasis treatment depends on the disease severity and the degree of quality of life impairment. In mild to moderate disease the treatment is made with topical formulations, from emollients until formulations containing corticosteroids, vitamin D analogues, coal tar and dithranol. Dithranol is an anti-psoriatic drug, presenting a yellow color, which oxidizes easily by contact with light, air and high temperatures [3]. The emulsions with silicones in the external phase show advantages in relation to the same formulations containing other excipients in the oily phase. These formulations can also be formulated without water in the internal phase, which can be replaced by an alcohol or polyalcohol, representing an advantage when it is intended to incorporate an active substance easily oxidizable in aqueous medium [4].

The main objectives of this work were the development of an anhydrous silicone emulsion containing dithranol for topical application, their characterization and study of stability in accelerated conditions by mechanical stress and in real time by rheological and textural analysis, during one month. In addition, the color was evaluated over time, in samples maintained at different conditions, namely, protected from light, exposed to light and exposed to high temperature (37°C). The same formulation without dithranol was also prepared and tested.

Both formulations (with and without dithranol) showed physical stability over time and in mechanical stress conditions. The formulations also showed appropriate characteristics for cutaneous application, namely, pseudoplastic behavior. In relation to color evaluation it was found that the silicone emulsion containing dithranol, stored protected from light and at room temperature, showed less variation of $L^*a^*b^*$ parameters. The formulations maintained at 37°C presented a greater color variation.

It can be concluded that the anhydrous silicone emulsion is a promising dermatological base for cutaneous application of dithranol, however it should be stored in a cool place and protected from light.

References:

- [1] Gelfand, J.M., Troxel, A.B., Lewis, J.D., et al. (2007), The risk of mortality in patients with psoriasis: Results from a population-based study. *Archives of Dermatology*, 143(12),1493-9.
- [2] Walters, K.A. and Roberts, M.S. (2002), The Structure and Function of Skin. In: Walters, K.A. (ed). *Dermatological and Transdermal Formulations*. 119. New York: Marcel Dekker, Inc. ; 2002, pp. 1-41.
- [3] Chaul, A., Duarte, A.A., Gontijo, B., Martins, G.A., Duarte, I.G., Machado-Pinto, J., et al. (2009), *Consenso Brasileiro de Psoríase*. Rio de Janeiro: Sociedade Brasileira de Dermatologia.
- [4] Somasundaran, P., Mehta, S.C. and Purohit, P. (2006), Silicone emulsions. *Advances in Colloid and Interface Science*, 128–130(0),103-9.

Alkyne-azide “click” chemistry in designing sugar-based bioactive compounds

P. Cardoso^{1,2}, M. Correia-da-Silva^{1,2,3}, E. Sousa^{1,2,3} and M. Pinto^{1,2,3}

¹ Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP). ² Laboratório de Química Orgânica e Farmacêutica, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. ³ Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal.

Considering carbohydrates, their intrinsic merits such as conformational flexibility, high biocompatibility and low toxicity, as well as the abundantly affordable, made them interesting scaffolds for sugar-based drug discovery [1]. In this work the synthesis of new galactosyl-based compounds by “green click” chemistry is reported (Fig. 1).

A simple and effective synthesis of tetraacetate β -galactosyl azide, by the reaction of commercially available tetraacetate α -galactosyl bromide with sodium azide in aqueous acetone as solvent, was performed in only 2 hours at room temperature (r.t.) with good yield (**1**, 70%). Propargylation of a heterocyclic compound, under basic conditions in acetone, afforded a *bis* alkyne (**2**, 60%) after 3 hours in reflux. Using the “click” building blocks **1** and **2**, the copper catalyzed alkyne-azide cycloaddition was applied and the sugar-based product (**3**) purified by silica flash chromatography [2]. The deprotected compound was obtained in 1 hour after treatment with MeONa solution at r.t (**4**, 90%). Structure elucidation was performed by IR and ¹H and ¹³C NMR.

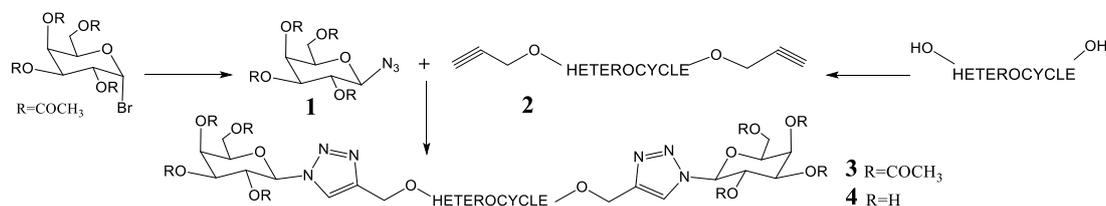


Figure 1. Synthesis of triazole-linked heterocyclic galactosides.

In conclusion, new *bis* triazole-linked heterocyclic galactosides (protected and deprotected) were effectively obtained. These molecules are predictable to have interesting *in vivo* half lifes, associated to the influence of the glycosaminoglycans moieties. Future studies will involve the characterization of biological effects of these synthetic triazole-linked heterocyclic galactosides.

Acknowledgments: FCT, PEst-OE/SAU/UI4040/2011, FEDER, POCI, POPH/FSE/QREN for financial support and for the post-doctoral grant to M. Correia-da-Silva (SFRH/BPD/81878/2011).

References:

[1] Meutermans, W., G.T. Le, B. Becker (2006), *Carbohydrates as Scaffolds in Drug Discovery*, ChemMedChem, 1 (11), 1164-1194.

[2] Chen, Q., et al. (2010), *Glucosamine hydrochloride functionalized tetraphenylethylene: A novel fluorescent probe for alkaline phosphatase based on the aggregation-induced emission*. Chemical Communications, 46 (23), 4067-4069.

Acetabuloplasty procedures: Objectives, Indications and Results

M. Cruz¹, G. Costa²

¹ Faculty of Medicine, University of Porto, Portugal.

² Chairman and Professor of Orthopaedic and Trauma, Faculty of Medicine, University of Porto, Portugal; Director of Childrens Orthopaedics Department, Hospital São João, Porto, Portugal.

Acetabuloplasty is a surgical technique which leads to the increasing of the acetabular roof by inserting a bone graft, thus improving the coverage of the femoral head [1,2].

Objectives: Evaluate, in the X-ray image, the improvement of the coverage of the femoral head by the acetabulum obtained with the surgery. Thus, we determined the acetabular index (AI) and the percentage of femoral head covered by the acetabulum (PFHA), before and after an acetabuloplasty, in patients aged at 1 to 14 years.

Methods: This is a retrospective observational study, in which we included 20 patients who underwent an acetabuloplasty. The AI and PFHA were measured in the X-ray image. Fifteen patients underwent a shelf acetabuloplasty. At the time of surgery, patients had a mean age of 8,7 years.

Results: Only 4 patients had a higher value of AI postoperatively (AIPO) compared with the preoperative AI (AIPR). Twelve patients had an AIPO value of less than 20°. The PFHA postoperatively (PFHA-PO) value, of all patients, exceeded 75%. Undergoing an acetabuloplasty led to a statistically significant difference of the AI and the PFHA values.

Conclusion: There was a clear improvement in the radiographic parameters after an acetabuloplasty.

References:

- [1] Daly, K., Bruce, C. and Catterall, A. (1999), *Lateral shelf acetabuloplasty in Perthes' disease. A review of the end of growth*, Journal of Bone and Joint Surgery - British Volume, 81 (3), 380-384.
- [2] Sales de Gauzy, J. (2010) *Pelvic reorientation osteotomies and acetabuloplasties in children. Surgical technique*, Orthopaedics & Traumatology: Surgery & Research, 96 (7), 793-799.

Determination of macrophage distribution at the feto-maternal interface during *Toxoplasma gondii* infection and implications on pregnancy outcome using the mice model

QM. Lin¹, N. Teixeira¹, M. Borges¹

¹ Biological Sciences Department, Faculty of Pharmacy, University of Porto, Portugal.

Congenital Toxoplasmosis is caused by *Toxoplasma gondii* and is a prevalent disease worldwide, with serious implications for the fetus as mental retardation, blindness, cerebral palsy, stillbirth and miscarriage. Macrophages have a key role in innate immunity, but also participate as an effector cell population in the adaptive immune response.

In this work, we intend to determine the localization of macrophages in the fetoplacental units from *T. gondii* infected pregnant Balb/c mice at different time points of gestation and if it correlates with the outcome of pregnancy. We have used pregnant Balb/c mice and a *T. gondii* line, derived from Me49 (a type II strain) stably expressing green fluorescence protein. Systemic infection was confirmed by the detection of the parasite in the liver, spleen, kidney and fetoplacental units using fluorescence microscopy. The localization pattern of macrophages in fetoplacental units from *T. gondii*-infected or non-infected BALB/c mice was studied by evaluating the expression of F4/80 cell marker, by immunohistochemistry. It was detected F4/80+ cells in the anti-mesometrial decidua and in the metrial gland of the fetoplacental units from infected animals, indicating the presence of macrophages in fetoplacental units during *T. gondii* infection. Moreover, histological analysis of fetoplacental units suggested a delay in the placenta development and an alteration of the resorption process during *T. gondii* infection. These results will allow pursuing the study to determine the relationship between macrophages and the pathology associated with congenital toxoplasmosis.

Birth in Portugal: a national descriptive study

R. Loureiro¹, J. Bernardes², P. Freitas³

¹ Department of Obstetrics and Gynecology, Faculty of Medicine, University of Porto, Portugal.

² Department of Obstetrics and Gynecology, Faculty of Medicine, University of Porto, Portugal.

³ Department of Behavioural Sciences, Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

Introduction: Recent transformations have influenced the circumstances of birth and the experience of motherhood. We assessed the actual characteristics of pregnancy and birth giving in Portugal.

Material and methods: Quantitative, observational, descriptive transverse study. The studied population were the 96925 puerperal women of year 2007 and the sample the 2505 women from 19 hospitals from all over the country, who answered a questionnaire.

Results: The puerperal women were on average 29.4 years old and 16.7% were over 35. The average number of children was 1.56, with 89.5% of deliveries in the North and Centre of the country and in Lisbon. 73.4% of pregnancies were planned and only 0.2% were not medically supervised. Complications occurred in 21.3% of the cases, namely miscarriage (4.0%) and diabetes (3.0%). In 21.4% of pregnancies there was training for childbirth. Delivery was performed by a physician in 53,9% of cases, at 38.8 weeks average gestational age. Eutocic delivery occurred in 54.5% of cases and caesarean section in 34.7%. Epidural analgesia occurred in 74.7% of cases and 74.5% of eutocic births had the presence of a support person.

Discussion and conclusion: There is evidence of a tendency for desertification of some regions, with a late age for having children, an average number of children close to one and a high rate of births from non-married couples. More than half of the deliveries were performed by a physician with a high caesarean section rate and a wide national availability of epidural analgesia and of a support person for the mother.

Comparison of the fertility, protein content and allergenicity between transgenic and conventional *Zea mays* pollen.

Marta A. Sousa¹, Helena Ribeiro², Susana Pereira^{1,3} & Ilda Abreu^{1,2}

¹Department of Biology, Faculty of Sciences of the University of Porto, Portugal

²Geology Centre of the University of Porto, Portugal

³BioFIG – Center for Biodiversity, Functional and Integrative Genomics, Portugal

Plant genetic engineering offers opportunities for the creation of insect-resistant plants by insertion and expression in plant of entomopathogenic proteins. Transgenic *Zea mays* is a variant of maize that has been genetically altered to express one or more proteins from the *Bacillus thuringiensis* bacteria (Bt). The protein is poisonous to certain insect pests and is widely used in organic gardening.

In this work, we intended to assess the fertility rate, protein content and allergenicity of transgenic maize pollen in comparison to conventional pollen samples (control). The pollen fertility was evaluated by the viability test using several vital stain tests namely Alexander's stain, Trypan Blue Dye, FDA (fluorescein diacetate), X-gal test and lugol solution. Biochemical and immunochemical assays were performed in order to analyze changes in the protein content, polypeptide profile by SDS-PAGE and IgE reactive profiles using sera from sensitized individuals.

The results based on the comparison of transgenic maize and control pollen revealed a decrease on fertility and differences on protein content. Differences on polypeptide profiles of pollen soluble proteins were not observed; however most of the tested sera revealed increased IgE reactivity to proteins of Bt *Zea mays* compared with the non-transgenic one.

A reliable method for testing pollen viability is essential for the study of pollination biology. Also, evaluation of protein content and allergenicity of transgenic pollen are two important aspects of risk assessment and biosafety study for transgenic plants, especially those produced to be ingested by other organisms.

Searching for cyanobacterial natural antifouling compounds against *Mytilus galloprovincialis* settlement

S. Cruz^{1,2}, J. R. Almeida² and V. Vasconcelos^{1,2}

¹ Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal.

² CIIMAR/CIMAR- Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal.

In aquatic environments, every natural or artificial underwater surface is subjected to a successional colonization by micro and macroorganisms in a process called biofouling. Biofouling represents one of the current issues in the marine environment due to the biocidal properties of the effective antifouling (AF) agents inducing toxic responses in organisms (*e.g.* tributyltin (TBT), booster biocides) and also due to the strong negative impact on the economical level leading to large investments worldwide estimated at 150 billion USD per year. Thus, there is a growing need of alternative non-toxic and environmentally friendly AF coatings based on natural bioactive compounds.

Cyanobacteria is a group of prokaryotic microorganisms that produce a wide range of secondary metabolites with recognized bioactivity towards a wide range of biological responses and although not so explored in terms of potential AF properties. Regarding this, this work aims to test several strains of cyanobacteria for potential AF properties using anti-settlement bioassays with adult, post-larvae and plantigrade larvae of the biofouling mussel *Mytilus galloprovincialis*.

Organisms were exposed for 15 hours to different cyanobacterial extracts-enriched media using experimental sets adapted to each developmental stage and post-exposure settlement was determined by the production/non-production of byssal threads by each test individual, determining the potential of each strain to inhibit mussel adhesion. Promising extracts were then fractionated by a gradient of polarity and new assays were performed testing each obtained fraction. Results showed some promise cyanobacteria crude extracts and fractions with anti-settlement activity of both adults, post-larvae and plantigrade larvae of *Mytilus galloprovincialis*.

In conclusion, this bioassay-guided screening showed effectiveness in the detection of new potential natural AF agents.

Annual profile of planktonic community of Crestuma-Lever reservoir

L. Pires¹, M.N.Vieira^{1,2}, S.C.Antunes^{1,3}

¹Department of Biology, Faculty of Sciences, University of Porto, Portugal.

²CIMAR/CIIMAR-Interdisciplinary of Marine and Environmental Research, University of Porto, Portugal

³Centre of Environmental and Marine Studies (CESAM), Campus of Santiago, University of Aveiro, Portugal

The Water Framework Directive (WFD) 2000/60/EC of the Parliament and of the Council of 23 October 2000 was transposed to national juridical order, through Water Law for Decree Law n° 58/2005 of 29 December and for Decree Law n° 77/2006 of 30 March. The WFD establishes a framework for community action in the field of water policy, in order to protection, conservation and sustainable management of water and its quality for all states members until 2015.

The reservoirs set in this regime and according to WFD, and the phytoplankton is one of the biological indicators, belonging to the water column, for classification of water mass [1]. Bearing this in mind, the main goal of this study was to assess the water quality of Crestuma – Lever reservoir taking account the physical and chemical parameters proposed by the WFD and the profile of the planktonic communities over a year. The Crestuma-Lever reservoir is exposed to a different kind of anthropogenic activities with high values of diffuse contamination [3]. According to these, two sampling points were selected to conduct this study (Marina and Crestuma). *In situ*, a several parameters were measured (pH, conductivity, temperature, Secchi disc, dissolved oxygen, TDS, ORP), and on laboratory, some nutrients proposed by WFD were quantified (ammonia, phosphates, nitrates, nitrites, BOD₅, chlorophyll *a*, turbidity and DOC). In the same sampling sites, zoo and phytoplankton samples were collected, and immediately transported to the laboratory for quantification analysis [2].

The results of physical and chemical parameters revealed that water status of Crestuma-Lever reservoir, according to WFD namely with chlorophyll *a* concentration, is classified as “less than good” in almost sampling periods. Relatively to the zooplankton matrix was possible to calculate the abundance relative of the *taxa* which occur in all sampling period. The results showed that predominant zooplankton species over a year were *Bosmina sp.* and *Moina sp.* (cladocerans) and copepods from the *Cyclopoida* group, for both sampling sites. These organisms are characteristic of polluted water, which is in accordance to the WFD classification for Crestuma-Lever reservoir [4].

References:

[1] European Commission. Directive (WFD) 2000/60/EC of the Parliament and of the Council of 23 October 2000. Official Journal of European Communities of 22 December 2000.

[2] Instituto da Água. *Manual para avaliação da qualidade biológica da água em lagos e albufeiras segundo a Diretiva Quadro da Água – Protocolo de amostragem e análise para fitoplâncton.* (2009) Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional.

[3] Barros, M.C.; Mendo, M.J.M.; Negrão, F.C.R. *Surface water quality in Portugal during a drought period.* (1995) Instituto da Água. The Science of the Total Environment, p. (69-76)

[4] Brito, S.L.; Barbosa, P.M.M.; Coelho, R.M.P. *Zooplankton as an indicator of trophic conditions in two large reservoirs in Brazil.* (1995) Lakes & Reservoirs Research and Management, 2011, p. (253-269)

Ecological adaptation in *Littorina fabalis*

Sofia Abrunheiro^{1,2}, **Graciela Sotelo**¹, **Diana Costa**^{1,2}, **Rui Faria**^{1,3}

1-CIBIO, Research Center in Biodiversity and Genetic Resources, InBio, University of Porto, Vairão, Portugal; 2-Departement of Biology, Faculty of Sciences, University of Porto, Porto, Portugal; 3-IBE, Institute of Evolutionary Biology (UPF-CSIC), Universitat Pompeu Fabra, PRBB, Barcelona, Spain.

The interface between terrestrial and marine ecosystems is one of the most extreme environmental transitions in nature, forming a very unique and dynamic habitat. The flat periwinkle *Littorina fabalis* is a marine gastropod commonly found in intertidal rocky shores throughout the Northern Hemisphere in subarctic and temperate regions. Different forms/ecotypes have been described, at least two in Northern Europe and three in the Iberian Peninsula, inhabiting different parts of the intertidal zone [1,2,3] where they face different selective pressures related with abiotic factors, such as temperature, wave force and desiccation, as well as with biotic factors such as predation and competition; making this species a good model system to study the processes of ecotype formation and ecological speciation [4,5]. Since 2011, we have sampled several rocky shores in the Iberian Peninsula in order to characterise the distribution of the *L. fabalis* ecotypes described for this region. Data for abiotic parameters (air and water temperatures, tide cycle, wave height and period) were collected from public databases for some of the European shores in order to identify those factors potentially driving *L. fabalis* ecotype formation. We also implanted temperature, humidity and wave force loggers in several shores to add data from the field and complement the available information. Concerning biotic parameters, experiments of crab predation and mating behaviour were initiated in the lab to understand how these factors could explain some of the differences between *L. fabalis* ecotypes. Our preliminary findings based on these different approaches will be presented and the evolutionary forces underlying ecotype evolution in *L. fabalis* will be discussed.

References:

- [1] Rolán, E. and Templado, J. (1987), *Consideraciones sobre el complejo Littorina obtusata-mariae (Mollusca, Gastropoda, Littorinidae) en el Nordeste de la Peninsula Iberica*. Thalassas, 5: 71-85.
- [2] Williams, G. A. (1990), *The Comparative Ecology of the flat Periwinkles, Littorina obtusata(L.) an L. mariae Sacchi et Rastelli*. Field Studies, 7: 469-482.
- [3] Tatarenkov, A. and Johannesson, K. (1998), *Evidence of a reproductive barrier between two forms of the marine periwinkle Littorina fabalis (Gastropoda)*. Biological Journal of the Linnean Society, 63: 349-365.
- [4] Tomanek, L. and Helmuth, B. (2002), *Physiological Ecology of Rocky Intertidal Organisms: A Synergy of Concepts*. Integrative and Comparative Biology, 42: 771-775.
- [5] Rolán-Alvarez, E. (2007), *Sympatric speciation as by-product of ecological adaptation in the galician Littorina saxatilis hybrid zone*. Journal of Molluscan Studies, 73: 1-10.

Area and edge effects on leaf-litter decomposition in a fragmented Subtropical Dry Forest

M.L. Moreno^{1,2,3}, **M.L. Bernaschini**^{2,3}, **N. Pérez-Harguindeguy**² and **G. Valladares**^{2,3}

¹ Department of Biology/CIBIO, Landscape, Faculty of Science, University of Porto, Portugal

² Institute for Multidisciplinary Plant Biology (IMBIV), University of Córdoba, Argentina

³ Center of Cordoba Entomological Research (CIEC), University of Córdoba, Argentina

South American tropical and subtropical dry forests are disappearing at an alarming rate, with 80 % deforestation of the original area being recorded in the last decade. Deforestation involves habitat loss and fragmentation, i.e. the transformation of a forested area to a number of smaller forest remnants embedded in a matrix with different land use, usually agricultural. In forest remnants, changes in soil environment (i.e. the sum of soil physicochemical and biological properties, and microclimate) could have significant effects on key ecosystems processes. In this work, we experimentally analyzed leaf-litter decomposition in a fragmented Chaco Serrano forest in central Argentina. We asked whether decomposition can be affected by forest area and edge effects. By using a common substrate (from native plants) we avoided effects mediated by changes in litter quality, thus focussing on effects of *in situ* conditions for decomposition. We collected freshly senesced leaf material of two abundant native species and constructed 72 litter-bags. The litter-bags were incubated during 120 days in the edge and interior of 12 forest remnants (0.57-1000 ha). We analyzed by linear mixed models if the decomposition rate (% dry weight mass loss) of a common substrate varied with fragment size and between forest edge and interior. Decomposition declined with fragment size, with no significant effects of location (edge/interior). Our results suggest that *in situ* conditions for decomposition may change as a consequence of forest fragmentation, specifically as a result fragment size. The results also suggest that the mechanisms underlying such effects may involve changes in decomposer/detritivores biota.

A new approach for the discovery of antivirals against norovirus

J. Rocha-Pereira ¹, L. Neves ¹, N. Ribeiro ¹,
M.S.J. Nascimento ¹

¹ Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Portugal

Human noroviruses are the leading cause of foodborne outbreaks and sporadic cases of gastroenteritis worldwide. Despite their impact in public health there are no antiviral drugs to treat or prevent this important illness. Human norovirus are not cultivable which hampers the use of classic infectivity assays to identify antivirals for norovirus. The Norwalk virus (NV) replicon-bearing cell line is today the closest model to human norovirus available and constitutes an innovative approach to find new antivirals against norovirus.

The aim of the present study was to implement and optimize an antiviral assay based on the NV replicon model and use it to evaluate the anti-norovirus activity of 10 chemical compounds, namely (i) the nucleoside analog 2'-C-methylcytidine [2CMC]; (ii) two benzo[b]thiophene-2-carboxylates [4MBC and 2FBC]; (iii) six cysteine protease inhibitors and (iv) the broad spectrum antiviral drug favipiravir [T-705].

The HG23 cells (Norwalk virus replicon-bearing Huh-7 cells) were cultivated in the presence of increasing concentrations of G418 (geneticin) in order to maximize the expression of the replicon. The antiviral activity of compounds was assessed through the quantification of NV genome copies by quantitative reverse transcription-PCR (qRT-PCR) with the MiniOpticon (BioRad). Ribavirin was used as a reference compound to validate the antiviral assay. The cytotoxicity of the compounds was evaluated by the MTT colorimetric assay and by qRT-PCR for the housekeeping gene β -actin.

2CMC was able to reduce the levels of the Norwalk virus RNA in a dose-dependent manner. The effective dose necessary for 2CMC to reduce NV genome copies to 50% (EC₅₀) was 18 μ M. 4MBC reduced levels of Norwalk virus RNA with an EC₅₀ of 72 μ M but the maximum reduction obtained was 70%. 2FBC did not display any antiviral effect against the Norwalk replicon. None of the six cysteine protease inhibitors presented antiviral activity in the Norwalk replicon up to the tested concentrations of 100 μ M. T-705 showed an inhibitory effect with an EC₅₀ of 31 μ M, but was unable to inhibit > 90 % of the Norwalk virus replicon.

In conclusion, we here successfully implemented a Norwalk replicon-based antiviral assay to evaluate the inhibitory effect of compounds against human norovirus. We identified 3 compounds with anti-norovirus activity. Among these, 2CMC showed the strongest inhibitory effect and should be further explored in the design and development of anti-norovirus drugs.

Acknowledgements:

Kyeong-Ok Chang (Kansas State University, USA) for the Norwalk replicon-bearing cells. FCT for the PhD grant of J. Rocha-Pereira (SFRH/BD/48156/2008). IJUP Projectos Pluridisciplinares 2012 for the funding.

References:

Chang KO et al.(2006) Virology 353:463-473

Effects of coloured and non-coloured phenolics of *Echium plantagineum* L. bee pollen in Caco-2 cells

P. Monteiro, E. Moita, P. Valentão, P. B. Andrade and C. Sousa

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Echium plantagineum L. bee pollen is a rich source of non-coloured [1,2] and coloured [3] phenolic compounds. A hydromethanolic extract rich in kaempferol derivatives, previously studied, revealed to have antioxidant (cell-free systems) and anti-inflammatory potential in lipopolysaccharide-insulted RAW 264.7 macrophages [2].

In this work, three phenolic extracts of *E. plantagineum* bee pollen were characterized by HPLC-DAD and tested in the colorectal adenocarcinoma Caco-2 cells: coloured phenolics extract rich in anthocyanins, namely petunidin derivatives, non-coloured phenolics extract containing kaempferol derivatives and a whole extract presenting both flavonols and anthocyanins. Only the whole extract displayed toxicity towards Caco-2 cells at 20 mg mL⁻¹, as assessed by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release assays (Fig. 1). As so, the extracts at non-toxic concentrations are being tested for its antioxidant potential in *tert*-butyl hydroperoxide insulted Caco-2 cells.

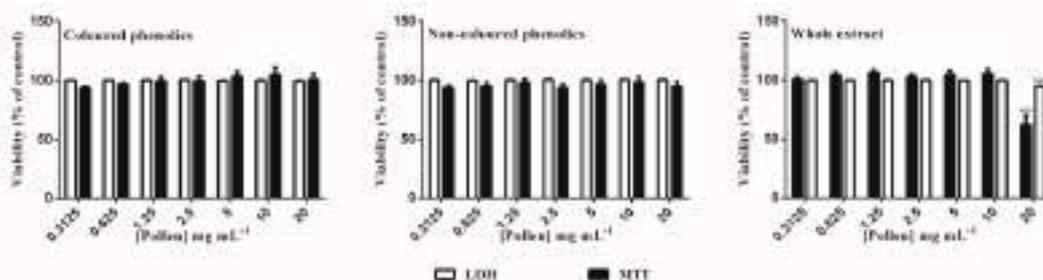


Fig. 1. Effect of *E. plantagineum* bee pollen extracts on the viability of Caco-2 cells exposed to the extracts for 24 h, assessed by MTT reduction and LDH release assays. Values show mean + SEM of at least three independent assays, performed in triplicate. *** $p < 0.001$.

Acknowledgments: The authors are grateful to QREN (NORTE-07-0124-FEDER-000069).

References:

- [1] Ferreres, F., Pereira, D. M., Valentão, P. and Andrade, P. B. (2010), *First report of non-coloured flavonoids in Echium plantagineum bee pollen: differentiation of isomers by liquid chromatography/iontrap mass spectrometry*, Rapid Communications in Mass Spectrometry, 24, 801-806.
- [2] Moita, E., Gil-Izquierdo, A., Sousa, C., Ferreres, F., Silva, L. R., Valentão, P., Domínguez-Perles, R., Baenas, N. and Andrade, P. B. (2013), *Integrated analysis of COX-2 and iNOS derived inflammatory mediators in LPS-stimulated RAW macrophages pre-exposed to Echium plantagineum L. bee pollen extract*, PlosOne, 8(3), e59131.
- [3] Di Paola-Naranjo, R. D., Sánchez-Sánchez, J., González-Paramás, A. M. and Rivas-Gonzalo, J. C. (2004), *Liquid chromatographic–mass spectrometric analysis of anthocyanin composition of dark blue bee pollen from Echium plantagineum*, Journal of Chromatography A, 1054, 205–210.

Antibacterial activity of leaf and fruit essential oils of *Schinus areira* L. from northwestern Argentina

L. Celaya^{1,2}, C. I. Viturro¹ and L. R. Silva²

¹ Laboratory of Natural Products PRONOA, Faculty of Engineering, National University of Jujuy, Argentina.

² REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Schinus areira L. (molle) is a tree native from South America and widely distributed through northwestern Argentina. Different parts of this plant are used in traditional medicine as antimicrobial, antiviral, repellent, antiseptic or diuretic [1,2]. The fruits are aromatic and used like pepper flavor in traditional foods [2].

The interest of the present study is focused in the determination of antibacterial activity of essential oils (EO) from *S. areira* growing in Jujuy province (Argentina) against Gram-positive and Gram-negative bacteria. For this purpose, EO was obtained by distillation of four dried samples of leaves and fruits (HN12, HN13, HN14 and HN16). EO chemical composition was characterized by GC-MS and GC-FID.

The results showed that EO isolated from leaves and fruits have higher antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus cereus*). On the other hand, high concentration of EO was needed to have bacteriostatic effect on Gram-negative bacteria (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*), the last strain being the most resistant

The chemical composition of EO from *S. areira* fruits and leaves revealed to be similar, with a major percentage of β -phelandrene, α -phelandrene, limonene, mircene and sabinene. In general way, extracts with higher amounts in β -phelandrene and limonene showed a great antibacterial activity (samples HN12 and HN14).

These results confirm the antimicrobial potential of EO of *S. areira* to inhibit the growth of different bacteria and contribute to the valorization of a native plants from Argentina.

References:

- [1] Bras C., Domínguez S., Codón S., Minetti A., Ferrero A. (2010). Consequences of subchronic exposure to ethanolic extract from fruits and leaves of *Schinus molle* var. *areira* L. in mice. *J Ethnopharmacol.*, 132, 321–327.
- [2] Alabrudzińska M. H., Celaya L. S., Molina A. C., Viturro C. I. and Moreno S. (2013). Antibacterial activity of essential oils obtained from leaves and fruits of a native plant from South America *Schinus areira*. EUTOBIOTECH 2013, October 2013, Krakow, Poland. Abstract Book ISSN 0001 527X.

In vitro* studies to assess the biological potential of *Endopleura uchi

R. B. Pereira , B. Schindler, R. Teixeira and L. R. Silva

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Endopleura uchi is an Amazonian tree and its bark is used in the preparation of teas against arthritis, cholesterol, diarrhea and cancer [1]. In spite of its large use, the antidiabetic, anti-cholinesterase, antioxidant and cytotoxic effect on human colorectal adenocarcinoma Caco-2 cells have not been assessed before and its chemical composition is scarcely known.

This study was conducted in order to assess the metabolite profile and biological activities of ethanol:water and infusion extracts of *E. uchi* barks. Phenolic compounds were analyzed by HPLC-DAD. Antioxidant, anti-cholinesterases, antibacterial and α -glucosidase inhibition capacity were checked. Additionally, infusion was tested against Caco-2 cells.

Five phenolic compounds were determined, bergenin being the major one. The extracts showed a dose-dependent response against DPPH[•], superoxide and nitric oxide radicals. The same effect was observed in acetylcholinesterase, butyrylcholinesterase and α -glucosidase inhibitory assays. Antibacterial capacity of both extracts investigated against Gram-positive and Gram-negative bacteria revealed to be more effective against the first. The concentrations of infusion tested revealed that it is non-toxic for intestinal cells. The bioactivities observed may be due, at least partially, to the presence of the determined phenolic compounds, particularly to bergenin and its derivatives. The results suggest that the extracts of *E. uchi* may be interesting for incorporating in pharmaceutical preparations for human health, since it can suppress hyperglycaemia and inhibit cholinesterases, and/or as food additive due to its antiradical and antibacterial activities.

References:

[1] Politi FAS, Mello JCP, Migliato KF., Nepomuceno ALA, Moreira RRD, Pietro RCLR (2011). Antimicrobial, cytotoxic and antioxidant activities and determination of the total tannin content of bark extracts *Endopleura uchi*. *Int. J. Mol. Med. Sci.*, 12(4), 2757-2768.

Influence of low isoflurane anaesthesia on the recall of object recognition task in adult mice – a pilot test

J. Ferreira^{1,2}, I. A. S Olsson², and A.M. Valentim².

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal

² Laboratory Animal Science group, Instituto de Biologia Molecular e Celular, University of Porto, Portugal

Anaesthesia can be described as a reversible process of intoxication where the individual undergoes a state of unconsciousness from which s/he can recover without harm. Over the last decade, concern has been raised over long-term effects of anaesthesia. We have previously found that low-concentration of anaesthesia with isoflurane (1%) impaired spatial task learning in mice [1, 2]. The influence of this anaesthetic on non-spatial tasks and on other processes of memory has not been studied. Here we present a pilot study preparing for a larger experiment to study the effect of 1% of isoflurane on the memory stage recall, where the individual retrieves information from the past, using the object recognition test in adult mice.

Ten C57BL/6 male mice were randomly divided into two groups: control group (non-anesthetized animals) and ISO group where mice were anesthetized for 1 hour with 1% of isoflurane concentration. After 3 days of arena habituation, each mouse was placed in the arena with two identical objects and allowed to explore for 10 minutes- sample phase. On the morning of the next day, anaesthesia was given to the ISO group. Seven hours afterwards, each mouse was placed in the arena with one object previously shown (familiar) and with a new object (unfamiliar), and allowed to explore for 10 minutes-choice phase. Video recordings from the test will be analysed with *Observer*. Statistical analysis will be performed using unpaired Student's t-test for parametric data or Mann-Whitney test for non-parametric data.

The data will soon be analysed so the results will be presented in the IJUP 2014. It is expected that the control group explore more the unfamiliar than the familiar object in the choice phase, indicating an intact recognition memory. The discrimination index (difference between the time of exploration of new and familiar object) will be compared between the two treatment groups.

A low number of animals were used because this is only a pilot test. This pilot will allow us to be sure that seven hours of interval between anaesthesia procedure and test would be enough for the ISO animals to move normally, with no external signs of sedation. Furthermore, if the variability within groups is low, it would be possible to have an indication if 1% isoflurane affects the recall of a non-spatial task.

References:

- [1] Valentim, A.M., Alves, H.C., Olsson, I.A.S and Antunes, L. M. (2008) *The effects of depth of isoflurane anesthesia on the performance of mice in a simple spatial learning task*. Journal of the American Association for Laboratory Animal Science 47(3):16-19.
- [2] Valentim A., Di Giminiani P., Ribeiro P., Rodrigues P., Olsson I. A.S, Antunes L. (2010) *Lower isoflurane concentration affects spatial learning and neurodegeneration in adult mice compared with higher concentrations*. Anesthesiology 113:1099-1108.

***Cystoseira usneoides* (Linnaeus) M. Roberts effects on the viability of the neuroblastoma cell line SH-SY5Y and inhibition of cholinesterases' activity**

J. Costa, M. Barbosa, B. R. Pinho, P. Valentão, P. B. Andrade and C. Sousa

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

The metabolic profile of an ethanol extract of the brown algae *Cystoseira usneoides* (Linnaeus) M. Roberts was established by GC-MS after derivatization with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). The extract was characterized by the presence of proline (amino acid), phloroglucinol (phenolic compound), mannitol (sugar), palmitic and stearic acids (saturated fatty acids) and fucosterol (sterol) [1]. In addition, the extract displayed activity against acetylcholinesterase (AChE) from electric eel and butyrylcholinesterase (BChE) from equine serum [1]. Based in these promising results, the extract was assessed for its effect in the viability of human neuroblastoma cell line SH-SY5Y and capacity to inhibit both AChE and BChE obtained from this cell line [2]. Cellular viability, as evaluated by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release assays, was significantly affected only for the extract concentration of 1 mg/ml (Fig. 1). The inhibitory effects of AChE and BChE previously observed were not confirmed using the enzymes from SH-SY5Y cells.

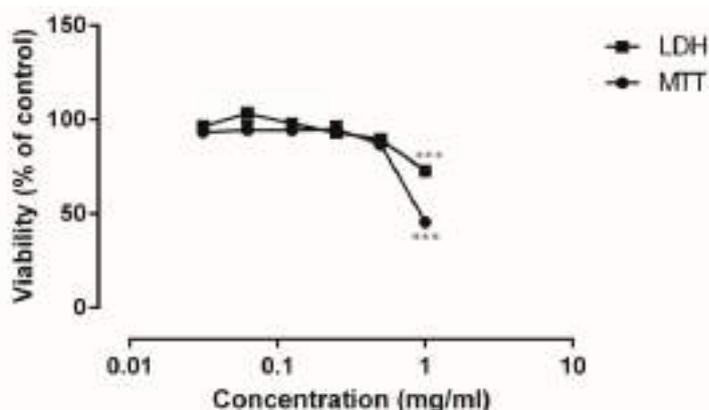


Fig. 1. Effect of *C. usneoides* ethanol extract in SH-SY5Y cells viability exposed to the extract for 24 h. Values show mean + SEM of five independent assays, performed in triplicate. *** $p < 0.001$.

Acknowledgments: The authors are grateful to QREN (NORTE-07-0124-FEDER-000069).

References:

- [1] Andrade, P. B., Barbosa, M., Matos, R. P., Lopes, G., Vinholes, J., Mouga, T. and Valentão, P. (2013), *Valuable compounds in macroalgae extracts*, Food Chemistry, 138, 1819–1828.
- [2] Taveira, M., Sousa, C., Valentão, P., Ferreres, F., Teixeira, J. P., and Andrade, P. B. (2014), *Neuroprotective effect of steroidal alkaloids on glutamate-induced toxicity by preserving mitochondrial membrane potential and reducing oxidative stress*, Journal of Steroid Biochemistry and Molecular Biology, dx.doi.org/10.1016/j.jsbmb.2013.12.013 (in press).

Evolution of post-zygotic barriers in a scenario of non-allopatric ecological speciation

R. Pereira¹, M. Martínez –Fernandez^{2,3}, C. Lopez-Fernandez⁴, Z. Ferreira¹, A. Sá-Pinto¹, J. Gosálvez⁴ and E. Rolán-Alvarez^{2,5}

¹CIBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos, Vairão, Portugal

²Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidade de Vigo, Vigo, Spain

³Unidad de Oncología Molecular, Dpto. Investigación Básica, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Madrid, Spain

⁴Departamento de Biología, Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain

⁵Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA

Non-allopatric speciation is an unlikely speciation scenario and knowledge on the evolution of distinct reproductive barriers in non-allopatric speciation and their importance for these processes is scarce [1].

Littorina saxatilis is one of the best studied cases of non-allopatric ecological speciation [2]. In some exposed Galician shores this species presents two ecotypes (RB and SU), which are morphologically different and occur at distinct shore areas [3] meeting at mid-shore, occasionally hybridising and forming apparently fertile hybrids [3]. Pre-zygotic barriers play a major role restricting gene flow between these two ecotypes [2, 3]. Much less evidence is known supporting the existence of post-zygotic barriers [4].

In several species, Sperm DNA Fragmentation (SDF) rates are negatively correlated with fertilisation and birth rates [6, 7] and positively correlated with abortion rates [8]. Therefore, in order to test the existence of post-zygotic barriers in this specie, we analysed the degree of SDF of individuals morphologically classified as RB, SU and hybrids. According to our results, hybrids showed significantly higher SDF rates than RB and SU males in one location and significantly higher variances in both locations. These results suggest the existence of an incipient post-zygotic barrier in a non-allopatric scenario, which strength may vary across the Galician shore. Moreover, these result highlight the potential of SDF analyses for speciation research.

References

1. Coyne, J.A. and Orr, H.A. (2004), *Speciation*, Sinauer Associates.
2. Rolán-Alvarez, E., et al. (1999), *Mechanisms of incomplete prezygotic reproductive isolation in an intertidal snail: testing behavioural models in wild populations*, *Journal of Evolutionary Biology*, 12(5) p. 879-890.
3. Rolán-alvarez, E., et al. (2004) *Nonallopatric and parallel origin of local reproductive barriers between two snail ecotypes*. *Molecular Ecology*, 13(11), p. 3415-3424.
4. Rolán-Alvarez, E., (2007) *Sympatric speciation as a by-product of ecological adaptation in the Galician *Littorina saxatilis* hybrid zone*. *Journal of Molluscan Studies*, 73(1), p. 1-10.
5. Cruz, R. and García, C. (2001) *Disruptive selection on female reproductive characters in a hybrid zone of *Littorina saxatilis**. *Evolutionary Ecology*, 15(3), p. 167-182.
6. Gwo, J.-C., et al. (2003), *Evaluation of damage in pacific oyster (*Crassostrea gigas*) spermatozoa before and after cryopreservation using comet assay*. *CryoLetters*, 24(3), p. 171-180.
7. Gosálvez, J., et al. (2011), *Sex-sorted bovine spermatozoa and DNA damage: II. Dynamic features*. *Theriogenology*, 75(2), p. 206-211.
8. Pérez-Cereales, S., et al. (2010), *Fertilization capacity with rainbow trout DNA-damaged sperm and embryo developmental success*. *Reproduction*, 139(6), p. 989-997.

SOCIAL INTERACTIONS IN A CAPTIVE PACK OF IBERIAN WOLVES (*Canis lupus signatus*)

S. Lino¹, H. de Boer² and L. Cesariny Calafate¹

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² GaiaZOO, Kerkrade, The Netherlands.

Wolves (*Canis lupus* Linnaeus, 1758) are carnivores which form familiar units (packs), usually consisting of a breeding pair and their offspring from different years (Mech and Boitani, 2003). The Iberian wolf, *Canis lupus signatus*, was described by Cabrera in 1907. It's a subspecies of *Canis lupus* endemic to the Iberian Peninsula and differs morphologically from other subspecies due its smaller size, white marks in the upper lips and black marks in their fore paws, along the tail and around their back, forming a "saddle".

In this study we analysed the social interactions between members of a captive pack of Iberian wolves, from GaiaZOO (Kerkrade, The Netherlands). The main purpose was to inquire the dominance hierarchy of the adult wolves of the pack, being a breeding couple and their offspring from different litters. Agonistic interactions within adult dyads were used to construct the dominance hierarchy, and affiliative interactions were used to quantify the existence and strength of social bonds between the whole pack. The chosen features to be analysed for the dominance hierarchy were Humbleness and Flight/Fight displays. The behavioural elements in these categories were assumed to be neutral or to signal dominance or submission. Using SOCPROG 2.4 software for MatLAB, both agonistic and affiliative interactions were analysed.

Results showed a hierarchy in which the offspring was always submissive to the breeding pair, and whose dominance relationships appeared as a sub sex hierarchy. The breeding pair acted mostly in an agonistic way towards each other and presented low values of affiliative interactions. They also had sporadic affiliative interactions with some of their offspring. With further research these results could be applied while choosing adequate pack structure and captive breeding efforts for wolves, being decisive in improving the welfare of the animals in captivity.

References:

[1] Mech, L.D. and Boitani, L. (2003), *Wolves: behavior, ecology and conservation*, University of Chicago Press, Chicago.

Phenolic profile and *in vitro* anti-cholinesterase activity of *Piper betle* leaves

A. P. Oliveira¹, J. Ferreira¹, S. Ribeiro¹, F. Ferreres², A. Gil-Izquierdo², C. Belo¹, P. Valentão¹ and P. B. Andrade¹

¹ REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

² Research Group on quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), Murcia, Spain.

Piper betle Linn. is a widely distributed plant in the tropical and subtropical regions, its leaves being largely consumed as masticator and mouth freshener [1]. In this work, the phenolic profile of *P. betle* leaves aqueous and ethanol extracts was characterized by HPLC-DAD-ESI/MSⁿ and twelve phenolic compounds, comprising a phenylpropanoid, five cinnamoyl and six flavonoids derivatives were identified (Fig. 1). Hydroxychavicol was the major compound in both extracts; however, the aqueous one presented a higher diversity of compounds.

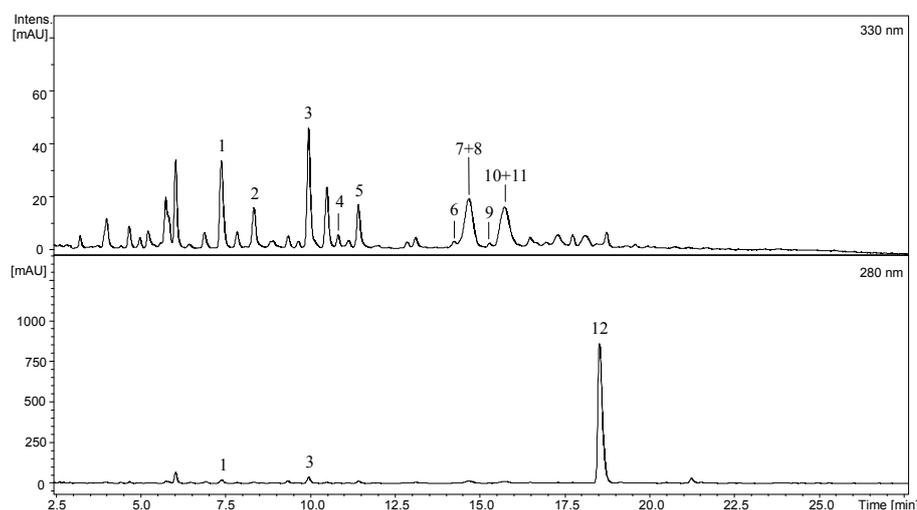


Fig. 1. HPLC phenolic profile of aqueous extract from *P. betle* leaves. (1) Caffeoyl derivative; (2) 5-caffeoylquinic acid; (3) *p*-coumaroyl derivative; (4) 4-*p*-coumaroyl quinic acid; (5) 5-*p*-coumaroyl quinic acid; (6) 8-*C*-hexosyl-luteolin; (7) 2''-*O*-hexosyl-6-*C*-hexosyl-luteolin; (8) 2''-*O*-rhamnosyl-6-*C*-hexosyl-luteolin; (9) 8-*C*-hexosyl-apigenin; (10) 2''-*O*-hexosyl-8-*C*-hexosyl-apigenin; (11) 2''-*O*-rhamnosyl-8-*C*-hexosyl-apigenin; (12) hydroxychavicol.

Regarding the cholinesterases, both extracts showed a strong activity against both acetyl- and butyrylcholinesterase, which can be due, at least partially, to the phenolic composition. The results suggest that the consumption of *P. betle* leaves as infusion can have a positive impact in the prevention and treatment of neurodegenerative diseases.

References:

[1] Guha, P. (2006), *Betel leaf: The neglected green gold of India*, Journal of Human Ecology, 19 (2), 87-93.

Xantho incisus Leach and *Macropipus puber* L.: crabs as source of bioactive compounds

A. P. Oliveira, A. C. Lopes, M. Silva, P. Valentão and P. B. Andrade

REQUIMTE/ Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

During the last decades, numerous novel compounds have been isolated from marine organisms and many of these have been reported to have biological activities, some of which are of interest from the point of view of potential drug development. Nevertheless, some marine organisms are still underexploited [1]. Thus, in order to improve the knowledge of some marine organisms, *Xantho incisus* Leach and *Macropipus puber* L., two crab species found in the Portuguese coast, were studied in this work. The carotenoids profile of the ethanol extract of both crab species was analyzed by HPLC-DAD. In a general way, both species presented a similar profile. Furthermore, their amino acids, fatty acids, sterols and triterpenes profiles are being explored by GC-MS. Regarding their biological activities, the capacity of both crab species to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) was also evaluated. A dose-dependent response against both cholinesterases was only observed with the *X. incisus* extract (Fig. 1), being more active against BuChE ($IC_{25} = 1.336$ mg/mL).

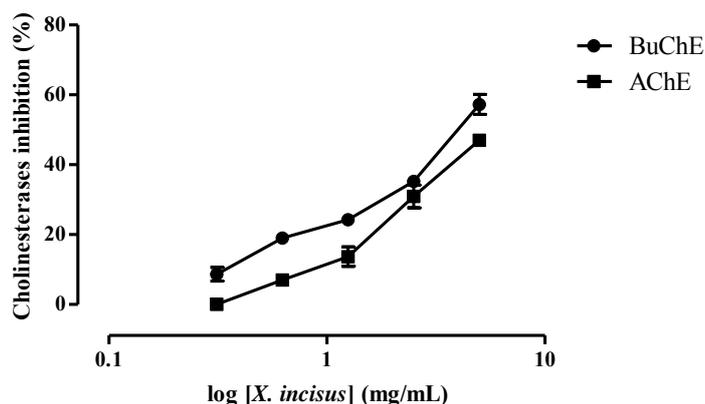


Fig. 1. AChE and BuChE inhibitory activities of *X. incisus* ethanol extract. Values show percentage of mean \pm SEM from three experiments performed in triplicate.

This activity may be due, at least partially, to the presence of the different metabolites determined in the extract.

References:

- [1] Blunden, G. (2001), *Biologically active compounds from marine organisms*, Phytotherapy Research, 15, 89-94.

Effect of different growth conditions in brown macroalgae carotenoids profile

T. Ribeiro¹, F. Fernandes¹, I. Sousa-Pinto^{2,3}, P. Valentão¹ and P. Andrade¹

¹ REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

² Interdisciplinary Centre for Marine and Environmental Research (CIIMAR/CIMAR), Portugal.

³ Faculty of Sciences, University of Porto, Portugal.

In order to survive in a highly competitive environment, seaweeds developed defence strategies that resulted in a tremendous diversity of compounds from different metabolic pathways. Among these compounds, carotenoids are highlighted. These constitute a class of metabolites widely distributed in nature, with more than 750 structures documented [1]. They are primarily responsible for the colour of some algae and are important defence compounds, for their antioxidant potential and UV protection. Seaweeds can synthesize many kinds of carotenoids, which can be used as chemotaxonomic markers. In spite the Portuguese coast being home to a great diversity of seaweed, most of them are not yet studied.

This work aimed to study the effect of different growth conditions in carotenoids profile of several kelps seaweeds species from the north Portuguese coast. For this purpose HPLC-DAD was used. Several carotenoids were found in the studied species, fucoxanthin being the most representative one (Fig. 1-A). Quantitative differences in the fucoxanthin content along the depth were observed (Fig. 1-C).

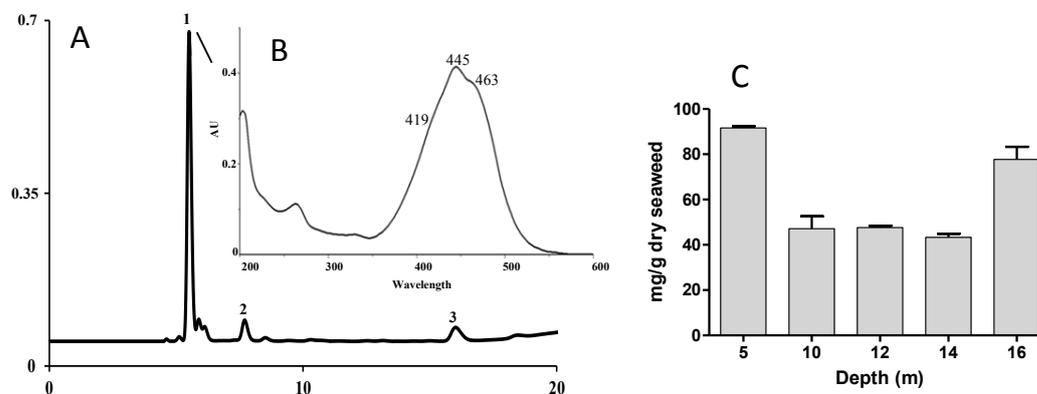


Fig. 1. (A) HPLC-DAD chromatogram of the acetonetic extract of *Laminaria ochroleuca*. Detection at 450 nm. (1) fucoxanthin; (2) unknown; (3) unknown. (B) Fucoxanthin UV-Vis spectrum. (C) Fucoxanthin content of the acetonetic extract of *Saccharina latissima* collected at different depth.

Acknowledgments: The authors are grateful to QREN (NORTE-07-0124-FEDER-000069).

References:

[1] Mohamed, S., Hashim, S.N., Hafeedza, A.R. (2012), *Seaweeds: A sustainable functional food for complementary and alternative therapy*. Trends in Food Science & Technology 23, 83-96.

Flavonoids as anti-inflammatory and anti-allergic agents

A. P. Oliveira, A. Cabeças, F. Carvalho, S. T. Silva, P. Valentão and P. B. Andrade

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Inflammation and allergy are among the most common worldwide diseases, often being present concomitantly. Recently, the attention has been directed to phytochemicals that can modulate the inflammatory and allergic responses. In this context, natural products from several sources can be interesting for the development of modern therapeutic drugs. Among them, flavonoids are recognized for their benefic health effects [1]. However, human beings are exposed to a huge variety of flavonoids, rendering difficult to know which are the most effective and whether it is a sole compound or the mixture of several of them that is responsible for the overall effects. Furthermore, the biological activities of these compounds seem to be related with structural variations among each class. Therefore, the anti-inflammatory and anti-allergic activities of several flavonoids, representative of different classes and with different substitution patterns (quercetin, quercetin-3-*O*-rutinoside, apigenin and luteolin) are being assessed by *in vitro* chemical systems. Quercetin-3-*O*-rutinoside was the most active compound ($IC_{50} = 0.184$ mg/mL) in terms of nitric oxide scavenging, a reactive nitrogen species involved in inflammation (Fig. 1).

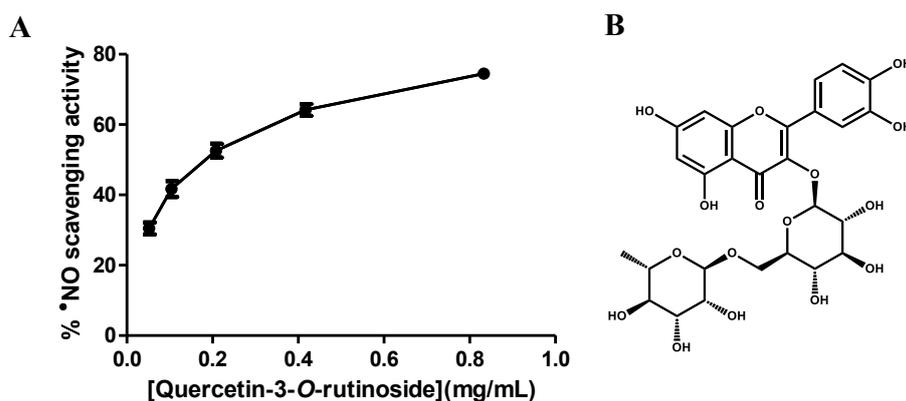


Fig. 1. (A) % *NO scavenging activity of quercetin-3-*O*-rutinoside. Values show percentage of mean \pm SEM from three experiments performed in triplicate. (B) Chemical structure of quercetin-3-*O*-rutinoside.

Furthermore, the structure-activity relationships are being established in order to investigate the existence of possible synergic or antagonic effects.

References:

- [1] Middleton, Jr.E., Kandaswami, C., Theoharides, T.C. (2000), *The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer*. Pharmacological Reviews, 52, 673-751.

Discovering microalgae: *Scenedesmus obliquus* (Turpin) Kützing

F. Fernandes, A. Surreira, A. Teixeira, C. Azevedo, C. Nunes, D. Lourenço, J. Barbosa and R. F. Gonçalves

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Aquatic organisms' compounds have been utilized in medicine and their use is a tendency for the future. This is because they live under severe conditions, like low temperatures, low light and high pressure, that make them produce secondary metabolites, some with strong pharmacologic properties. In this work, *Scenedesmus obliquus* (Turpin) Kützing, a microalgae whose literature is mostly absent, was studied.

HPLC-DAD analysis (Fig. 1) showed that the ethanol extract has carotenoids, like lutein, as well as chlorophylls. By GC-MS several amino acids, fatty acids and sterols were detected.

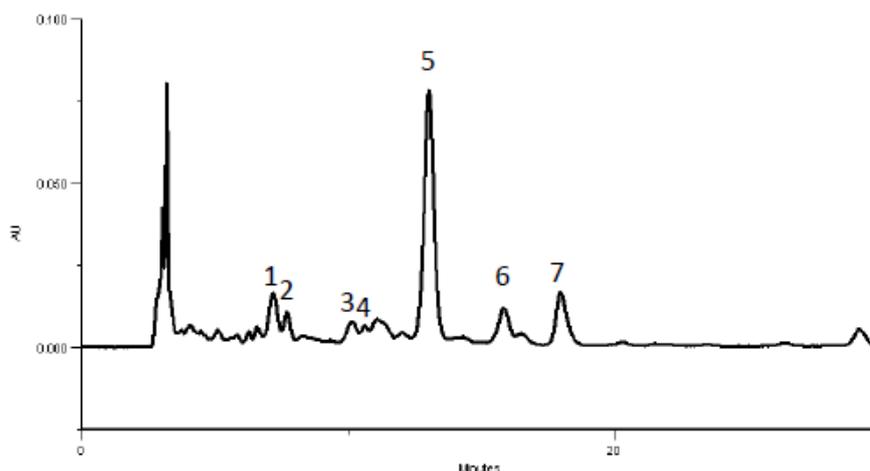


Fig. 1. HPLC-DAD chromatogram of the ethanol extract of *S. obliquus*. Detection at 450 nm. 1, 2: carotenoids; 3, 6, 7: chlorophyll *a* isomers; 4: chlorophyll; 5: lutein.

The ethanol extract showed no DPPH• scavenging activity and a modest one against nitric oxide. In order to evaluate the antibacterial activity, the extract was also tested regarding its ability to inhibit the growth of *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli* and *Salmonella typhimurium*. The last two grown at all concentrations, but the growth of *S. aureus* e *M. luteus* was reduced at the highest concentration. More studies, with different extracts of *S. obliquus*, can be done to search for different activities.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5th year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head) and Patrícia Valentão.

***Polybius henslowii* crab: An approach to crustaceans**

A. P. Oliveira, A. Alves, A. Borba, A. Teixeira, C. Seabra, J. Mendes, M. Patrão and P. Teixeira

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

An ethanol extract of the crab *Polybius henslowii* Leach collected in the Portuguese west coast was studied. Primary and secondary metabolites were determined, as was also its antioxidant and antibacterial capacity.

HPLC-DAD analysis allowed the identification of one carotenoid (fucoxanthin), while several classes of compounds were detected by GC-MS, with emphasis on amino acids and fatty acids (Fig. 1).

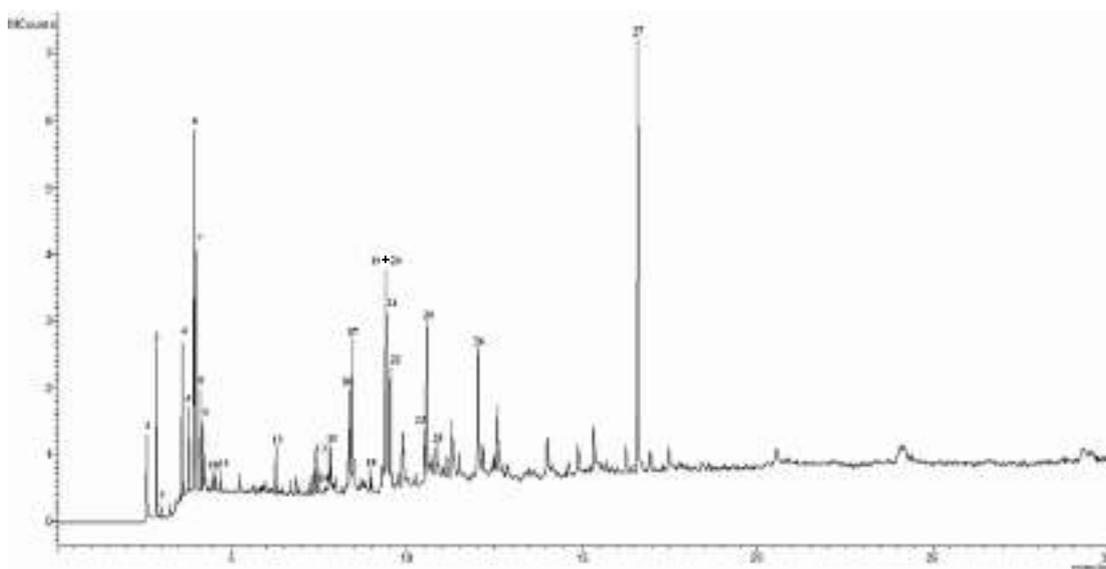


Fig. 1. GC-MS profile of the ethanol extract of *Polybius henslowii*. (1) lactic acid; (2) alanine; (3) glycine; (4) valine; (5) urea; (6) glycerol; (7) leucine; (8) isoleucine; (9) proline; (10) serine; (11) threonine; (12) phenylalanine; (13) tetradecanoic acid; (14) tyrosine; (15) pentadecanoic acid; (16) palmitelaidic acid; (17) hexadecanoic acid; (18) heptadecanoic acid; (19) linoleic acid; (20) linolenic acid; (21) oleic acid; (22) octadecanoic acid; (23) arachidonic acid; (24) eicosapentaenoic acid; (25) *cis*-11-eicosaenoic acid; (26) docosahexaenoic acid; (27) cholesterol.

The extract displayed a dose-dependent activity against nitric oxide, though no protective effect was observed against selected Gram-positive and Gram-negative bacteria strains. Other extracts could be prepared in order to find more interesting effects.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5th year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head) and Patrícia Valentão.

Development of factorial design for a phenolic-rich extract of *Chelidonium majus* L.

J. Lima, M. Sampaio, Patrícia Valentão, Paula B. Andrade and C. Grosso

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Chelidonium majus L. is characterized by its high content of alkaloids and few information is available on its phenolic composition (Fig. 1). Therefore, a Box-Behnken factorial design was developed with the aim of obtaining an extract of *C. majus* rich in phenolic compounds. Four parameters were combined at three different levels, in order to study their influence on extraction yield and phenolic composition (extraction time - 90, 120, 150 min.; extraction temperature - 25, 42.5, 60 °C; extraction solvent - water, water:methanol, methanol; and solid/solvent ratio - 1:50, 1:100; 1:150).

Methanolic extracts afforded the lowest extraction yields and this parameter is proportional to time of extraction and solid:solvent ratio. On the other hand, the highest yields were obtained for the aqueous extracts, but these contained the lowest amount of phenolic compounds. In general, the content of phenolic compounds is lower when extractions are performed at 60 °C. The best compromise between extraction yield and phenolics content was observed for extractions performed with a mixture of methanol:water.

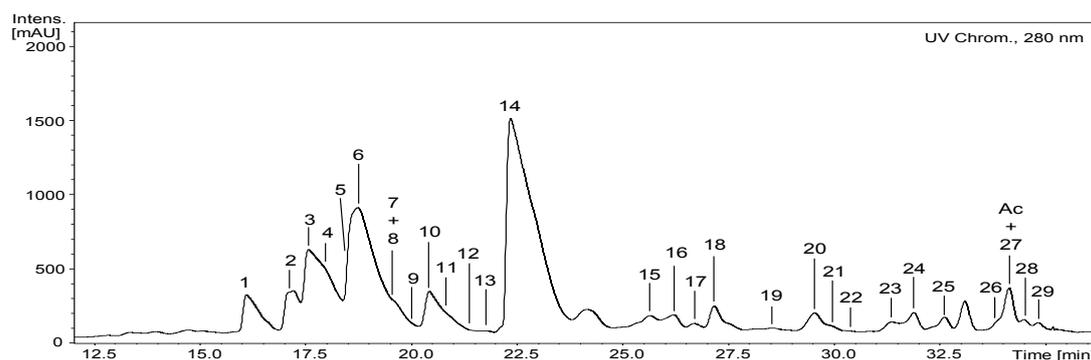


Fig. 1. HPLC-UV chromatogram of the hydromethanolic extract from *C. majus*. 1 - Dihydroberberine; 2 - Protopine; 3 - Allocryptopine; 4 - Chelidonine; 5 - Alkaloid A; 6 - Coptisine; 7 - Alkaloid B; 8 - Alkaloid C; 9 - Caffeoyl threonic acid; 10 - Tetrahydrocoptisine; 11 - Tetrahydroberberine; 12 - Caffeoyl glyceric acid; 13 - Caffeoyl malic acid; 14 - Berberine; 15 - Norchelidonine; 16 - Alkaloid D; 17 - Alkaloid E; 18 - Chelerythrine; 19 - Kaempferol-3-glucosyl-7-rhamnoside; 20 - Quercetin-3-rhamnosyl(1→6)glucoside; 21 - Quercetin-3-glucoside; 22 - Quercetin-3-glucuronide; 23 - Syringoyl caffeic acid; 24 - Syringoyl caffeic acid isomer; 25 - Kaempferol-3,7-di-rhamnoside; 26 - Quercetin-3-rhamnoside; 27 - Kaempferol-3-glucoside; 28 - Isorhamnetin-3-glucoside; 29 - Isorhamnetin-3-rhamnosyl(1→6)glucoside.

Acknowledgements: The authors are grateful to FCT for grant no. PEst-C/EQB/LA0006/2011 and to “Programa Operacional Regional do Norte ON.2” (P23-NORTE-01-0124-FEDER-000070. Clara Grosso thanks FCT for the Post-Doc fellowship (SFRH/BPD/63922/2009).

Relationship between Alzheimer's disease and type 2 diabetes: anticholinesterase and anti- α -glucosidase activity of *Pilocarpus pennatifolius* Lem. herbal tea

A. Fernandes, P. Valentão, P. B. Andrade and C. Grosso

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Recent evidences point to a possible link between type 2 diabetes and Alzheimer's disease (AD), being also suggested that AD is a different type of diabetes, type 3 [1]. Since *Pilocarpus pennatifolius* Lem. is known for its antidiabetic properties, the ability of the herbal tea prepared from a commercial sample to inhibit α -glucosidase and cholinesterases (acetyl- and butyrylcholinesterase, AChE and BuChE), enzymes involved in the pathogenesis of type 2 diabetes and AD, respectively, was undertaken. The results revealed that this extract is a better α -glucosidase inhibitor ($IC_{50} = 0.3$ mg/mL) than cholinesterases inhibitor ($IC_{50} = 1.7$ mg/mL for both AChE and BuChE) (Fig. 1A-C).

Although the morphology of the commercial plant material used seems to indicate that it is *P. pennatifolius*, its chemical composition rise some doubts. This species is supposed to contain pilocarpine and other imidazole alkaloids up to 1% of dry matter weight [2] but negative results were obtained in the general test for alkaloids detection. Moreover, the dominant compound found was quercetin-3-*O*-rutinoside, a compound never described before for this species. In this way, the authenticity of this species is under investigation.

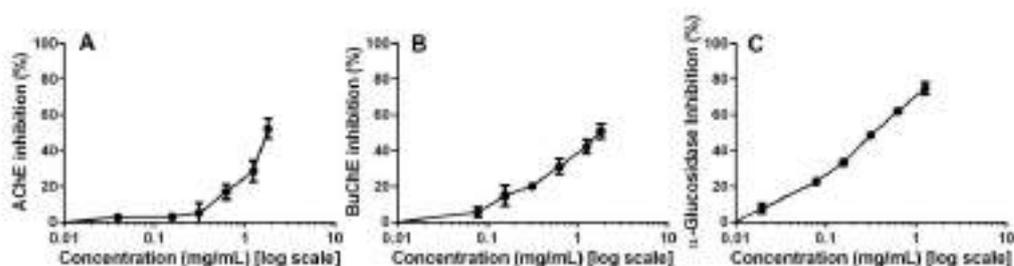


Fig. 1. *P. pennatifolius* herbal tea inhibition of AChE (A), BuChE (B) and α -glucosidase (C). Results show mean \pm SEM of three experiments performed in triplicate.

Acknowledgements: The authors are grateful to FCT for grant no. PEst-C/EQB/LA0006/2011 and to "Programa Operacional Regional do Norte ON.2" (P23-NORTE-01-0124-FEDER-000070. Clara Grosso thanks FCT for the Post-Doc fellowship (SFRH/BPD/63922/2009).

References:

- [1] Kroner, Z. (2009), *The Relationship Between Alzheimer's Disease and Diabetes: Type 3 Diabetes?*, *Alternative Medicine Review*, 14 (4), 373-379.
- [2] Sawaya, A.C.H.F., Vaz, B.G., Eberlin, M.N. and Mazzafera, P. (2011), *Screening Species of Pilocarpus (Rutaceae) as Sources of Pilocarpine and other Imidazole Alkaloids*, *Genetic Resources and Crop Evolution*, 58 (3):471-480.

***In vitro* antidepressant activity of medicinal plants extracts and mixtures**

A. C. Andrade, P. Valentão, P. B. Andrade and C. Grosso

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Depression is a psychiatric disease that affects over 350 million people worldwide and many patients do not respond positively to the treatment used. Therefore, there is a need for more efficient antidepressants. Monoamine oxidase A (MAO-A) inhibitors is one of the classes of antidepressants used in the treatment of depression. Thus, some herbal teas known for their antidepressant effects and mixtures containing two extracts were tested against *in vitro* MAO-A activity. Individually, *Cereus grandiflorus* Miller, *Annona muricata* L. and *Trichilia catigua* A. Juss. displayed IC₅₀ values of 437 (Fig. 1A), 23 (Fig. 1B) and 8 µg/mL, respectively. However, a dual effect was verified for *T. catigua* extract: for concentrations above 15.5 µg/mL a stimulatory rather than inhibitory effect was observed on MAO-A activity. This behaviour was also detected for all mixtures containing this extract. Concerning the mixture composed by *A. muricata* and *C. grandiflorus*, the IC₅₀ value against MAO-A was 33 µg/mL (Fig. 1C) and according to the median effect equation, the combination index (CI) [1] indicates a slight synergism until 40% inhibition (CI between 0.86 and 0.90) and nearly additive effect between 40 and 70% inhibition (CI between 0.90 and 0.95). The composition of this promising mixture is being analysed by HPLC-DAD and compared with those of the individual herbal teas that compose the mixture.

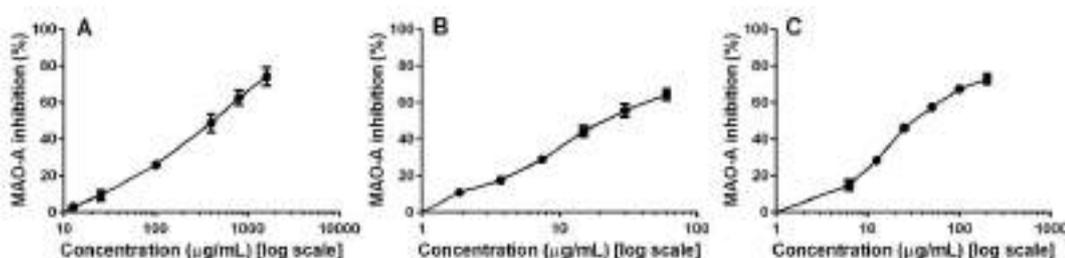


Fig 1. MAO-A inhibition activity of *C. grandiflorus* (A) and *A. muricata* (B) herbal teas and of *C. grandiflorus* and *A. muricata* mixture (C). Results show mean \pm SEM of three experiments performed in triplicate.

Acknowledgements: The authors are grateful to FCT for grant no. PEst-C/EQB/LA0006/2011 and to “Programa Operacional Regional do Norte ON.2” (P23-NORTE-01-0124-FEDER-000070). Clara Grosso thanks FCT for the Post-Doc fellowship (SFRH/BPD/63922/2009).

References:

[1] Chou, T.-C. (2010), *Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method*, Cancer Research, 70 (2), 440-446.

An ultrastructural study of ejaculated spermatozoa from three patients presenting total sperm immotility

M. Sousa¹, M. Gouveia^{1,2}, L. Ferraz³, A. Barros⁴, E. Oliveira¹, A. Alves¹, R. Sá¹

¹ Lab of Cell Biology, UMIB, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

² Department of Biology, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal

³ Department of Urology, Hospital Centre of Vila Nova de Gaia/Espinho, EPE, 4400-129 V. N. Gaia, Portugal

⁴ Centre for Reproductive Genetics Alberto Barros, 4100-009 Porto, Portugal

Sperm motility is fundamental for migration through the female genital tract as well as to penetrate the zona pellucida of the oocyte. In cases of absolute immotility, the ultrastructural study of spermatozoa is the only tool available to provide a diagnosis and thereafter to search for the presence of specific genetic mutations and give a prognosis to the patient and family. We studied by transmission electron microscopy (TEM) semen samples from three patients with total sperm immotility in order to obtain a diagnosis for this problem.

Under informed written consent, semen was collected, allowed to liquefy, washed with SPM, mixed with sodium cacodylate buffer, 0.1 M, pH 7.2 (1:1) and centrifuged for 10 min at 1500 rpm. The pellet was then fixed with Karnovsky for 2h, washed, post-fixed with 2% osmium tetroxide in buffer for 2h at 4°C, washed, serially dehydrated in ethanol, equilibrated in propylene oxide, serially impregnated with propylene oxide and Epon and embedded in Epon. Semithin and ultrathin sections were prepared with a diamond knife on a LKB ultramicrotome. Ultrathin sections were collected on 300 mesh copper grids, contrasted with aqueous uranyl acetate and Reynolds lead citrate, and observed in a transmission electron microscope operated at 60 kV.

At the ultrastructural level, Patient 1 presented multiple defects attaining the midpiece, the fibers and the microtubules of the axoneme. In Patient 2 it was observed disruption of the axonemal microtubules. Patient 3 showed disorganization of the midpiece and fibers, with an intact axoneme except for the absence of dynein arms.

In Patient 1 no definitive diagnosis could be given as the anomalies found were multiple. For Patient 2 as the main feature was disorganization of the axoneme it may be related with mutations in CCDC (Coiled-coil domain) genes. For Patient 3, the absence of dynein arms indicates mutations in DNAH (Dynein heavy chain) genes. These patients may get fathering by the use of intracytoplasmic sperm injection, as selection of a near normal head will indicate correct centriole positioning as observed in all cases. To get further insight into the genetic causes, patients should be screened for mutations of the above cited genes as well as for the outer dense fibers (ODF genes) and fibrous sheath (AKAP genes) genes.

- H.E. Chemes and V.Y. Rawe; *Human Reproduction Update*, 9 (2003) 405-428.
- E. Horowitz, et al.; *Molecular Human Reproduction*, 11 (2005) 307-317.
- K. Inaba; *Molecular Human Reproduction*, 17 (2011) 524-538.
- M. Cabral, et al.; *Revista Internacional de Andrologia* (2012) 156-159.

Metabolome constituents, antimicrobial and antioxidant potential of the brown seaweed *Laminaria ochroleuca*

G. Lopes, A. Costa, D. Costa, F. Azevedo, F. Durães, J. Marques, J. Vilarinho and N. Gomes

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Marine ecosystems are considered to have great phylogenetic diversity, with high biotechnological potential. Extreme temperature, light and salinity conditions subject marine species to strong competitiveness, leading them to produce different and new molecules, which have been gaining attention in the past few years.

For this work the ethanol extract of the brown seaweed *Laminaria ochroleuca* Bachelot de la Pylaie was considered. The HPLC-DAD analysis revealed the presence of carotenoids, namely fucoxanthin, and chlorophylls, mainly chlorophyll *a* isomers. Fatty acids, amino acids and sterols were determined by GC-MS, among other compounds from distinct classes.

Its antioxidant capacity was assessed against DPPH• radical and against a reactive nitrogen species with biological significance (nitric oxide, •NO). A concentration-dependent protective effect was observed for both radicals (Fig.1). The determined compounds, most certainly, contribute for the overall scavenging ability of *L. ochroleuca*.

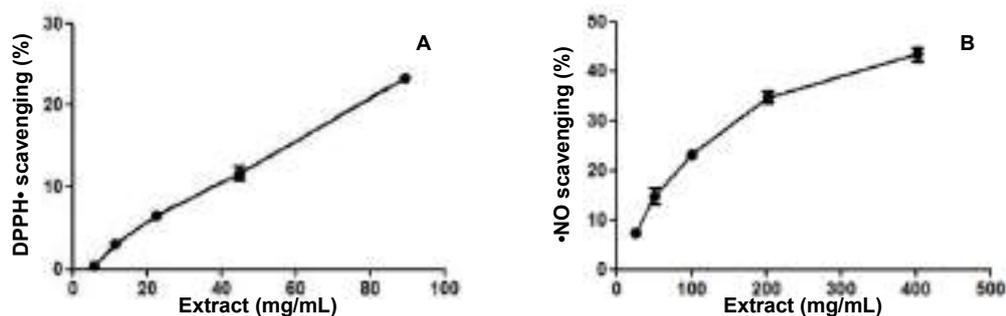


Fig. 1. Scavenging ability of an aqueous extract of *Laminaria ochroleuca* against DPPH• (A), and •NO (B). Results show mean \pm standard error of three determinations.

The antibacterial activity was also evaluated against a set of Gram+ and Gram- species, the minimum inhibitory concentrations revealing to be higher than 807.52 mg/mL.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5th year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head) and Patrícia Valentão.

Development and characterization of a co-culture two-dimensional blood-brain barrier for the study and correlation of drug permeation

B. Mendes^{1,2}, C. Marques¹, P. Costa¹, D. Ferreira¹, B. Sarmiento²

¹ Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Portugal.

² INEB, Institute of Biomedical Engineering, University of Porto, Portugal

The most crucial limitation in diagnosis and treatment of the neurodegenerative diseases is the unique and complicated environment imposed by the central nervous system barriers, mainly the Blood-Brain Barrier (BBB). This interface is formed by specialized endothelial cells (ECs), paving the luminal side of brain microvessels in close association with basement membrane and neighbouring cell types within the neurovascular unit. The microvascular endothelium at the BBB is characterized by the presence of tight junctions between adjacent endothelial cells, lack of fenestrations, and minimal pinocytotic vesicles [1, 2]. At present, no *in vitro* model can faithfully reproduce all the properties and characteristics of the *in vivo* BBB model since the latter has several types of cells and junctions that give rise to unique properties. So, the development of a close *in vitro* cell system is a difficult task and an important first step in the evaluation of new drugs and drug delivery systems to cross the BBB [3].

Our work aims at developing a two-dimensional *in vitro* cell model co-cultured using semi-permeable plate filters. In order to study the induction of a functional BBB, human brain ECs are seeded on the luminal side (on the top side of the membrane), while the human astrocytes (HA) on the abluminal side.

Firstly, it was studied the endothelial monoculture by monitoring the transendothelial electrical resistance (TEER) using different cell seeding densities. At $4,6 \times 10^4$ cells/cm² it was achieved the more interesting values, with the highest value acquired by the seventh day. Besides the TEER measurements, it was studied the membrane integrity by observation of the membrane markers using immunocytochemistry and optical microscopy giemsa technique and by the permeability assay. The proposed *in vitro* model reflected an intact and functional barrier, although the hCMEC/D3 monolayer had lower TEER values.

As future work, it will be added the HA to improve the characteristics of the BBB model. Particularly, it is expected to augment the physical integrity of the barrier. Also, it is intend to study specific endothelial markers, such as claudin-5, to better understand the tight junction's importance for the robustness of the barrier. Finally, our main goal is to be able to use the developed BBB model in order to perform transport assays, using camptothecin as model drug, and camptothecin-loaded solid lipid nanoparticles.

References:

[1] Abbott, N.J. (2013), *Blood-brain barrier structure and function and the challenges for CNS drug delivery*, Journal of Inheret Metabolic Disease, 1-13.

[2] Cardoso, F. (2010), *Looking at the blood brain barrier: Molecular anatomy and possible investigation approaches*. Brain Research Reviews, 64(2), 328- 363.

[3] Cucullo, L. (2008), *Immortalized human brain endothelial cells and flow-based vascular modeling: a marriage of convenience for rational neurovascular studies*, Journal of cerebral blood flow and metabolism, 28(2), 312-328.

***Spodoptera littoralis* larvae: beyond the damage**

M. Taveira, A. Almeida, A. Guerra, C. Garcia, L. Quiamin, P. Ribeiro, S. Oliveira, S. Pisco and T. Pires

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

Spodoptera littoralis Boisduval in its larval stage causes much damage to many vegetal species of commercial interest. In this work larvae of *S. littoralis*, fed with tomato plant leaves from “cherry” variety were studied regarding chemical composition and bioactivity and their potential applications hypothesized.

The ethanol extract was characterized by HPLC-DAD. Several carotenoids and one chlorophyll were detected, lutein being identified. Using GC-MS, numerous primary metabolites, such as long chain fatty acids (Fig. 1) and amino acids, were detected. Glycerol, as well as sterols like cholesterol and β -sitosterol were also noticed.

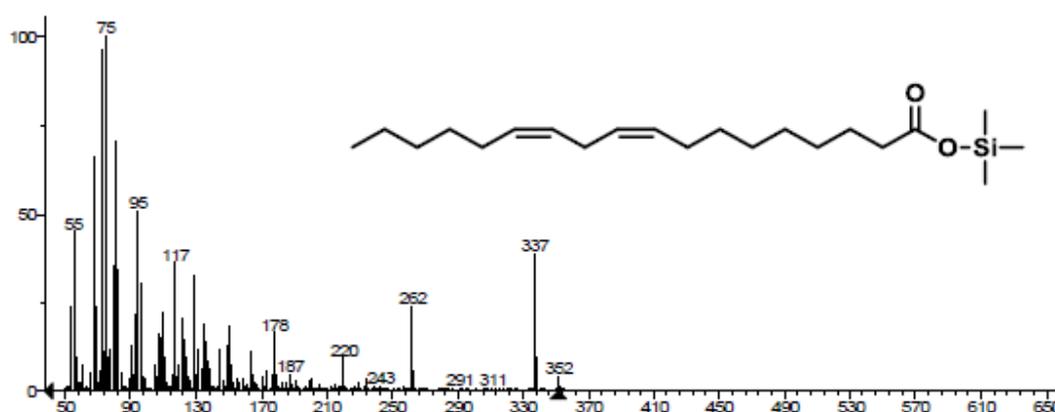


Fig. 1. Mass spectrum of linoleic acid trimethylsilyl derivative.

To assess the antioxidant potential, DPPH \bullet and \bullet NO scavenging assays were performed. The extract revealed low dose-dependent activity.

The extract was also studied regarding its antimicrobial capacity, bacteriostatic activity against *Staphylococcus aureus* and *Micrococcus luteus* being observed.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5th year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head) and Patrícia Valentão.

Spodoptera littoralis/Lycopersicon esculentum: ecological duo

M. Taveira, I. Costa, M. Costa, M. Ferreira, P. Valentão, P. B. Andrade

REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal..

Spodoptera littoralis (Bois.) is a common pest from several important crops in the world, with especial impact on *Lycopersicon esculentum* Mill. (tomato plant). In fact, tomato plant has economic and health impact worldwide, so the losses caused by this pest justified the chemical characterization. The metabolic profile of several *S. littoralis* materials (larvae, pupae, adults and excrements) and the host plant material (leaves) were characterized in terms of phenolics, alkaloids (Fig. 1), carotenoids, amino acids, fatty acids and sterols. These metabolites were determined by HPLC-DAD and GC-MS methods. Phenolics were detected only in *L. esculentum* leaves, being absent in *S. littoralis* materials.

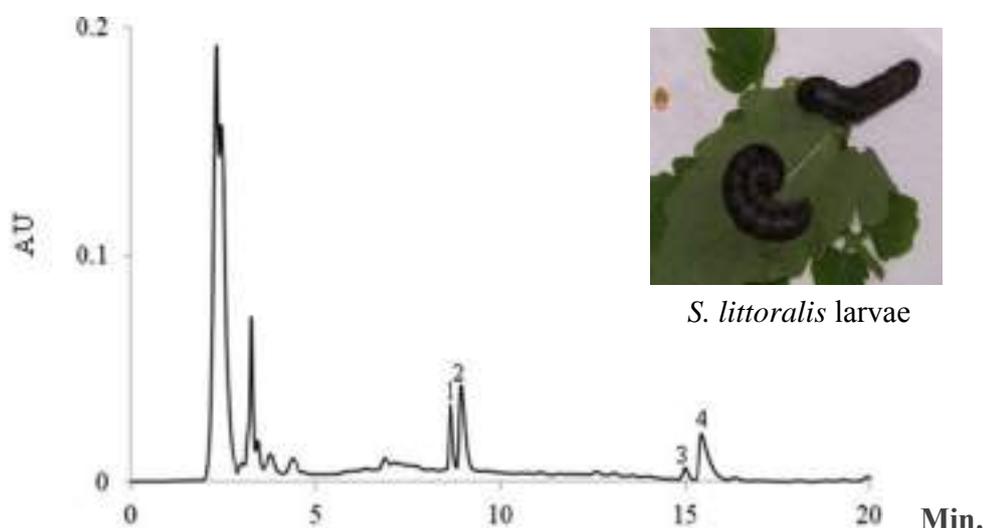


Figure 1. HPLC-DAD steroidal alkaloids profile of *L. esculentum* leaves. Detection at 205 nm. (1) dehydrotomatine; (2) α -tomatine; (3) tomatidenol; (4) tomatidine.

Steroidal alkaloids were identified in all matrices, with especial emphasis on tomato plant leaves and *S. littoralis* excrements. *S. littoralis* seems to present a detoxification process for this class of compounds. Results suggest that amino acids and fatty acids are bioaccumulated by *S. littoralis* larvae. Carotenoids were mainly identified in leaves, being also present in *S. littoralis* larvae and excrements.

Acknowledgements: The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT) for Grant No. PEst-C/EQB/LA0006/2011.

Application of Urban Biodiversity Index in O'Porto City

L. Machado¹, P. Santos^{1,2} and J. Lameiras³

¹Department of Biology, Faculty of Sciences, University of Porto, Portugal.

²CIIMAR, Interdisciplinary Centre of Marine and Environmental Research

³Department of Geosciences, Environment and Territorial Planning, Faculty of Sciences, University of Porto, Portugal.

The importance of the role of cities and local authorities in biodiversity conservation had international recognition through the Convention on Biological Diversity (CBD), during the ninth meeting of the Conference of the Parties (COP9) where the implementation of an index that measures the urban biodiversity was proposed [1]. In 2010, CBD approved an index of biodiversity to cities, City Biodiversity Index (CBI), which would act as an international indicator to measure urban biodiversity; to support national governments and local authorities creating “milestones” in order to conserve biodiversity; to assist in evaluating progress to decrease the rate of biodiversity loss in urban ecosystems; to help measuring the ecological footprint of cities; to assist in the development of guidelines to prepare an Action Plan for biodiversity in cities in order to achieve the three main objectives of the Convention: "Conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding."; and make the cities aware about the lack of information regarding the biodiversity in their areas [1].

The CBI is still developing a final methodology which means that it still has some degree of subjectivity associated mainly with the rating scales of some of the 23 indicators that compose it. Thus, the objective of this work is to propose scales of valuation of some of the indicators of the CBI.

The methodology used was based on the adaptation of User's Manual for the CBI [2]. For the sub-indicators 2, 3, 9, 11, 12, 15 and 16 scales were developed with the minimum value of 1 and a maximum of 2, as advised in the manual.

The scales obtained match a non-integer value between 1 and 2 the values obtained from analysis of the information collected respectively for “connectivity measures or ecological networks to counter fragmentation”, “native biodiversity of bird species in built-up areas”, “proportion of protected natural areas”, “regulation of quantity of water”, “climate regulation: carbon storage and cooling effect of vegetation”, “budget allocated to biodiversity” and “number of biodiversity projects implemented by the city annually”. The new scales will allow a more objective and reproducible assessment of the Urban Diversity Index.

References:

[1] CBD (2011). City Biodiversity Index (or Singapore Index).

Http://www.cbd.int/en/subnational/partners-and-initiatives/city-biodiversity-index, consulted in June 2013;

[2] CBD (2012). User's Manual for the CBI. *Http://www.cbd.int/authorities/doc/User's%20Manual-for-the-City-Biodiversity-Index18April2012.pdf*, consulted in June 2013.

Increased expression of P2X7 receptors in the neocortex of patients with drug-resistant Mesial Temporal Lobe Epilepsy

A.L. Fonseca¹, A. Barros-Barbosa¹, S. Guerra-Gomes¹, J.M. Cordeiro¹, M.G.Lobo¹, F. Ferreira¹, A. Santos², R. Rangel³ and P. Correia-de-Sá¹

¹ Laboratório de Farmacologia e Neurobiologia, UMIB, Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, ²Serviço de Patologia Forense, Instituto Nacional de Medicina Legal – Delegação do Norte (IML-DN), ³Serviço de Neurocirurgia, Centro Hospitalar do Porto – Hospital Geral de Santo António (CHP – HGSA), Portugal

Epilepsy is a common chronic brain disorder affecting ~50 million people worldwide. Forty percent of epilepsy cases adulthood are drug refractory and the majority of these suffer from mesial temporal lobe epilepsy (MTLE). Only a subset of patients meet the criteria for surgical ablation of damaged tissue as last resource treatment, leaving remaining patients with an unmet medical need. This calls for researching new potential pharmacological targets able to control seizures and/or epileptogenesis. During epileptic discharges extracellular ATP concentration is significantly raised to the level of activation of low-affinity ATP receptors like P2X7. The P2X7 receptor is an ATP-gated non-selective cation-permeable ionotropic receptor selectively expressed in neurons and glia in the brain.

Previous functional studies carried out in our laboratory showed that P2X7 receptor activation downmodulates Na⁺-dependent high affinity GABA and glutamate uptake in the human cadaveric neocortex. Furthermore, we demonstrated that downmodulation of GABA and glutamate transport by P2X7 receptors is increased in the neocortex of MTLE patients. In this study, we tested whether there is a relationship between functional data and the amount of P2X7 receptors expressed in the neocortex of MTLE patients as compared with cadaveric controls.

The expression of P2X7 purinoreceptors was evaluated by Western Blot analysis in cadaveric (control) specimens and in samples collected from patients with drug-resistant MTLE submitted to surgery for epileptic focus removal (amygdalahippocampectomy). All procedures were approved by the Ethics Committee of CHP-HGSA, IML-DN and ICBAS. Samples were separated in a 10% SDS-PAGE gel and transferred onto a PVDF membrane. The anti-P2X7 receptor antibody (Alomone, 1:200) incubation was followed by the incubation with horseradish peroxidase-conjugated anti-rabbit secondary antibody (Abcam, 1:70.000). The membranes were developed with chemiluminescent reagent Immun-Star Western Kit and quantitatively analyzed by the ImageJ software.

Data show that P2X7 receptors expression in the neocortex of MTLE patients is increased roughly by 3 times as compared to cadaveric controls. These results suggest that the negative control of GABA and glutamate uptake by P2X7 receptors in MTLE patients' neocortex may be significantly enhanced as a consequence of the increased P2X7 receptors expression in the epileptic tissue.

Work supported by FCT (FEDER, PIC/IC/83297/2007; UMIB-215/94; FSE-POPH-QREN; Barros-Barbosa A. is in receipt of a PhD Studentship SFRH/BD/79259/2011), Univ.Porto/Santander Totta and Tecnifar.

***Fractonotus caelatus* (Marcus, 1928) and *Echiniscus quadrispinosus brachyspinosus* Bartoš, 1934, two rare Tardigrades from Portugal**

M. Sá¹ and P. Fontoura^{1,2}

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

² Eco-Ethology Research Unit (FCT 331/94)

Tardigrades are a group of micrometazoans (body length about 500 µm), close related with the Arthropods, with about 1200 known species widely distributed throughout marine, freshwater and terrestrial environments. In limno-terrestrial ecosystems they are very common in litter, mosses and lichens. The scientific study of tardigrades was for long time neglected but the amazing abilities to undergo cryptobiosis that can rely on DNA repairing mechanisms, justify the recent increasing interest on tardigrades.

Since the first note about Portuguese tardigrades in 1937, only a few studies dealing with this phylum have been carried out in Portugal. As a result of these studies, a final number of 70 limno-terrestrial tardigrade species have been identified in our country. However, during the last years, enhanced by the improvement of the technical means of analysis, the taxonomy of the group underwent considerable changes and very probably there are some inaccuracies and mis-identifications in the abovementioned list of Portuguese tardigrades.

In this work, some morphological features of two rare species *Fractonotus caelatus* (Marcus, 1928) and *Echiniscus quadrispinosus brachyspinosus* Bartoš, 1934, are described and pictured according to the modern taxonomic standards. Specimens of both species have been extracted from dried moss samples collected in Northern Portugal, Amarante (41° 16' 24'' N; 8° 03' 48'' W), in September and October 2013. Tardigrades were mounted in Hoyer's medium. Measurements and photographs were made under oil immersion, using a Zeiss Phase Contrast Microscope equipped with digital camera and using Axiovision 4.7 Imaging System.

Fractonotus caelatus is known from several localities in Europe, from Australia and Africa (Cameroon). In Portugal it has been recorded only once in Vouzela (Viseu) in 1984. Formerly considered a subspecies *Calohypsibius ornatus caelatus* (Marcus, 1928), it was elevated in 1989 to the level of species, *Calohypsibius caelatus*, and later assigned to a new monospecific genus *Fractonotus* Pilato, 1998. Probably *Calohypsibius ornatus* (Richters, 1900) is a complex of species because the intraspecific variability is considered extremely high, justifying the importance of the description of *Fractonotus caelatus* presented in this work.

Echiniscus quadrispinosus brachyspinosus is recorded for the first time from Portugal (mainland) in this study. The subspecies was referred from the Carpathians (Slovakia), Azores, Vietnam and North America. The nominal species *Echiniscus quadrispinosus quadrispinosus* Murray, 1907, probably cosmopolitan, is also common in Portugal. The differences between the two subspecies are focused on the shape and length of the dorsal and lateral appendages. According the results of this work, the possibility to elevate both subspecies to the level of species cannot be excluded.

Assessment of disturbance in coastal systems based on analysis of benthonic organisms with AMBI software

¹ M. Venâncio and ¹ P. Santos

¹ Department of Biology, Faculty of Sciences, University of Porto, Portugal.

The aim of this study is the evaluation of the environmental status of Aterro beach, based on the diversity of benthonic macroinvertebrates.

Benthonic organisms are animals that live in bottom sediments. They are used as environmental indicators because: their mobility is limited, being more sensitive to local disturbances; have long life cycles and are in direct relation with sediment, where they are exposed to the contaminants accumulated by the sediment; and each species has a different sensitivity to disturbances, which leads to different response to stress^[1,2].

The AZTI's Marine Biotic Index (AMBI) allows classifying the ecological status of sites based on sensitivity/tolerance of species to disturbance. The AMBI software contains a database that serves as a benchmark for assigning an ecological group for each taxon identified. The ecological groups are ordered from I to V, ranging between species very sensitive to environmental disturbance to first-order opportunistic species, respectively. The AMBI is calculated using the following formula which gives us a number from 0 to 7, where 0 represents undisturbed and 7 represents extremely disturbed^[3]:

$$\text{AMBI} = \frac{[(0 \times \%GE I) + (1.5 \times \%GE II) + (3 \times \%GE III) + (4.5 \times \%GE IV) + (6 \times \%GE V)]}{100} \quad (1)$$

Sampling was carried out between March and November 2013 in two locations at Aterro beach, Matosinhos. In each location two replicas were taken. Samples were always collected in the low-tide period using a corer. The organisms were later identified under a magnifying glass, and sometimes under a microscope, using adequate keys.

A total of 27 taxa were identified. For both sampling sites the AMBI average value is 3 and the dominant ecological group is group III (tolerant species to disturbance). The results are constant over the sampling period in each of the points, except in March at the riverside outfall, where the AMBI is lower compared with the other months (0,3), corresponding to “undisturbed”. The main conclusion is that both sites at Aterro beach are slightly disturbed.

References:

- [1] Piló, D., Leitão, F., Ben-Hamadou, R., Range, P., Chicharo, M. and Chicharo, L. (2011), *Macrobenthic Response to Sewage Discharges in Confined Areas from Coastal Lagoons: Implication on the Ecological Quality Status*, *Vie et Milieu – Life and Environment*, 61 (2), 107-118.
- [2] Pinto, V.C., Costa, M.J and Costa, J.L (2009), *Ecologia e qualidade ecológica de comunidades de macroinvertebrados bentónicos em zonas costeiras e estuarinas: abordagem comparativa*, Faculdade de Ciências da Universidade de Lisboa, Lisboa.
- [3] Borja, A., Franco, J. and Pérez V. (2000), *A Marine Biotic Index to Establish the Ecological Quality of Soft-Bottom Benthos Within European Estuarine and Coastal Environments*, *Marine Pollution Bulletin*, 40, 1100-1114.

Cytotoxicity of CALND pentapeptide coated gold nanoparticles in human intestinal epithelial Caco-2 cells

C. Pereira, M. Fernandes, S. Fraga, H. Carmo

REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal

Nanoparticles (NPs) of natural origin such as those from aerosols released in volcanic eruptions are ubiquitous. Nowadays, manufactured Nps represent an emerging area that raises increasing concern regarding their impact in living organisms. Gold has traditionally been considered an inert and biocompatible metal. However, the toxicological profile of gold nanoparticles (AuNPs) remains controversial, significant efforts have been made to develop different surface coatings that may grant higher biocompatibility and efficiency for their expected biomedical applications [1]. Peptides, for example, are considered versatile surface capping agents and are also advantageous since they can increase the stability, biocompatibility and solubility of AuNps in water [2]. Though, changing the surface coating may produce significant modifications in their biokinetics in the human organism. After ingestion, the gastrointestinal system is the first barrier offering resistance to any xenobiotic, and is therefore determinant for bioavailability. On the other hand, the intestinal epithelium is also one of the first targets for toxicity after exposure. In this work, we used an *in vitro* model of the human intestinal epithelium, the Caco-2 cell line, established from human epithelial colorectal adenocarcinoma cells, that has been extensively used to predict the absorptive and defensive properties of the intestinal mucosa, to evaluate the cytotoxic effects of 23 nm CALND-capped AuNps. After incubation with different concentrations of AuNps (1, 3, 10, 30, 60, 100 μ M), morphological alterations (evaluated by light microscopy), cellular viability and metabolic activity (evaluated by the calcein assay and the MTT reduction assay), plasma membrane integrity (evaluated by the propidium iodide incorporation assay), reactive oxygen species production [evaluated by the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay] and lysosomal membrane integrity (evaluated by the neutral red incorporation assay) were investigated at different time-points (6, 24, 48, 72h). All data showed that in this *in vitro* system the tested CALND-capped AuNps did not produce any signs of cytotoxicity or oxidative stress and corroborated previous data obtained with other capping agents including, the mercaptoundecanoic acid and peptides CALNN and CALNS, in other cellular models [1]. Performing complementary cytotoxicity assays gives additional support to these conclusions since several interferences in classical cytotoxicity *in vitro* tests have been noted with Nps raising concern on the accuracy of the *in vitro* data obtained when testing the toxicity of AuNPs for biomedical applications.

References:

- [1] Fraga, S. Faria, H., et al. (2013), *Influence of the surface coating on the cytotoxicity, genotoxicity and uptake of gold nanoparticles in human HepG2 cells*, Journal of Applied Toxicology, **33**: p. 1111-1119.
- [2] Morais, T., Soares, M.E. et al. (2012), *Effect of surface coating on the biodistribution profile of gold nanoparticles in the rat*, European Journal of Pharmaceutics and Biopharmaceutics, **80**: p. 185-193.

Predicting the neurotoxicity of mixtures of piperazine drugs of abuse using the SH-SY5Y neuroblastoma cells

A.M. Ferreira¹, D. Dias da Silva^{1,2}, H. Carmo^{1,2}

¹ Faculty of Pharmacy, University of Porto, Portugal.

²REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal.

Drug abuse remains a critical socio-economic problem. In an attempt to overcome legal restrictions, new drugs that are misleadingly marketed as safe and capable of causing similar effects to those of illegal drugs are becoming increasingly popular. These drugs are currently known as legal highs. However, for many of these drugs, knowledge on their possible harmful effects is currently inexistent or very limited [1].

The derivatives of piperazines, in particular 1-benzilpiperazine (BZP) and 1-[3-(trifluoromethyl)phenyl]piperazine (TFMPP), fall into this category of drugs and were disclosed as capable of causing similar effects to 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) when consumed in combination. Despite having been recently banned, there are still reports of their use, and little is known about their neuronal toxicity, particularly in what concerns detrimental combination effects [1].

The present study aimed to evaluate the in vitro neurotoxicity of these drugs in combination using the neuroblastoma SH-SY5Y cell line, as well as to determine whether the well-known pharmacological models of concentration addition (CA) or independent action (IA), that have been previously applied to mixtures of amphetamine derivatives [2], could predict the mixture effects of these piperazine derivatives. For this purpose, the cytotoxicity of each compound was tested individually, in a wide range of concentrations, to produce single concentration-response curves, using the MTT reduction assay. With these data and applying the CA and IA models, concentration-response curves of the expected combination effects of BZP and TFMPP were produced, and three different mixtures were designed and experimentally tested. The three combination ratios were chosen based on either the EC₅₀ value of the individual substances, their EC₀₁ values, or the most frequent combination used by consumers (1 TFMPP : 2 BZP).

The results demonstrated that, in these cells, the TFMPP derivative (EC₅₀ 0.21 mM) is far more toxic than BZP (EC₅₀ 3.74 mM). The combination effects of all three mixtures were well predicted by the CA model as observed by the close match between the predicted and the experimentally obtained curves. Of special interest, the significant cytotoxic effect observed when the drugs were mixed at individually non-cytotoxic concentrations demonstrated that when these drugs are consumed in combination a dramatic increase in toxicity may occur. Therefore, combination effects must be evaluated to prevent underestimating the real hazard associated with the abuse of these drugs.

References:

[1] Arbo, M., Bastos, M. and Carmo, H. (2012), *Piperazine compounds as drugs of abuse*. Drug Alcohol Depend., 122, 174–85.

[2] Dias da Silva, D., Carmo, H. and Silva, E. (2013), *The risky cocktail: what combination effects can we expect between ecstasy and other amphetamines?*, Arch.Toxicol. 87(1):111-22.

On the anti-tumoral properties of Port Wine polyphenols and their bioavailability

H. Oliveira, I. Fernandes, M. Araújo, V. de Freitas, N. Mateus*

Centro de Investigação em Química, Faculdade de Ciências da Universidade do Porto, Porto, Portugal

*nbmateus@fc.up.pt

Several studies have already demonstrated the preventing properties of polyphenols against cancer. Port wine is a fortified wine produced with grapes from the demarcated region of Douro, with an historic and economical value to Portugal, appreciated worldwide.

Table 1. Values of IC₅₀ for the three cell lines.

	Port wine fractions/ cell lines	IC ₅₀ (mg/mL)		
		Caco-2	HT-29	MKN-28
Vintage	FI	0,251 ± 0,005	> 0,6	0,240 ± 0,003
	FII	0,176 ± 0,006	0,331 ± 0,003	0,120 ± 0,003
	F0	0,229 ± 0,012	0,324 ± 0,023	0,119 ± 0,010
20 years	FI	0,245 ± 0,001	> 0,6	0,290 ± 0,016
	FII	0,262 ± 0,007	0,363 ± 0,001	0,278 ± 0,005
	F0	0,296 ± 0,003	0,430 ± 0,009	0,376 ± 0,002

In this study we investigated the cytotoxic effects of two different Port wine polyphenolic extracts (Vintage Port and Twenty-year-old) on human stomach cancer cell line MKN-28, human colon cancer cell line HT-29 and small-intestine cancer cell line caco-2, using Sulforhodamine B (SRB) assay. Furthermore the antioxidant properties of

Vintage Port and Twenty-year-old aged Port wines polyphenolic extracts were analyzed using Ferric Reducing Antioxidant Power (FRAP) and 1,1-Diphenyl-2-picryl-hidrazyl (DPPH) assays. The obtained polyphenolic fractions of both Port wines used were also characterized by HPLC-DAD and the total phenols of each fraction were quantified by Folin assay. We also evaluated the absorption of anthocyanins at stomach level using MKN-28 cell line, in the perspective of understanding the bioavailability of this compounds using Transwell assay. It was made a comparison between the presence and the absence of ethanol, a crucial element in Port Wine.

Vintage Port wine polyphenolic extracts were found to be more efficient with a lower IC₅₀ in the three cell lines used (table 1). Indeed, Vintage Port is a younger wine compared with 20 years old, so the polyphenolic compounds present in this, are likely to have a simpler structure. The more susceptible cell line were found to be MKN-28, which is, interestingly, the first cell line to be in contact with Port wine, in a context of gastrointestinal tract.

On the bioavailability of anthocyanins, Malvidin-3-glucoside, were found to be the most absorbed anthocyanin by MKN-28 cells, both in the presence and absence of ethanol. The results also showed that ethanol seems to have a synergic effect on the absorption of these compounds at gastric level. These results are only preliminary but they also suggest that the polyphenols of very old Port wines remain somehow bioactive.

Thanks: The authors thanks the company Sogevinus Fine Wines Group for providing the Port Wines.

Using phosphoproteomics to elucidate the role of the phosphatase Sit4p in mitochondrial function and lifespan yeast

C. Pereira¹, Andreia T. Pereira¹, H. Osório³, P. Moradas-Ferreira^{1,2}, V. Costa^{1,2}

¹IBMC, Instituto de Biologia Molecular e Celular, Grupo de Redox Cell Signal, Porto, Portugal.

²ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Departamento de Biologia Molecular, Universidade do Porto, Porto, Portugal.

³ IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal.

Saccharomyces cerevisiae Isc1p, an orthologue of the mammalian neutral sphingomyelinase-2, regulates sphingolipid metabolism affecting mitochondrial function, oxidative stress resistance and lifespan [1, 2]. *SIT4*, which encodes for the catalytic subunit of a ceramide-activated serine-threonine protein phosphatase, is critical for *isc1Δ* phenotypes since its absence restores mitochondrial function, increasing the longevity of these mutants [3]. Therefore, the identification of Sit4p downstream targets is important for understanding the cross-talk between sphingolipid metabolism, mitochondria function and lifespan.

In this work, potential Sit4p targets were evaluated by two-dimensional gel electrophoresis of proteins extracted from isolated mitochondrial fractions. Alterations in the mitochondrial phosphoproteome in *sit4Δ* cells relative to the wild-type cells were then evaluated by western-blot.

The analysis of total protein patterns showed that the deficiency in Sit4p led to alterations in the levels of 21 proteins (11 upregulated and 10 downregulated). The most significantly affected cell functions were related to carbohydrate metabolism and energy production. From the analysis of the phosphoproteome, 9 protein spots were detected as being hyperphosphorylated in *sit4Δ* cells. These spots were identified by mass spectrometry and correspond to Atp1p, Atp2p, Qcr2p, Ilv5p, Sod2p, Ssc1p, Hsp26p and Por1p, proteins with distinct functions in mitochondria. Our data suggest that Sit4p, directly or indirectly, modulate expression and phosphorylating patterns of mitochondrial proteins. The contribution of these alterations for mitochondrial function, and lifespan is under assessment.

This work was financially supported by FEDER (Fundo Europeu de Desenvolvimento Regional) through the program “Programa Operacional Fatores de Competitividade-COMPETE”, by FCT (Fundação para a Ciência e Tecnologia) and by “Programa Operacional Regional do Norte (ON.2 – O Novo Norte)”, through the projects NORTE-07-0124-FEDER-000001 and FCOMP-01-0124-FEDER-028210. C.P. (PTDC/BBB-BQB/1850/2012) is supported by a FCT fellowship.

[1] Almeida, T., Marques, M., Mojzita, D., Amorim, M.A., Silva, R.D., Almeida, B., *et al.* (2008) *Isc1p plays a key role in hydrogen peroxide resistance and chronological lifespan through modulation of iron levels and apoptosis*. *Mol Biol Cell* 19: 865–876.

[2] Kitagaki, H., Cowart, L.A., Matmati, N., Vaena de Avalos, S., Novgorodov, S.A., Zeidan, Y.H., *et al.* (2007) *Isc1 regulates sphingolipid metabolism in yeast mitochondria*. *Biochim Biophys Acta* **1768**: 2849–2861.

[3] Barbosa AD, Osorio H, Sims KJ, Almeida T, Alves M, *et al.* (2011) *Role for Sit4p-dependent mitochondrial dysfunction in mediating the shortened chronological lifespan and oxidative stress sensitivity of Isc1p-deficient cells*. *Mol Microbiol* 81: 515–527.

Semi-feral cats: the human influence on circadian activity

Carla Fafiães^{1,2} & Liliana de Sousa²

¹ Faculty of Science, University of Porto, Portugal.

² Grupo de Estudos em Etologia (GEE) do Departamento de Ciências do Comportamento do Instituto de Ciências Biomédicas Abel Salazar (ICBAS)

This study's main objective was to realize how *Felis catus* behaviors change at different times of the day. Generally, as nocturnal animals, cats are more active during early morning, afternoon and evening, but human influence makes this routine change [1]. The sample that was chosen was of semi-feral cats living free but benefiting from the presence of humans to access food. Naturalistic observation technique was used with video recording to register cats' behaviors (individual and social) which allowed constructing an ethogram. The study was made during 5 days in a 10 minutes record for each of 4 periods of the day (early morning, late morning, afternoon and evening). Statistical data analysis regarding frequencies and duration differences of their behaviors according to those 4 day periods were accomplished with SPSS 17.0. Results lead to conclude that the circadian rhythms of the studied cats were affected by humans once there were no remarkable differences between the analyzed behaviors in the 4 different periods of the day. Results point out that this population was somehow influenced by human activity, which indicates that cats can change their nocturnal habits if factors that influence this change are present.

Preference: POSTER.

References:

[1] Turner DD and Bateson P (2000), *The Domestic Cat, the biology of its behaviour*, Cambridge University Press, pp. 9-18.

Molecular characterization of the *SFBB* genes in *Malus* S2-, S3-, S9-, and S28-haplotypes

A. Araújo^{1,2}, B. Aguiar², M. I. Pratas², J. Vieira², and C. Vieira²

¹ Faculty of Sciences, University of Porto, Portugal. ²IBMC - Institute for Molecular and Cell Biology

In flowering plants, a widespread mechanism to prevent inbreeding is gametophytic self-incompatibility (GSI), where the self-incompatible phenotype of the pollen is determined by its haploid genome. In Solanaceae, Plantaginaceae, and Rosaceae (both in *Prunus* and Pyrinae), the pistil specificity is determined by a ribonuclease, called *S-RNase*. The pollen specificity is determined by one F-box gene in *Prunus* (called *SFB*, *S*-haplotype specific *F*-box gene), and multiple genes in Pyrinae (called *SFBBs*, *S*-locus *F*-box brothers genes), *Petunia* (Solanaceae), and *Nicotiana* (Solanaceae; called *SLFs*, *S*-locus *F*-box). The allelic combination of the pistil and pollen genes is named *S*-haplotype.

Under the multiple *S*-pollen genes system, *SFBB* (in Pyrinae)/*SLFs* (in Solanaceae) genes are expected to be in linkage with the *S-RNase* and to have pollen expression only. In this system, non-self *S-RNases* are targeted and marked for degradation by multiple *SFBBs*, that recognize a subfraction of *S-RNases* proteins in a population. This model is called non-self recognition by multiple factors.

The high intra-haplotypic diversity of *SFBB* together with evidence for positive selection when intra-haplotype *SFBB* genes are analyzed using codon models support the non-self recognition by multiple factors model. Nevertheless at present few *S*-haplotypes have been characterized preventing a detailed working model. In this work, we will characterize four *Malus* *S*-haplotypes (*S2*, *S3*, *S9* and *S28*). Genomic DNA from Golden Delicious (*S2*, *S3*) and Red Delicious (*S9*, *S28*) cultivars are being used in PCR reaction using primers that amplify large number of *SFBB* genes. All the amplification products will be cloned. On average, 80 colonies will be sequenced for each individual. A F1 progeny of Golden Delicious x Red Delicious, is being used to show linkage of the *SFBB* genes and the *S-RNase*.

Diversity at the *Fragaria S-RNase* lineage genes

I. Silva^{1,2}, **B. Aguiar**², **M. I. Pratas**², **J. Vieira**², and **C.P. Vieira**²

¹ Faculty of Sciences, University of Porto, Portugal.

² IBMC - Institute for Molecular and Cell Biology

In flowering plants, a widespread mechanism to prevent inbreeding is gametophytic self-incompatibility (GSI), where the self-incompatible phenotype of the pollen is determined by its haploid genome. The gene responsible for determining female specificity is a ribonuclease, called *S-RNase* in Rosaceae, Solanaceae, Rubiaceae and Plantaginaceae species. Phylogenetic analyses of the *T2-RNases* suggest that *S-RNase*-based GSI has evolved before the split of the Asteridae and Rosidae. *S-RNases* belong to the *T2-RNase* gene family, where genes other than the *S-RNase*, have been associated with responses to wounding, pathogen invasion, and leaf senescence. In this family sequences not involved in GSI, having the same origin than the *S-RNase*, are called *S-lineage* genes. In contrast to other *T2-RNases*, *S-RNase* lineage sequences have just one or two intron. Furthermore, four amino acid patterns (patterns 1 and 2 which are exclusively found in *S-RNases*, pattern 3 which is found mainly in *S-lineage* genes, and 4 which is not found in any of the *S-RNases*) allow the distinction of *S-RNases* from other *T2-RNase* proteins. The *S-RNase* have also an isoelectric point above 8. Because of frequency dependant selection acting at the *S*-locus *S-RNase* gene is expected to present high levels of polymorphism. In this study we will characterize at molecular level the *S-lineage RNases* genes of *Fragaria vesca* (*F.vesca* scf0513144.1, *F.vesca* scf0513159.1, *F.vesca* 22609, and *F.vesca* scf0513063.1) that show these features, in four *Fragaria* self-incompatible species - *F. viridis*, *F. nipponica*, *F. pentaphylla*, and *F.mandshurica*, in order to identify the *Fragaria S*-pistil gene.

Application of a High-Throughput Microarray Technique for screening stem cells behavior in 3D Alginate Matrices

D.B. Gomes^{1,2}, **S.C. Neves**^{1,2}, **C.C. Barrias**¹

¹ INEB - Instituto de Engenharia Biomédica, Divisão de Biomateriais, Rua do Campo Alegre, 823, 4150-180, Porto, Portugal

² FEUP - Universidade do Porto, Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e de Materiais. Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Stem cells are highly influenced by their niche, in particular by the extracellular matrix (ECM) where they are embedded in. The cross-talk between ECM and stem cells can influence their “stemness” as well as their differentiation behavior. In order to analyze this cross-talk, our group has previously synthesized a range of peptide-modified alginate to create multifunctional 3D matrices that mimic some features of the native ECM [1]. These hydrogels are easy to handle, and their biochemical (type and density of each peptide) and biophysical (viscoelasticity) properties can be tuned in a straightforward manner. The development of microarrays of cell-loaded hydrogels, combining numerous cell-matrix formulations into miniaturized specimens, will certainly contribute to enhance the throughput of these studies [2]. Here, microarrays are being prepared by Laser-assisted bioprinting (LAB) a versatile, rapid and readily adaptable bioprinting technique [3,4,5]. Through a computer-assisted high yield process, LAB may be used to print cell-hydrogel microdroplets of multiple compositions along a slide in a spatially addressable manner. Using a rapid prototyping workstation equipped with an IR pulsed laser ($s = 30$ ns, $k = 1064$ nm, $f = 1 - 100$ kHz), galvanometric mirrors (scanning speed up to 2000 mm s^{-1}) and micrometric translation stages (x, y, z) [3], we were capable of printing peptide-alginate solutions at different concentrations, combined with crosslinking agents to promote in-situ hydrogel formation, and loaded with different densities of mesenchymal stem cells (MSCs). The produced microarrays remained stable for 7 days under standard cell culture conditions, and cell viability was maintained. Concomitantly, all the gel formulations were analyzed for their mechanical properties and gelation time, in an attempt to optimize the properties of these peptide-alginate solutions as bioinks, and also to subsequently correlate cell behaviour with matrix stiffness. Different *in situ* immuno-stainings are currently being optimized to better characterize cell behaviour. In summary, LAB seems to be a promising technique to obtain microarrays of 3D cultured cells, which will provide a valuable platform for the high-throughput analysis of MSC-matrix interactions.

[1] Fonseca, K.B. et al., *Acta Biomater.* 2011 Apr;7(4): 1674-82

[2] Fernandes TG et al., *Anal Chem.* 2008 Sep 1;80 (17): 6633-9

[3] Guillemot F et al., [Acta Biomater.](#) 2010 Jul;6(7): 2494-500

[4] Guillemot F et al., *Nanomedecine.* 2010 Apr; 5: 507-515

[5] Bordenave, L et al., *Biomaterials.* 2010 Oct; 31: 7250-7256

Paternal Lineages in Macau and Shanghai: Enriching the Knowledge of Chinese and East Asiatic Populations

J. Guilherme Alexandre^{1,2}, Qi Huang³, A. L. Parra³, E. Arroyo³, A. Amorim^{1,2}, L. Alvarez², M. J. Prata^{1,2}

¹ Department of Biology, Faculty of Science, University of Porto, Portugal.

² IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal.

³ Faculty of Medicine, Complutense University of Madrid, Spain.

East Asia, the geographical region bordered by the Plateau in the southeast, by the Bering strait in the northeast, and extending into island southeast Asia, much uncertainty still persists about when and how the first settlements of humans were established in the area, being also scarcely known the demographic history of extant human populations, especially in the region now known as China, where currently 56 ethnic groups are recognized.

This work aimed at investigating the male-mediated genetic background of the Shanghai and Macau populations in the Chinese populations context, as well as to enrich the historical knowledge of the region, combining genetics with ethnic patterns, linguistic affiliation and historical record. [1] Furthermore, we compared the results obtained with data from other available East Asian populations to evaluate whether signs were retained of the different geographical, historical and cultural factors that might have shaped the Y-chromosome diversity in a broader context.

We have sampled 135 unrelated males, 85 from Shanghai and 50 from Macau. After DNA extraction, samples were tested for 25 Y-SNPs using two multiplex systems and a singleplex system for the Yap element. Samples were also genotyped for 17 Y-STR markers using the AmpF ℓ STR[®] Yfiler[®] PCR Amplification Kit (Applied Biosystems).

As typically found in East Asia, the large majority of the Y lineages in the two samples here studied belonged haplogroup O (M175): 89.5% in Macau and 81.5% in Shanghai. Furthermore, high haplogroup diversity was detected in both populations. Levels of haplotype diversity were also very high, reaching values commonly found in other Chinese populations, among which the lowest diversities up to now reported were in the Muslim and Tibetan populations, probably due to a certain isolation of these populations due to religious beliefs and ethnic history, respectively. [2]

Further analyses revealed a remarkable degree of population substructure in China, and genetic distance between populations was found to have no correlation with geographical distance.

References:

[1] PRB, World Population Data Sheet. Population Reference Bureau, 2007

[2] Wang, C.-C.a.L., Hui, *Inferring human history in East Asia from Y chromosomes*. Investigative Genetics, 2013. 4(11): p. 1-10

Development of a new pyruvate-selective electrode for wine control

Estudante: Joel Oliveira*

Supervisores: Célia Amorim, Alberto Araújo, Conceição Branco

REQUIMTE/Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, R. Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

As with other processed foods, wine making requires real-time control of several critical chemical and biological parameters. Such is the case of pyruvate which influences the final properties of wine, including aroma, color, flavor and stability. The use of chemical sensors, in this context, is desirable because it allows lower processing costs and control the quality of the final product.

Among known chemical sensors, are those with potentiometric transduction which stand to be economical, easy to use, and allowing selective responses in real time in a broad range of concentrations. .

It has been shown that the metalloporphyrins have the ability to selectively bind organic acids and can thus integrate the constitution of selective membranes used in potentiometric sensors. So. this project explores the use PVC membranes using 5,10,15,20-Tetraphenyl-21H,23H-porphine manganese (III) chloride as ionophore . The optimization of membrane composition will help to achieve the best sensor for real analysis.

*Estudante do Núcleo de Iniciação à Investigação

INDEX

“ <i>Nos Copains les Légumes!</i> ” – Effects of an intervention program on the consumption and appreciation of vegetables in preschool and primary school French children.....	434
“O POETA DESTROI-VOS”: body and writing as resistance in Cesariny and Gullar.....	30
“ <i>Your past counts</i> ”: Leaders’ (il)legitimacy as a guide to reaction to deviance.....	349
A computational study to investigate the binding of new potential antitumor xanthone derivatives to human serum albumin.....	566
A meta-analysis of obesity, and overweight/obesity in 10 year old Portuguese children.....	327
A microcosm of light and beauty: the Chapel of Nossa Senhora da Conceição.....	537
A mouse model for infection with Group B Streptococcus that mimics the human neonatal disease.....	18
A new approach for the discovery of antivirals against norovirus.....	585
A new selective activator of PKC α discovered using a yeast-based assay.....	158
A questionnaire adjustment and validation for Portuguese language about the perceptions of pre-service student teachers about the educational process: final results.....	458
Accuracy throwing on different launching distances of Boccia athletes.....	310
Acetabuloplasty procedures: Objectives, Indications and Results.....	577
Acorns as a food resource.....	469
Acoustic emission experiments in different types of rocks.....	462
Activity of <i>Thymus caespitosus</i> against <i>Candida</i> species.....	532
Addition of spent yeast extract on the production of cooked ham- Compositional and Sensory evaluation.....	231
Adoption of older children: Waiting period, transition, adaptation and integration. Exploratory Study.....	549
Adult Scoliosis.....	233
Aerobiology of <i>Populus</i> and effects of different O ₃ levels on its pollen fertility, proteins and allergenicity.....	384
Aged female diet anti-oxidant supplementation: effect on placental bed and reproductive outcome.....	157
Aging: perspectives and representations and intergenerational solidarity.....	346
Alkyne-azide “click” chemistry in designing sugar-based bioactive compounds.....	576
Almada, the invention of the poet.....	32
Alpha 1a adrenoceptor antagonist suppresses bladder pain and urothelial disruption in a model of bladder pain syndrome/interstitial cystitis.....	189
Alpha 1-antitrypsin deficiency caused by rare alleles: haplotype characterization and history.....	479
Amino acid characterization of wild duck (<i>Anas platyrhynchos</i>) meat.....	264
An example of a summation error and ways of dealing with it.....	94
An ultrastructural study of ejaculated spermatozoa from three patients presenting total sperm immotility.....	602
Analysis of malt volatile compounds by headspace solid-phase microextraction coupled with GC-MS.....	266
Analysis of the Morphology and Dynamic Behaviour of populations of particles of FeSi in Heavy Media Separation.....	357
Analysis of the yearly sunspot number with the Empirical Mode Decomposition.....	367
Anatomical region differences and age-related changes on calcium and magnesium levels in the human brain.....	107
Annual profile of planktonic community of Crestuma-Lever reservoir.....	582
Antarctic Dry Valleys: geochemical soil properties and microbial communities.....	75
Anti-angiogenic properties of a palladium derivative compound, the Pd2Spm.....	555
Antibacterial activity of leaf and fruit essential oils of <i>Schinus molle</i> L. from northwestern Argentina.....	587
Antimicrobial activity of synthetic and natural compounds.....	554
Antimicrobial resistant coliform bacteria on beach sands in the North coast of Portugal.....	505
Antioxidant activity and bioactive compounds of honey.....	563
Antioxidant activity and total phenolic compounds of leaf lettuce (<i>Lactuca sativa</i> L., var. <i>crispa</i>) and cape gooseberry (<i>Physalis peruviana</i>) mix.....	447
Antioxidant analysis of green-leaf vegetables:.....	463
ANTIOXIDANT CAPACITY OF TROPICAL FRUITS.....	465
Antioxidant potential of <i>Asteraceae</i> species with botanical relevance in Portugal.....	399
Application of a High-Throughput Microarray Technique for screening stem cells behavior in 3D Alginate Matrices.....	618
Application of an Fe ^{III} dimeric porphyrin as catalyst for oxidative desulfurization.....	423
Application of gas-diffusion microextraction for high-performance liquid chromatographic analysis of volatile compounds in bread.....	408
Application of Urban Biodiversity Index in O’Porto City.....	607
<i>Aquatipia</i> as stratigraphic painting.....	37
Architectural textured surfaces panels for optical purposes.....	536
Area and edge effects on leaf-litter decomposition in a fragmented Subtropical Dry Forest.....	584
Assessment of amylin’s nociceptive effect in an animal model of neuropathic pain.....	105

Assessment of children exposure to PM ₁₀ and PM _{2.5} levels at an Oporto school	540
Assessment of disturbance in coastal systems based on analysis of benthonic organisms with AMBI software	610
Assessment of nitrite and nitrate contents and antioxidant capacity of Portuguese fresh vegetables	464
Assessment of the antioxidant properties of blueberry pomace as potential food preservative	539
Astrophysical Constraints on the BSBM Model	206
Attachment, caregiving, emotional regulation and caregiver burden in partners of women with breast cancer: a comparative study	214
Auto dealers in Presidente Prudente, Brazil	147
Automated software for prosody assessment in European Portuguese	175
Balanced and vegetarian diets: comparison in macro and micronutrients composition	446
Behavior of osteoblasts and osteoclasts cultured on a nanophased hydroxyapatite/collagen construct	497
Behavior of prostate cancer cells in a 3D bone scaffold of Nanohydroxyapatite/collagen	525
Benzene sorption isotherms of soils contaminated with biofuels	379
Between Architecture and Plastic Arts	39
Between the Digital and the Analogic project	535
Between two worlds: Narratives about psychotherapists' Countertransference	133
BeWo cells differentiation: Syncytialisation induction and anandamide effects in cell viability	488
Bioaccumulation of cyanotoxins in microalgae and plants – A laboratorial approach towards risk assessment of toxic cyanobacteria	380
Biological evaluation of new steroidal compounds: aromatase inhibition and effects in MCF-7aro cells viability	516
Biomechanical study of cervical spine	541
Biomonitoring of coastal contamination using a biomarker approach: the use of gooseneck barnacle as a sentinel species	90
Birth in Portugal: a national descriptive study	579
Body image in amputation: relationship with depression, anxiety, satisfaction with social support and self-esteem	215
Body image in infertility	131
Body image in persons with spinal cord injury: relationship with depression, anxiety, satisfaction with perceived social support and self-esteem	174
Brewer's spent grain valorisation: extraction and characterization of natural antioxidants	427
Brewers' spent grains characterization and its potential as raw material for bioenergy	293
Cannibalism in the brown shrimp <i>Crangon Crangon</i>	44
Carbapenem-resistant <i>Pseudomonas aeruginosa</i> in imported fruit	529
Cardiac damage induced by mitoxantrone: a time and age-dependent effect involving oxidative stress	323
Cardiac P2X4 purinoceptors predominantly decrease atrial rate with only minor effects on ventricular active tension in the rat	562
CARDIORESPIRATORY RESPONSE OF AN ELITE ATHLETE OF TAEKWONDO DURING A SIMULATED COMBAT	258
Carob seed germ meal as a partial fish meal substitute in diets for meagre (<i>Argyrosomus regius</i>): growth and health status	88
Carotenoids content of cherry tomato fruits: A comparative study	553
<i>Castanea sativa</i> by-products: a new sustainable source of bioactive compounds	467
Cavity ring-down for remote sensing	208
Celebrating the golden anniversary of Merrifield's Solid Phase Peptide Synthesis	411
Cetacean monitoring between Continental Portugal and Madeira Island	499
Challenges of the "almost Psychologist": the effects of personal and social skills in Psychology entails in how well psychology students tend to respond to requests for support or counseling or speeches that reflect on unadaptive beliefs regarding the psychologist and exercise psychology.	351
Characterization of cholinesterase activity in three biofouling invertebrate species of NE Atlantic	86
CHARACTERIZATION OF PROCEDURES AND FORENSIC PSYCHOLOGICAL APPRAISAL OF CHILDREN AND YOUNG VICTIMS OF MALTREATMENT: 10 YEARS OF EVALUATION IN GEAV	348
Characterization of Proteolysis in Specialty malts by chromatographic methods	138
Characterization of Tie1 expression in human erectile tissue and its relation with aging and cardiovascular risk factors	483
Characterization, treatment proposal and metal recovery in <i>Active Implantable Medical Devices</i>	391
Chemical characterization of fennel (<i>Foeniculum vulgare</i>) extracts obtained by salting-out assisted liquid-liquid extraction	405
Chemometric discrimination of commercial squids based on fatty acids, vitamin E and cholesterol contents	419
Chiral xanthenes: Synthesis, biological evaluation and molecular docking as inhibitors of P-glycoprotein (Pgp), phospholipase A2 (PLA2) and cyclooxygenases (COX-1 and 2)*	431
Cholesterol and sphingomyelin are myelin-associated lipid inhibitors that modulate axonal regeneration following injury	57
Chromium and vanadium in the human brain: A post-mortem study of anatomical region differences and age-related changes	442
Citizens' Perceptions of Private Security Agents	248
City of Professions, Project <i>Grow and Show Up</i> . Early intervention for the academic and professional success.	81

Clinical Training: Effect on Empathic Communication Skills Acquired in the Second Year. Relationship with Emotion Recognition Ability and Alexithymia.....	546
Collective action as model for social-ecological systems management: the Douro region in a climate change scenario..	164
comparison between several preservation methods used on vegetable samples.....	463
Comparison of different methods of extraction to quantify vitamin E in rainbow trout (<i>Oncorhynchus mykiss</i>) muscle.....	397
Comparison of the fertility, protein content and allergenicity between transgenic and conventional <i>Zea mays</i> pollen.....	580
Computational Study of Electronic and Structural Properties of Chemical Reactions Inside Carbon Nanotubes.....	66
Consistency tests of the stability of fundamental couplings and unification scenarios.....	365
Control of a Swimming Ergometer.....	240
Copper phytoremediation by <i>P. australis</i> enhanced by autochthonous bioaugmentation.....	122
Corporate Ecological Footprint – Methodological Discussion and Application to STEF.....	457
Correlates of Vigorous Physical Activity in Children. A Multilevel Analysis.....	256
Correlation between the efficiencies of caffeic acid alkyl esters and their distribution in emulsions.....	114
Correlation between the habitats productivity and species richness (amphibians and reptiles) in Portugal, through remote sensed data.....	73
Cosmic Superstrings in the Planck Era.....	205
Country Embeddedness: An exploratory study about the strengths that connect portuguese young adults to Portugal ..	165
Cultivation of <i>Chlamydomonas sp.</i> in brewery wastewater supplemented with pentose sugars.....	291
Current situation of Architecture in Portugal- Economic crisis and the future business model.....	534
Cyclodextrin-encapsulated herbicides: A promising technology.....	400
<i>Cystoseira usneoides</i> (Linnaeus) M. Roberts effects on the viability of the neuroblastoma cell line SH-SY5Y and inhibition of cholinesterases' activity.....	590
Cytotoxicity of CALND pentapeptide coated gold nanoparticles in human intestinal epithelial Caco-2 cells.....	611
Cytotoxicity of gold nanoparticles in the Caco-2 human intestinal epithelial cell line.....	494
Decellularizing colorectal tumours: the role of macrophages and extracellular matrix.....	60
Degradation of UV-filters 4-methyl-benzylidene camphor, benzyl salicylate and phenyl salicylate in chlorinated waters.....	64
Delivery of indomethacin loaded liposomes to Arthritic Joints.....	192
Demographic characterization of the deaths occurring in Portuguese Public Hospitals between 2000 and 2010.....	300
Design of 3-hydroxy-4-pyridinone functionalized with hydrophilic ethoxylated chains.....	415
Design of a Web-based Application towards Improving Education in Clinical Anatomy.....	325
Detection of horse meat adulteration in processed foods using the PCR technique.....	314
Determinants of food choice at work: exploratory study in food service.....	301
Determination of HIF-1 α relevance in the immunopathology associated with <i>Mycobacterium avium</i> infection.....	159
Determination of macrophage distribution at the feto-maternal interface during <i>Toxoplasma gondii</i> infection and implications on pregnancy outcome using the mice model.....	578
Development and characterization of a co-culture two-dimensional blood-brain barrier for the study and correlation of drug permeation.....	604
Development and characterization of a tridimensional intestinal model to study intestinal absorption.....	527
Development and characterization of an oil-in-water cream containing honey.....	570
Development of a method for simultaneous determination of drugs in natural waters by SPE-UPLC-MS.....	396
Development of a new pyruvate-selective electrode for wine control.....	620
Development of a new species-specific PCR assay for the detection of horse meat.....	313
Development of a toothpaste containing roots of.....	567
Development of an automated analytical system for the calibration of micro-fiber electrodes sensitive to NO.....	428
Development of factorial design for a phenolic-rich extract of <i>Chelidonium majus</i> L.....	599
Development of Fail-Safe Control Methodologies for Multi-Motor Electric Vehicles: An Initial Feasibility Study.....	241
Development of formulations containing mud from saline of Santiago da Fonte, Aveiro, for dermocosmetics and dermo therapy applications.....	568
Development of glyceryl behenate (Compritol® E ATO) matrix tablets for sustained release of thiamine hydrochloride.....	235
Development of sustained release matrix tablets of vitamin B1 containing hydrogenated castor oil (Cutina® HR).....	556
Development, characterization and stability evaluation of silicone emulsions containing dithranol.....	575
D-Galactose aging model and antioxidant treatment effects in anxiety, learning and memory.....	441
Dietary supplements for cognitive function improvement: control of amino acid levels.....	493
Different Views of Cosmic Defect Evolution.....	364
Differential toxicity in the cerebellum of adolescent and aged rats following a MDMA neurotoxic regimen.....	489
DINAM – testing a prototype dynamometer for handgrip strength.....	243
Discovering microalgae: <i>Scenedesmus obliquus</i> (Turpin) Kützing.....	597
Discovery of a new small molecule inhibitor of the p53-MDM2 interaction.....	322
Dispersion of Carbon Nanotubes by Ionic Surfactants.....	395
Distribution of adenosine A _{2A} receptors in the kidney of hypertensive diabetic rats.....	561
Distribution of Resveratrol and its Antioxidant Efficiency in Stripped Corn Oil Emulsions.....	407
Diversity at the <i>Fragaria S-RNase</i> lineage genes.....	617
Does environmental exposure to xenoestrogens contribute to cardiometabolic risk during obesity in menopause?.....	478

DOES THE STATUS OF AN INGROUP DEVIANT AND AN EFFECTIVE GROUP CONTROL INFLUENCE SOCIAL COHESION ON ATHLETES?	170
Dynamometric analysis of swimming starts: 3D assessment of moments and forces	239
Dysphagia in the elderly and nutritional status	480
Eating well and better with <i>Nutri Ventures</i>	217
Ecological adaptation in <i>Littorina fabalis</i>	583
Economic Growth in Cape Verde and its Impact on Environmental Sustainability	393
Economic impact of food waste in a school centre	262
Ecotourism Potential in National Parks: a Case Study	222
Ecto-enzymes involved in the metabolism of adenine nucleotides in the rat ileum: distribution, enzymatic activity and neuromodulatory role.....	190
Effect of <i>Cordyceps militaris</i> methanolic extract in NCI-H460 tumor cells.....	518
Effect of dietary L-tryptophan supplementation on growth performance and feed utilization in <i>Dicentrarchus labrax</i> reared at two densities	87
Effect of different growth conditions in brown macroalgae carotenoids profile	595
Effect of hypertonic sodium chloride on intracranial pressure and cerebral perfusion pressure.....	98
Effect of juice treatment, urea addition and double distilling on ethyl carbamate formation in sugar cane spirit	315
Effect of serial repitching on proteolytic and bioactive properties of brewer's spent yeast extracts: storage stability evaluation	510
Effect of sunlight on cadmium containing nanoparticles (quantum dots) toxicity.....	220
Effect of thermal treatments on Gd ₅ Si _{1.3} Ge _{2.7} thin films.....	271
Effect of years of practice and different positions on visual memory in male junior volleyball players	308
Effects of chronic alcohol consumption and withdrawal on the density and somatic volume of NPY-containing neurons and on the cholinergic innervation of the hippocampal dentate hilus	491
Effects of chronic ethanol treatment and withdrawal on the vasoactive intestinal polypeptide content and cholinergic innervation of the rat somatosensory cortex	490
Effects of coloured and non-coloured phenolics of <i>Echium plantagineum</i> L. bee pollen in Caco-2 cells	586
Effects of epigenetic modulation upon mitochondrial dynamics and neurite outgrowth in developing neurons	283
Effects of high-fat diet, energy restriction, exercise and atorvastatin treatment on angiogenic factors expression in the aged rat myocardium	58
Effects of macroalgae invasive species and climate-driven stressors on estuarine sediments nitrogen biogeochemistry. .	125
Effects of marine bacteria on <i>Arabidopsis thaliana</i> growth	508
Effects of Ozone in <i>Salix atrocinerea</i> pollen.....	383
Efficiency of cAMP, forskolin or prostaglandin E2 in the decidualization of human endometrial stromal cells	530
Efficient oxidative desulfurization process catalysed by PW ₁₁ Zn@MIL-101(Cr)	422
ELASTO-PLASTIC ANALYSIS OF THIN STRUCTURES USING A MESHLESS METHOD	24
Electrochemical study of the DNA damage induced by hypochlorous acid and its protection promoted by dietary antioxidants.....	426
Electrodeposition of Bi ₂ Te ₃ Nanomaterials for Thermoelectric generators	272
Elephant foot yam (<i>Amorphophallus paeoniifolius</i> (Dennst.) Nicolson): The effect of processing on the amino acid profile	487
Enantioselective Synthesis and Biological Evaluation of Proline Mimetic of PLG based on (1 <i>R</i> ,3 <i>S</i>)-2-azabicyclo[2.2.1]heptane	416
Encounters: A Psychological Intervention Program with Older Adults	347
Epiphytic fungal communities in vineyards: characterization by culture and molecular techniques	471
Evaluating the effect of dietary nitrate supplementation on growth, oxygen consumption and reproductive performance of zebrafish (<i>Danio rerio</i>).....	496
Evaluation of adhesion and biofilm producing abilities of worldwide spread <i>Escherichia coli</i> uropathogenic clonal complexes	451
Evaluation of hydration efficacy of a hand cream containing a coffee silverskin extract.....	433
Evaluation of ionic composition of water as chemical barrier against the invasive <i>Artemia franciscana</i>	180
Evaluation of isoflavones content in hydro-alcoholic extracts of <i>Medicago</i> spp.....	466
Evaluation of school snacks and breakfast after the implementation of the project "Lancheirinhas Saudáveis"	437
Evaluation of the toxicity of textile effluents and its removal by cork adsorption	72
Evaporation of fluids in microstructured fibers	209
Evolution of eye development genes in mammals.....	179
Evolution of post-zygotic barriers in a scenario of non-allopatric ecological speciation.....	591
Evolution of the Fine-structure Constant in the BSBM Model.....	207
Evolutionary population genetics and historical demography: a new method of crossing surnames and Y-chromosome	342
Exercise preconditioning prevents left ventricular dysfunction and maladaptive remodeling secondary to pulmonary arterial hypertension in rats.....	285
Expanding our therapeutic options: β -blockers for colon cancer?	324
Expanding the chemical space of antitumor xanthenes: a new route paved by a chromene moiety	558
Exploiting DNA markers for saffron (<i>Crocus sativus</i> L.) authentication	312

Expression Regulation of Rho GTPases family members during oligodendrocytes differentiation and myelination	449
Extended-spectrum beta-lactamase producing <i>Escherichia coli</i> in river water of a rural area in the North of Portugal ...	378
Facilitation of acetylcholine release via the activation of adenosine A _{2A} receptors in rats with Experimental Autoimmune <i>Myasthenia Gravis</i>	560
Farmed pheasant (<i>Phasianus colchicus</i>) meat for human consumption: Amino acid profile characterization	414
Feasibility of using a TiO ₂ -contained in a paint coat to inactivate microorganisms in an aqueous system	290
Firing distance estimation through the analysis of the gunshot residue deposit pattern around the bullet entrance hole using FT-NIR spectroscopy.....	246
Firing distance estimation through the detection of gunshot residues in blowfly larvae (<i>Calliphoridae</i>): an experimental study	460
Flavonoids as anti-inflammatory and anti-allergic agents	596
Florence case: violation of privacy of the body and the State of Exception	454
Flower extracts of <i>Filipendula ulmaria</i> (L.) Maxim inhibit the proliferation of human tumor cell lines	514
Fluoxetine effects in zebrafish embryos, insights to psychopharmaceuticals toxicity.....	531
Foliar application effects of 24-epibrassinolide in <i>Solanum nigrum</i> L. exposed to high levels of Ni	225
Food Portion Sizes.....	196
Fostering Parent Involvement in Writing: Development and Empirical Test of an Intervention Program for Parents.....	216
<i>Fractonotus caelatus</i> (Marcus, 1928) and <i>Echiniscus quadrispinosus brachyspinosus</i> Bartoš, 1934, two rare Tardigrades from Portugal.....	609
Frequency and Material Dependence of the Cooling Power in a Solid State Magnetic Refrigerator	270
Functional activity of extracts from Goji berries (<i>Lycium barbarum</i>).....	492
Functionalized clays for catalytic esterification reactions	63
Fundamental Cosmology with ESPRESSO and ELT-HIRES	363
Gene screening for gluten detection in food	436
Genotoxic effects in <i>Oncorhynchus mykiss</i> after acute exposure to erythromycin	519
Geostatistics applied to electrical resistivity data FEUP Geophysical Experimental Field – A case study	359
Green synthesis of silver nanoparticles (AgNP) – an automatic approach	417
Growth Charts for Portuguese School Children. A Study in Leça da Palmeira Basic School	259
Growth performance, antioxidant and immune responses in European seabass fed dietary probiotic supplementation under different rearing temperatures.	140
Heavy metals levels are increased in samples of kidney tumoral tissue: a microscopy technique, SEM-XRM, analysis	564
Hepatitis E virus in wild boar for human consumption	202
High-resolution diving behaviour revealed by satellite tagging of blue sharks	91
Histopathological lesions associated to canine norovirus.....	509
Homozygosity for the c.1486T>C polymorphism in <i>toll-like receptor 9</i> gene is associated with a decreased inflammatory response in hemodialysis patients	481
How to assess combination effects between <i>ecstasy</i> and often co-consumed licit substances?.....	571
How to develop a food product	317
Hub and non-Hub Proteins in protein-protein interaction networks	440
Human salivary protein HPLC profile on different days: differences between genders	112
Hydraulic bulge test for stress-strain curve determination	238
Hydrogen generation from catalytic hydrolysis of sodium borohydride on a novel mini-reactor for portable applications	355
Hypoxia-dependent regulation of galectin-3 in mammary tumours	156
Identification of photographic processes: photography lab FBAUP	538
Identity and nutrient availability drive the physiological response of intertidal macroalgae to pollution	495
Images of glass: glass as a médium for printing	38
Immunocytochemical and functional characterization of interstitial cells of Cajal in the rat ileum	557
Immunohistochemical analysis of phosphorylated mammalian target of rapamycin (p-mTOR) in canine mammary carcinomas.....	517
Impact Assessment of the Possible Free Trade Agreement between EU and Mercosur.....	162
Impact of abiotic factors in the invasion process: the case of <i>Artemia parthenogenetica</i> from Aveiro and Rio Maior saltworks.....	178
Impact of Microcystin contaminated irrigation water on the photosynthesis and growth of carrot (<i>Daucus carota</i>) – implications in water management and crop production	382
Impact of Sexual Orientation on Parental Justification and Social Support	545
Impact of urbanization level on <i>Acer negundo</i> pollen: fertility, protein content and allergenicity.....	385
Importance of oxygen and nitrogen reactive species on the control of <i>Leishmania infantum</i> infection	55
In order to meet our goals, 34 interviews of biographical type were carried out in a first phase with students of the 9 th grade from public schools of North Portugal. From this group, 4 participants were invited to a second meeting to deepen the data. Among the criteria for being selected for the second phase, we took into account gender, age and interest (or lack of) in school activities. The collected material was analyzed according to the narrative approach [1] and through the use of content analysis techniques [2].	172
<i>In vitro</i> antidepressant activity of medicinal plants extracts and mixtures	601

<i>In vitro</i> neuroprotective and antioxidant effects of seaweed extracts	513
<i>In vitro</i> studies to assess the biological potential of <i>Endopleura uchi</i>	588
In vitro tools to explore the communication between injured bone and nervous system: the humoral pathway	338
Incommunicability and deliberation: the shaping of the jurors conviction in Brazilian and French courts	251
Increased expression of P2X7 receptors in the neocortex of patients with drug-resistant Mesial Temporal Lobe Epilepsy	608
Influence of adipocytes in prostate cancer cells proliferation, invasion and migration profile	432
Influence of low isoflurane anaesthesia on the recall of object recognition task in adult mice – a pilot test	589
Inhabit on the streets: a study about identity and survival of homeless people	130
Inhibitory effect of beer marinades on formation of heterocyclic amines (HAs) in chargrilled pork loin	136
Innovative Technologies for the Treatment of Winery Wastewaters: Combination of Biological and Advanced Oxidation Processes	71
Insights on the Nanostructuring of Ionic Liquids by Infrared Spectroscopy	117
Instrumentation for Surface Electromyography	354
Interaction between metallic NPs and the salt marsh plant <i>Halimione portulacoides</i>	386
Interaction between Sparfloxacin and biological membrane models: a multi-technique approach	500
Introduction of artificial magnetic flux pinning centers in MgB ₂ multifilamentary superconducting wire	358
Introgression of Iberian maternal lineages onto the Sephardic Jews gene-pool inferred from the mitochondrial DNA	515
Investigating the cell growth inhibitory effect of flower extracts of <i>Sambucus nigra</i> L. in human tumor cell lines	528
Invisible shapes: the influence of serif shapes in the legibility of long printed texts	36
Ionothermal synthesis of transition metal based metal-organic framework materials	418
Is it true that education and training courses can contribute to the transformation of the trainees' self-efficacy beliefs and career development? A case study in the area of table waiting	173
Labview controlled Aerotech Stages	268
Larval Development of the Barnacle <i>Balanus perforatus</i> Reared in Laboratory	501
Lead in lipstick Results from a study of products manufactured in Brazil	410
Let-7c expression in HPV-associated cervical lesions and cancer	281
LGB Identity and Fear of Intimacy: an exploratory study	79
Life and death in the Benedictine Community of the Monastery of the Salvador of Paço de Sousa (1625-1826)	144
Life cycle of an association, dynamics of participation and learning opportunities: CRACS (Coletividade Recreativa e de Ação Cultural de Sousela)	218
Location of caffeic acid and its alkyl esters in liposomes	113
LOWER LIMBS DEXTERITY IN ELDERLY PEOPLE. EFFECT OF AGE AND GENDER	260
Lung Cancer – The Role of Trace Elements	484
Lupin: Nutritional composition and applications in the food industry	477
Macrophage-targeted nanoparticles: a novel approach towards rheumatoid arthritis theranostics	191
Magnetic characterization of meteorites using a mobile facility	362
Magnetic susceptibility in contaminated soils by mineral extraction of São Pedro da Cova	392
Marine macroalgae: a source of excellence of natural compounds with beneficial effects for health - A review	569
Marine Spatial Planning – contributions for (future) legal regime in Portugal	249
MDM2 SNP309 TT homozygous are associated with increased risk of colorectal cancer development	280
Measuring the direct and indirect effect of the content area in the psychometric quality multiple-choice items– A case study in clinical anatomy	78
Memristive Ag ₂ S Synapses: towards Artificial Neural Networks	273
Metabolome constituents, antimicrobial and antioxidant potential of the brown seaweed <i>Laminaria ochroleuca</i>	603
Metazoan parasites of <i>Trachurus picturatus</i>	223
Mexican four-eyed octopus species: nutritional value and metal contaminants	425
Mitochondrial regulation of epigenetics and its role in human diseases	106
Mitoxantrone causes time- and concentration-dependent toxicity in H9c2 differentiated cells at pharmacological relevant concentrations	574
Modulation of angiogenic pathways by 8-prenylnaringenin	340
Modulation of right ventricle function by Neuregulin-1 - therapeutic implications in pulmonary hypertension	56
Modulation of the uptake of critical nutrients by breast cancer cells by lactate: impact on cell survival, proliferation and migration	20
Modulation of visceral adipose tissue through exercise in obese animals: analysis of oxidative and apoptotic signalling markers	326
Molecular characterization of carbapenem-resistant <i>Pseudomonas aeruginosa</i> clinical isolates from a Portuguese Hospital	299
Molecular characterization of the <i>SFBB</i> genes in <i>Malus</i> S2-, S3-, S9-, and S28-haplotypes	616
Molecular Design of Potential Chk1-targeting Anti-Cancer Drugs	65
Molecular mechanisms underlying the beneficial effects of Neuregulin-1 in the treatment of Pulmonary Arterial Hypertension	54
Motor memory: a study with young swimming athletes	306
Motor Readiness and Physical Education Classes	254

Municipalities and Military Orders in the Middle Ages. Relations of dependence and confrontation in XII-XIV centuries.....	145
Mussel news: <i>Mytilus galloprovincialis</i> Lamarck.....	522
Mutation analysis of genes involved in sperm motility: A study in patients with total sperm immotility.....	435
Nanotechnology: Some Applications in Architecture and Design.....	42
N-doped TiO ₂ photocatalytic activity towards diphenhydramine oxidation and <i>E. coli</i> inactivation.....	388
New 7 α -allylandrostanes as Aromatase Inhibitors: Biological Effects in Hormone-dependent and Hormone-resistant Breast Cancer Cells.....	155
New inhibitors of p53-MDM2 interaction with chalcone scaffold.....	559
Novel methodology to immobilize efficiently polyoxometalates into silica nano sized spheres catalytically active for oxidation reactions.....	420
Nutritional evaluation of the invasive bivalve <i>Corbicula fluminea</i>	502
Nutritional intake adequacy in fourth grade children of Porto municipality –socioeconomic and anthropometric influences.....	198
Ochratoxin A residues in soluble coffee and coffee substitutes.....	404
OLFACTORY MEMORY: PERFORMANCE IN OLFACTORY IDENTIFICATION, SERIAL EVOCATION AND OLFACTORY RECOGNITION TASKS.....	550
On the anti-tumoral properties of Port Wine polyphenols and their bioavailability.....	613
One Health: MAC granulomatous lesions in slaughtered swine as a public health problem.....	512
Opioid-induced hyperalgesia is mediated by a pain facilitatory area of the brain.....	16
Optimal Stochastic Control of Life Insurance and Investment in a Financial Market.....	97
Optimization of Chlorogenic Acid Extraction from Lamb's Lettuce.....	115
Oral health and related behaviours: comparing Turkish and Portuguese dental students population.....	297
Order and Chaos in Architecture.....	40
OXIDASES AND DEHYDROGENASES METABOLIZING MANNITOL AND OTHER ALCOHOLS IN THE DIGESTIVE GLAND OF GASTROPODS.....	339
Oxidative stress parameters in the brain and peripheral organs following exposure of adolescent rats to an MDMA neurotoxic regimen.....	193
Oxytetracycline degradation using an homogeneous photo-Fenton process mediated by ferricarboxylate complexes.....	70
Parenting and substance abuse: a comparative investigation in parents with alcohol, heroin and cocaine abuse.....	544
Paternal Lineages in Macau and Shanghai: Enriching the Knowledge of Chinese and East Asiatic Populations.....	619
Pedal reaction time of Track & Field athletes: effect of sex and competition distance.....	257
Peer Instruction in the appropriation of concepts in a structuring CU for the Teaching of Engineering.....	83
Perceptions of risk associated with the consumption of honey.....	473
Perceptions of risk connected to the fish production and consumption.....	302
Performance of monounsaturated oils during potatoes deep-frying.....	398
Persimmon and Passion Fruit Cultures on Vale do Ave: Agronomic, Economic and Financial Analysis.....	470
Personality, anxiety, depression and Atopic Dermatitis severity: a cross sectional study.....	282
Phase Behaviour and Heat Capacities of 1-Benzyl-3-methylimidazolium Ionic Liquids Series.....	116
Phenolic profile and <i>in vitro</i> anti-cholinesterase activity of <i>Piper betle</i> leaves.....	593
Photocatalytic disinfection of bioaerosols generated from wastewater treatment plants (WWTPs) (Projeto 183).....	389
Photodegradation of veterinary antibiotics in culture media when exposed to artificial light.....	390
Phylogeny of OAS genes and association with dengue fever.....	448
Phylogeography and population structure of the powdery mildew fungus (<i>Erysiphe necator</i>) from diverse grapevine (<i>Vitis vinifera</i>) cultivars grown in Portugal.....	472
Phylogeography of mtDNA haplogroup L2.....	49
Piezoelectric Nanogenerators: Feeding the Future.....	274
Planctomycetes as a food source for <i>Daphnia longispina</i> : preliminary findings.....	524
Plate waste and his causes in lunch at Hospital Center of Alto Ave.....	298
Pleiotropic effects of cardiovascular drugs: evaluation of the ability to prevent myoglobin oxidation mediated by peroxyl radicals.....	486
Police officers' perceptions about prostitution and its actors: an exploratory study.....	171
<i>Polybius henslowii</i> crab: An approach to crustaceans.....	598
Polyoxometalates: synthesis, characterization and incorporation into multilayer films with organic dyes.....	430
Polyphenol oxidase activity of <i>Valerianella locusta</i> plants along time after package opening.....	183
Portrayal of the African people into Castro Alves' <i>Os Escravos</i>	33
Portuguese Self-Initiated Expatriates and their Social Networks: composition, diversity and roles.....	166
Potential antitumour xanthenes: synthesis and pharmacokinetics.....	565
Potential hepatotoxicity caused by synthetic cathinones acquired in 'smartshops'.....	341
Predicting the neurotoxicity of mixtures of piperazine drugs of abuse using the SH-SY5Y neuroblastoma cells.....	612
Preliminary results about the assessment of the bycatch in the beam trawl fishery on the Portuguese North coast.....	89
Preparation and characterization of pH-sensitive liposomes for the treatment of Rheumatoid Arthritis.....	230
Preparation of gold nanostars and functionalization with the pentapeptide CALNN.....	62

PREVALENCE OF HUMAN PAPILLOMAVIRUS IN TISSUE SAMPLES FROM HEAD AND NECK CANCER PATIENTS	296
Progesterone: a hormone with a role in plant defence against stress?	121
Proline Dehydrogenase cDNAs characterization in <i>Solanum nigrum</i> L. and <i>Solanum lycopersicum</i> L.	224
Protective effect of intranasal immunization against <i>Neospora caninum</i> infection established through the gastrointestinal tract	181
Protein structure and sequence complexity relationship: effect on protein interactome.	108
Protozoa colonization in Portuguese population on peritoneal dialysis	199
Psychoactive substances present in <i>Salvia divinorum</i> acquired in smartshops or in the Internet.....	247
QM/MM studies on the catalytic mechanism of human renin	67
Quality control of geomembranes in waste landfills.....	543
Quality control of geotextiles in waste landfills	542
Quality evaluation of honey: diastase activity and 5-hydroxymethylfurfural	429
Quality of life and psychological state after Heller myotomy in achalasia: What do patients think?	104
Quantification of Biogenic Amines in Liquors by a DLLME based method.....	468
Quantification of sugars in Specialty malts by HPLC-IR	137
Reactivity of green zero-valent iron nanoparticles produced using citrus wastes	387
Reducing the salt content in meat rissoles and its acceptability by the consumer.	316
Regression Methods for Multiple Outcomes in Health Research	373
Relationship between Alzheimer's disease and type 2 diabetes: anticholinesterase and anti- α -glucosidase activity of <i>Pilocarpus pennatifolius</i> Lem. herbal tea	600
Relationship between Tactical Skills and Young Basketball Players with height stature	309
Research and knowledge networks in the <i>European Network for Housing Research</i>	148
Resistance to biocides in <i>Salmonella</i> from Portugal: a multilayered approach	439
Response of microorganisms from constructed wetlands to veterinary drugs	123
Revaluation of Real Estate Assets Motivations and Consequences	163
Robotic Construction of Free Form Walls using Standard Cork Bricks	41
Role of adrenaline in the development of β_2 -adrenoceptors	188
Role of PKG-related Pathways in the Diastolic Response to Acute Myocardial Stretch Under Ischemic Conditions	234
Safety concerns regarding Plant Food Supplements	474
<i>Scaffolding</i> on Productivity and Quality of Life Narratives of Children in Institutional Care	213
School experiences and <i>queer</i> youth cultures: different ways of building (homo)sexual citizenships in schools	128
Schooling as experience: narratives of Portuguese 9 th grade students	172
Screening of virulence factors in swine <i>Enterococcus faecium</i> strains with zoonotic potential	197
Search for early TTR-related biomarkers in a transgenic AD mouse model	284
Searching for cyanobacterial natural antifouling compounds against <i>Mytilus galloprovincialis</i> settlement	581
Selection of antibiotic resistant bacteria: a role for mercury?.....	445
Semi-feral cats: the human influence on circadian activity	615
Sensitivity studies using analytical and numerical methodologies on variables influencing sheet metal Press Brake bending operations.....	22
Sensory profiling of duck rice using conventional and rapid methods	263
Sheet metal mechanical characterization using biaxial test and numerical modelling.....	242
Signalling role of avian barred plumage: a test with common waxbills	46
SOCIAL INTERACTIONS IN A CAPTIVE PACK OF IBERIAN WOLVES (<i>Canis lupus signatus</i>)	592
<i>Solanum nigrum</i> L.'s Glutathione-S-transferase (GST) and Gamma-glutamyl cysteine synthetase (γ -ECS) evaluation in response to two steroidal hormones	120
<i>Solanum nigrum</i> L.'s Glutathione-S-transferase plays a role in the detoxification of paracetamol	45
Solid lipid nanoparticles as a vehicle for brain-targeted delivery of resveratrol.....	343
Southern Europe's Welfare-State Model – arm in arm with ideology?.....	29
Spatial variations of the fine-structure constant in symmetron models	204
<i>Spodoptera littoralis</i> larvae: beyond the damage.....	605
<i>Spodoptera littoralis</i> / <i>Lycopersicon esculentum</i> : ecological duo	606
Springback prediction in Sheet Metal Forming	23
Stimuli-responsive lysine-based surfactants: synthesis and supramolecular self-assembly.....	412
Street-vending foods: a vehicle of pathogenic bacteria and clinically relevant antibiotic resistance genes.....	444
Stress alleviation capability of exogenous brassinosteroids applied to <i>Solanum nigrum</i> L. exposed to high levels of Zn	381
Study of food waste in primary schools and kindergartens	201
Study of patterns of movement and connectivity in <i>Emys orbicularis</i> inside the PAVT	523
Study of the microvesicles populations in <i>Leishmania infantum</i> exoproteome	506
Study of the regulation of a zinc transporter in <i>Leishmania infantum</i>	475
Sustainability assessment in agrosystems, combining terrestrial fauna simple information.	394
Sustainability Evaluation of Biodiesel from Microalgae <i>Chlamydomonas sp</i> grown in Brewery Wastewater	289
Sustained release dosage forms: a thiamine hydrochloride tablet formulation based ethylcellulose	154
Synthesis and application of novel molecularly imprinted material for determination of an aromatase inhibitor.....	401

Synthesis and characterization of MIL-101-type metal-organic frameworks with different metallic centres	413
Synthesis and characterization of μ -oxo-bridged Fe(III) complex of <i>meso</i> -tetrakis(pentafluorophenyl)porphyrin	421
Synthesis of new chiral diamines as potential bidentated ligands	406
Synthesis of new <i>N</i> -acyl homoserine lactones	403
Synthesis of novel serine-based surfactants as potential bioactive systems	402
Syphilis hospitalizations in Portugal over the last decade	200
Systematic characterization of attack coverage formations in high-level men's volleyball	459
Teaching Communication Skills to medical students: association between Empathy, Emotion Recognition Ability and Alexithymia	547
TelePalestra – A better way to share knowledge	356
Temperature tolerance limits of <i>Synechocystis</i> sp. PCC 6803 and its improvement using compatible solutes	521
Testing universality in complex systems	95
Tetralogy of Fallot: a cardiopathy with different physiopathology and evolution	476
The “Imperial” Mcdonaldization: glocal crossings in a ancient coffee shop in Porto	28
The 2014 FIFA World Cup as a Job Creator	167
The anticarcinogenic effect of the dietary compound kaempferol in a human breast cancer cell line is dependent on inhibition of glucose cellular uptake	59
The Associative Movement of Parents Educational Dimensions of Participation in Associations (O Movimento Associativo de Pais Dimensões Educativas da Participação nas Associações)	551
The Church of Nossa Senhora da Atalaia: an approach to the scientific methods of the Renaissance	334
The concept of cultural district: reflection about the form and the function	374
The Corporate Social Responsibility - A lever for sustainability? A case study: The Nestlé Group and cocoa plantations in Ivory Coast	375
The effect of Arabic gum in microbial growth	507
The Effect Of Sport (Karate And Football) Of Foot Dexterity In Under 15 Male	304
The Effect of Training Session on Simple Reaction Time in Children Pedal Practitioners Athletics	305
The effects of biased initial conditions on domain wall evolution	366
The efficiency of Portuguese Technology Transfer Offices and the importance of university characteristics	456
The entrance as element conceptual design	330
The environmental impacts from the Portuguese fish consumption patterns: assessing cod, hake, sardine, horse mackerel, chub mackerel and salmon	74
The evolution of Star and Star-related proteins in vertebrates	182
The evolution of the eye developmental genes in the barn owl (<i>Tyto alba</i>)	47
The evolution of the opsin gene family in cave fish (<i>Astyanax mexicanus</i>)	141
The Evolution of the Retinoid Orphan Receptor (ROR) in Lophotrocozoa Protostomes	526
The forensic psychological assessment in child custody and parenting capacity cases: characterization of the expertise (GEAV as an analyzer)	212
The IFC Standard as an Interoperability Agent in Data Exchange Between BIM Applications	25
The impact of literacy on mirror discrimination	82
The influence of Guinevere's royal legitimacy over the chivalry's supremacy	31
The influence of the incarceration experience in the development of social (re) integration perspectives in women serving sentences	132
The Influence of the Performance Management and Evaluation in the Behavior of Workers	80
The influence of the practice of physical activity on elderly's balance	307
The interplay of erythrocyte cytosolic peroxidases with the membrane under H ₂ O ₂ mediated oxidative stress	504
The invasive behaviour of <i>Cylindrospermopsis raciborskii</i> in Portuguese Freshwater Systems	139
The life experience of parenting in adoption	548
The matrix perturbation theory and the influence of the seminal paper of Von Neumann and Golsdstine (1947)	96
The Portuguese textile and clothing industry: How foreign events influenced its performance since 1973	455
The Red Fox (<i>Vulpes vulpes</i>) in Portugal: Variation in cranial size	503
The role of morphological complexity driving the invertebrate biodiversity of intertidal macroalgae	48
The role of oocyte-secreted factors in the human granulosa cell line - GC1a	438
The space of the School, a Silent educator	333
The threat of multidrug resistance bacteria to public health: when aquacultures raise more than fish	443
The use of a biosensor for the antioxidant determination in vegetables extract	424
The verb: a study on creativity and individuation	350
The viewer as a performer	331
The vision of the female coaches about their inclusion in leadership positions in sport	255
The works of João Antunes in Northern of Portugal	146
Therapeutic efficacy and safety of BNIPDaoct-loaded PLGA nanoparticles for the treatment of visceral leishmaniasis	573
Three-dimensional craniofacial reconstruction – An art or a reliable forensic method?	461
<i>Tongobriga's</i> Civic Center. Designed contributions for its conjectural reconstruction	335
Toxic effects of single and combined nickel and microplastics on juveniles of <i>Pomatoschistus microps</i>	377
Toxicity evaluation at different trophic levels of pharmaceutical drugs	376

Toxicity testing of emerging contaminants under single and combined exposure using zebrafish and sea urchin embryo bioassays	124
Toxicological Assessment of Marine Cyanobacterial Extracts in Human Cell Lines – Proteomic and Gene Expression Approach	17
Trace element imbalances in hemodialysis patients – final results of a 16-month monitoring study in a Portuguese population	109
Trace Elements and Cardiovascular Diseases	485
Tracking the ultrafast carrier dynamics in graphene using pump-probe spectroscopy: preliminary femtosecond pulse measurements using the d-scan technique	269
Treatment of a dye-containing effluent by heterogeneous Fenton-like oxidation in a continuous stirred tank reactor.....	292
Treatment of olive mill wastewater by phytoremediation with <i>Lemna minor</i> or biosorption with cork.....	221
Ultra-Pure H ₂ Production through Water-Gas Shift Reaction in a Packed-Bed Membrane Reactor	288
Uncoated tablets: influence of the technological excipients and compaction force on “in vitro” thiamine hydrochloride release.....	450
Universal Service, Competition and Regulation.....	250
Unraveling post-transcriptional regulation of alternative polyadenylated <i>MCL1</i> isoforms by miRNAs in human T Cells	482
Urban floods, flash floods and wave-overtopping in the city of Santa Cruz, Madeira: affected areas and frequency.....	372
Urban stray dog (<i>Canis familiaris</i>) packs	511
Use of Noninvasive sampling in to infer the evolutionary history of wild equids	498
Using phosphoproteomics to elucidate the role of the phosphatase Sit4p in mitochondrial function and lifespan yeast...	614
Validation of BPA and phthalates metabolites in urine samples by SPE-GC-MS.....	409
Validation of otolith daily increments in early juveniles of shanny <i>Lipophrys pholis</i>	520
Valorisation of Saffron by-products	552
Valorization of <i>Arbutus unedo</i> L. berries: a study on their nutritional and phytochemical composition.....	572
Visual Technologies applied to Human Rights: Photography on <i>Programa USP Diversidade</i> (University of São Paulo Diversity Program)	332
Vortex-assisted liquid-liquid micro-extraction coupled to spectrophotometric propofol determination in biological matrices.....	232
We built this city: Architecture in Espinho, Portugal (1900-1943)	336
Wild and farmed duck breast meat: comparison of amino acid profiles by HPLC-FLD	265
Women as commercial sex clients: an exploratory study about female patrons of male striptease	129
<i>Xantho incisus</i> Leach and <i>Macropipus puber</i> L.: crabs as source of bioactive compounds	594
Xenobiotics in Human Milk: Risks to Human Health	19