Centro de
Investigação
de Montanha

Sustainable Processes and Products

Project outputs

2013-2018

Processes and Product Engineering

Modeling and Development of Polymer Networks for Molecular Recognition of Drugs and Bioactive Compounds

KEYWORDS: Molecularly Imprinted Polymers | Molecular Recognition | Controlled Release | Bioactive Compounds

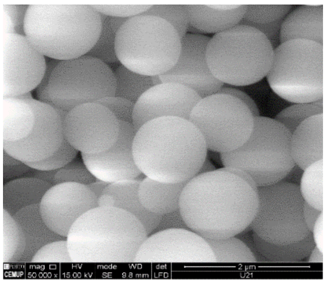
Achievements:

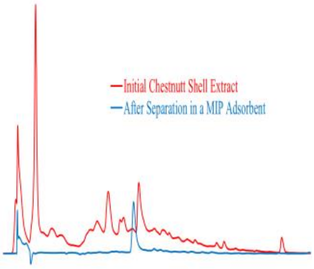
1. Functionalized and stimuli-responsive molecularly imprinted particles and hydrogels were produced using controlled radical polymerization.

2. Usefulness of these materials for molecular recognition of drugs and bioactive compounds, namely polyphenols, was evidenced.

3. Extraction with supercritical CO_2 was performed considering different vegetable resources (chestnut tree, cherry tree, olive tree, vineyard and cork) and separation/concentration of polyphenols was addressed through the developed molecularly imprinted polymers.

4. Hybrid natural/synthetic carriers (e.g. based on cellulose) for bioactive compounds were produced and initial results with polymersomes (mimicking natural cells) were obtained.





Objectives

The development of materials combining effective molecular recognition capabilities and variable swelling triggered by changes in the environment is a key issue to improve many kinds of separation processes (e.g. those involving biomolecules) and to design new drug delivery systems. In this research line, we consider responsive molecularly imprinted polymers (MIPs) to target specific molecules such as drugs (e.g. 5-fluorouracil used in cancer treatment) or bioactive compounds (e.g. polyphenols such as resveratrol or polydatin with known antioxidant activity). Different classes of functional monomers are used to exploit specific interactions with the target molecules. Polymer composition is also used as a design variable (e.g. changing the amount of crosslinker to synthesize soft molecularly imprinted hydrogels or stiff microparticles). Bulk, solution, suspension, inverse-suspension and precipitation polymerization processes are alternatively used in different kinds of reactors (batch, semi-batch, continuous flow micro-reactor) to tailor the morphology of the products.

Polymerization and extraction in supercritical CO_2 are also considered for molecular imprinting in more “green” conditions. Kinetics of the polymerizations and structural properties of the final materials are assessed using in-line/off-line FTIR-ATR and SEC with multiple detection (e.g. RI, MALLS, UV and viscosity).

Features of controlled radical polymerization mechanisms, namely of RAFT polymerization, are exploited to introduce stimuli responsive behavior in MIPs (e.g. triggered by changes in pH, temperature, ionic strength). Functionalization of micro-particles through surface grafting and synthesis of amphiphilic structures (e.g. core/shell architectures) leads to some improvement opportunities in this context. The assessment of the molecular recognition capabilities of the engineered responsive MIPs is performed through the measurement of their performance concerning the uptake and release of specific molecules (e.g. when used as stationary phase in chromatographic columns).

Having in view final applications in pharmaceutics, cosmetics, food industry or biomedicine, natural polymers like cellulose and alginates are also being used in the development of carriers for bioactive compounds. Hydrogels based on the chemical crosslinking of cellulose or on the physical gelation of alginates are considered within this purpose. Hybrid natural/synthetic networks with amphiphilic properties are also addressed through the grafting of polymer chains on cellulose backbone (esterification of the hydroxyl groups and RAFT polymerization are being considered).

RAFT polymerization is being also used to synthesize amphiphilic block copolymers and afterwards generate polymer vesicles (polymersomes), mimicking natural cells, to be considered as carriers for bioactive compounds.

Results

Polymer reaction engineering studies involving smart hydrogels formation were addressed in this period and computational tools to aid the tailoring of such materials were developed. Classical free radical polymerization [1] and RAFT polymerization [2,5], were both tried. Important discrepancies in the dynamics of gel formation were identified when FRP mechanism is replaced by the RAFT polymerization. The opportunity to control the swelling ratio of the hydrogels through the design of the amount of RAFT agent was proven and the stimulated release of drugs such as caffeine, ibuprofen or 5-fluorouracil from the synthetized materials was assessed.

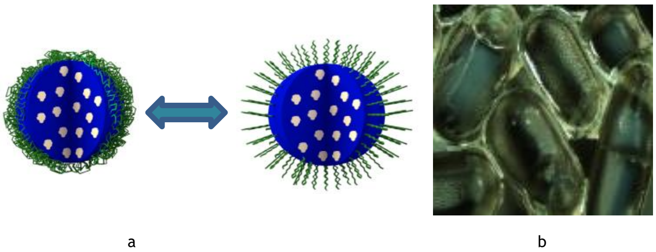
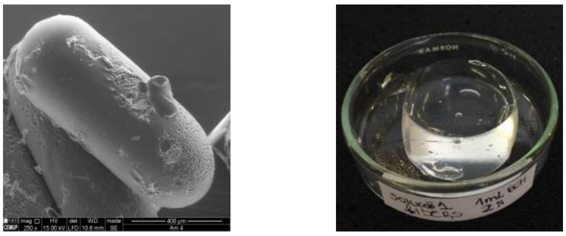


Figure 1. (a) Depiction of molecularly imprinted polymer particles with surface grafted polymer brushes (e.g. through RAFT polymerization). Improved stimulation to external changes and amphiphilicity are some advantages of such advanced molecular architectures. (b) Images of swelled acrylic acid hydrogel particles produced in continuous flow micro-reactor. Combination of different polymerization processes with controlled reaction mechanism (e.g. RAFT) is being considered to tailor the morphology and molecular architecture of the products.

The combination between the operation in continuous flow micro-reactor, RAFT polymerization and molecular imprinting was explored to synthetize hydrogel particles with simultaneous high swelling capacity and molecular recognition features [3]. The genotoxic impurity 3-aminopyridine was addressed in these studies considering an aqueous environment.

The development of molecularly imprinted particles combining molecular recognition capabilities in aqueous

environment and good conditions to be used as packing materials in continuous adsorption processes was target considering precipitation and inverse-suspension polymerization and a much higher crosslinker content than with hydrogel synthesis (stiffer materials were sought) [4]. Caffeine and 5-fluorouracil were considered as template molecules and the important impact of the molecular imprinting conditions and working conditions on materials performance was evidenced, namely due to the hydrophobic interactions. The superior performance of the inverse-suspension products was showed, probably due to a surface imprinting process enhancing the mass transfer mechanisms. A MIP for caffeine retention with imprinting factor ~17 and maximum retention capability ~146 µmol/g was thus achieved.
The grafting of functional brushes (pH or Temperature sensitive) on the surface of particles molecularly imprinted with 5-fluorouracil was considered to develop materials combining molecular recognition capabilities and stimulation by external conditions [5,6]. Improved sensitivity of the particles with MAA polymer brushes to the pH of the surroundings was proven through the enhancement of 5-FU release in alkaline conditions (pH = 10) as compared to acidic environments (pH = 2). A boost on drug release was also observed for particles with PNIPA grafted brushes at T=20 °C as compared to 40 °C.

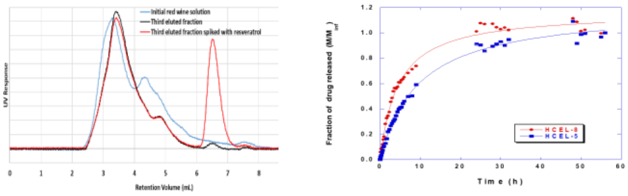


a

b

Figure 2. (a) SEM image of dried molecularly imprinted hydrogel particles produced in continuous flow micro-reactor. A swelling-induced process was designed for the selective retention of drugs such as 3-aminopyridine. Other kinds of molecularly imprinted microparticles were obtained through precipitation polymerization. These kinds of particles (with/without grafted functional brushes) are being used to target drugs and bioactive compounds (e.g. polyphenols). (b) Image of a cellulose based hydrogel piece. Natural polymers, such as cellulose and alginates, are also being used to develop carriers for drugs and bioactive compounds. Uploading of the target molecules in these polymer networks with supercritical CO_2 is also being assessed.

In a later stage, molecular imprinting studies were extended to the targeting of polyphenols present in plant extracts [7]. A MIP with polydatin as template molecule, resulting from an inverse-suspension process, showed an outstanding performance for polyphenols retention (e.g. polydatin, resveratrol). Plant extracts (e.g. from chestnut tree, cherry tree, vineyard) obtained with supercritical CO_2 were used in these studies. The development of carriers for such bioactive compounds based on cellulose, alginates or polymersomes (based on RAFT amphiphilic copolymers) was also already started and first results have been published [8].



a

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Figure 3. (a) HPLC chromatogram showing the usefulness of the produced MIP particles for separation and concentration of polyphenols (e.g. polydatin, resveratrol) present in natural extracts. (b) Fraction of drug (polydatin) release from two initially dried cellulose-based hydrogels after pouring into a 5% ethanol aqueous solution. Impregnation of the polydatin in the hydrogels was previously performed in supercritical CO_2 at T=40 °C and P=170 bar during 1 h.

Future Work

The following major issues will be addressed in future work of this research line:

- Improvement of the selectivity of MIPs for targeting polyphenols present in natural extracts. Designing of amphiphilic materials to address the non-specific hydrophilic/hydrophobic interactions is a major goal in this context. These materials should be useful to separate and concentrate polyphenols present in complex plant extracts, namely those arising from supercritical CO_2 extraction of agriculture and forest residues (e.g. chestnut, olive and cherry trees, vineyard, etc.).
- Design of new carriers for these kinds of bioactive compounds based on cellulose or its derivatives, alginates and amphiphilic block copolymers (polymersomes). Hybrid natural/synthetic materials resulting from the RAFT grafting of polymer brushes on cellulose will be specially addressed.

OUTPUTS

PhD Thesis

[1] Miguel Gonçalves, Synthesis and properties of polymers resulting from radical polymerization of multivinyl monomers, PhD degree in Chemical and Biological Engineering, FEUP, 2014.

Master Dissertations

[1] Carla Machado, Synthesis and Characterization of Smart Hydrogels for Controlled Drug Release Considering RAFT Polymerization and SEC with Tetra-Detection, Chemical Engineering, IPB, 2014

[2] Daniela Oliveira, Experimental Studies and Mathematical Modeling of Drug Release in Interpenetrating Polymer Networks, Biomedical Engineering, IPB, 2014.

[3] Patrícia Reitor, Preparation and Testing of Smart Hydrogels for Reversible Immobilization and Separation of Proteins and DNA, Biomedical Engineering, IPB, 2014.

[4] Filipa Freitas, Synthesis and Characterization of Molecularly Imprinted Polymer Particles (MIPs) for Biomedical Applications, Biomedical Engineering, IPB, 2015.

[5] Gayane Sadoyan, Development of Amphiphilic Adsorbents for the Stimulated Uptake and Release of Polyphenols, Chemical Engineering, IPB, 2017.

Selected Publications

[1] M.A.D. Gonçalves, V.D. Pinto, R.C.S. Dias, M.R.P.F.N. Costa, Journal of Nanostructured Polymers and Nanocomposites 9, 40 (2013)

[2] M.A.D. Gonçalves, V.D. Pinto, R.A.S. Costa, R.C.S. Dias, J.C. Hernándes-Ortiz, M.R.P.F.N. Costa, Macromolecular Symposia 333, 41 (2013)

[3] P. Kadhirvel, C. Machado, A. Freitas, T. Oliveira, R.C.S. Dias, M.R.P.F.N. Costa, Journal of Chemical Technology & Biotechnology 90, 1552 (2015)

[4] D. Oliveira, A. Freitas, P. Kadhirvel, R.C.S. Dias, M.R.P.F.N. Costa, Biochemical Engineering Journal 111, 87 (2016)

[5] D. Oliveira, R.C.S. Dias, M.R.P.F.N. Costa, Macromolecular Symposia 370, 52 (2016)

[6] D. Oliveira, C.P. Gomes, R.C.S. Dias, M.R.P.F.N. Costa, Reactive and Functional Polymers 107, 35 (2016)

[7] C.P. Gomes, G. Sadoyan, R.C.S. Dias, M.R.P.F.N. Costa, Processes 5, 72 (2017)

[8] C.P. Gomes, R.C.S. Dias, M.R.P.F.N. Costa, Polymer Reaction Engineering Tools to Tailor Smart and Superabsorbent Hydrogels, Book Chapter in "Cellulose-Based Superabsorbent Hydrogels", Polymers and Polymeric Composites: A Reference Series, ISBN 978-3-319-77831-0, Springer, (2018)

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FUNDING

LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017
LA LSRE-LCM Strategic Project, PEst-C/EQB/LA0020/2013, 2013-2014
AIProcMat@N2020, NORTE-01-0145-FEDER-000006, 2016-2019
Polymer Reaction Engineering, NORTE-07-0124-FEDER-000014, 2013-2015
Molecular Engineering, Synthesis and Testing of Water Compatible Smart Polymers, PTDC/EQU-EQU/098150/2008, 2010-2013
Research contracts with cork industry (Amorim&Irmãos), 2015/2016 and 2017/2018

Processes and Product Engineering

Tailored Polymer Networks for Energy Storage and Conversion Systems

KEYWORDS: Sulfur-based Polymer Networks | Lithium-Sulfur Batteries | Polymer Encapsulation | Phase Change Materials | Dye-Synthesized Solar Cells

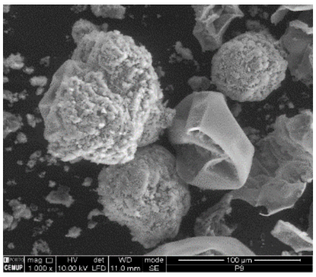
Achievements:

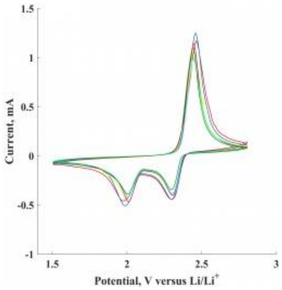
1. Tailored and functionalized polymer networks with applications in different kinds of energy storage and conversion systems were produced.

2. For the first time, sulfur networks have been synthesized through RAFT polymerization and used to make cathodes for lithium-sulfur batteries.

3. Vegetable and animal natural fats were tested as phase change materials and encapsulated in tailored polymer shells, aiming the thermal energy storage/release.

4. Tailored conductive polymers were tested in dye-synthesized solar cells, aiming the improvement of energy efficiency.





Objectives

The focus of our research is the reaction engineering of non-linear polymers with emphasis on both classical free-radical polymerization (FRP) as well as different kinds of reversible-deactivation radical polymerization (RDRP) mechanisms, namely, nitroxide-mediated polymerization (NMP), atom-transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization. Our goal is the development of materials with tailored properties through the computer-aided design of polymerization processes leading to hyperbranched polymers and networks. These lines of thought are used to improve and develop new materials with practical applications in different areas, namely for energy storage/conversion systems.

Indeed, many polymer materials play a key role in energy efficiency, which is nowadays a central issue for mankind. In this research line, we explore the development of polymer networks with useful properties for energy storage and conversion systems.

Tailored sulfur-based polymer networks have been chosen as a means to improve the performance of cathodes in lithium-sulfur (Li-S) batteries (or others). Indeed, in the last few years, Li-S batteries have been looked as a potential alternative to legacy lithium-ion systems, which are reaching their limit performance regarding energy density storage, thus restricting their application in systems with large power needs, such as electric vehicles. Nonetheless, different kinds of issues (polysulfides formation, poor conductivity of sulfur and increase of specific volume during discharge) lead to a poor stability of the cathodes causing the degradation of battery performance after some discharge-charge cycles. The transformation of elemental sulfur into a sulfur-rich polymer network is a possible way to avoid these shortcomings. So, we are developing a RAFT inverse-vulcanization process leading to networks with tailored structures, also improving the control of the involved polymerization mechanisms.

New synthesis pathways have been implemented, in particular the functionalization of the networks and the incorporation of conductive polymers such as poly(3,4-ethylenedioxythiophene) in the cathodes. Our work comprises the FRP and RAFT inverse-vulcanization of elemental sulfur, assembly of electrochemical cells with the produced cathodes (at the same time with lithium in the anode) and the testing of the batteries (e.g. by cyclic voltammetry and discharge-charge cycling analysis). Electropolymerization synthesis is another strategy to improve the conductivity of the networks.

Another research line is devoted to encapsulation of phase change materials (PCMs) for thermal energy storage/release applications. We have been considering different kinds of PCMs, including natural fatty acids from animal and vegetal sources, and also different encapsulation polymer networks by changing the monomers, crosslinkers and polymerization mechanisms involved. The effect of the polymerization technique used (FRP, ATRP, NMRP, and RAFT) on the encapsulation efficiency, size/morphology of the particles, thickness of the particle shell and final materials performance for thermal energy storage and release is assessed in our research. These studies comprise the encapsulation of the PCMs (e.g. in batch reactor operating with aqueous suspension), the analysis of the formed particles and encapsulating shell (SEM, FTIR, SEC) as well as the evaluation of their ability to be considered for energy efficiency applications (e.g. in building industries).

Figure 1. Elemental sulfur (1). Sulfur-rich polymer network synthesized by inverse-vulcanization (2). Produced cathode based on the sulfur polymer network (3). Assembly of a lithium-sulfur battery containing the produced cathode (4). Testing of the lithium-sulfur battery (5).

Results

Polymer reaction engineering studies involving networks formation were addressed in this period and new models, as well as, new computational tools to aid the tailoring of such materials were developed. Classical free radical polymerization and controlled radical polymerization, namely NMRP and RAFT polymerization, were dealt with [1-4]. The possibility for the synthesis of more homogeneous polymer networks when replacing FRP by NMRP or RAFT was assessed (e.g. through the synthesis of polymer networks with low dispersion of the molecular weight between crosslinking points). Styrene/divinylbenzene was selected as a model system in the experimental studies performed and the dynamics of gelation was measured for aqueous-suspension processes conducted in atmospheric or pressurized reactors (reaction temperatures up to 130 °C were considered in these synthesis) [1,2]. Results obtained have shown an improved control of network homogeneity with NMRP or RAFT but intramolecular cyclization occurs in a great extent even in bulk.

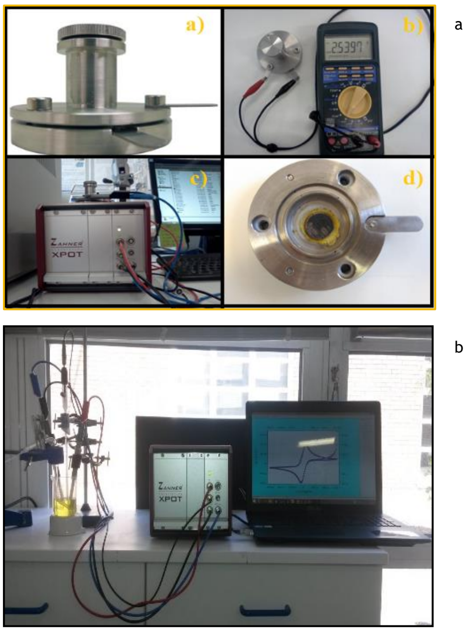


Figure 2. (a) Split test type device used to assemble electrochemical cells with lithium in the anode and a sulfur polymer network in the cathode. (b) Testing of the cells include discharge-charge cycling analysis and cyclic voltammetry. Cyclic voltammograms for electrochemical cells assembled with lithium in the anode and a sulfur polymer network in the cathode were thus obtained.

Population balances of sequences [3,4]. have been used to describe primary cyclization. Better predictive capabilities have been achieved but the need for additional developments was also identified. The performance of the modeling approach developed by this research group, based upon the use of population balance equations with generating functions, was compared with alternative methods, namely the kinetic Monte Carlo, and mathematically naive statistical/kinetic Flory/Tobita and numerical fractionation [5]. It yields reliable predictions for the full chain length distribution with non-linear polymerization, but its speed needs to be improved.

For the first time, sulfur networks have been synthesized through RAFT polymerization and used to make cathodes [6]. The crosslinker 1,3-diisopropenylbenzene was used in these synthesis and the electrochemical activity of the generated sulfur-rich materials was assessed. Testing of electrochemical cells assembled with lithium in the anode and the sulfur networks in the cathode has also been carried out, showing the usefulness of these new materials [6].
Phase change materials (e.g. n-octadecane) were successfully encapsulated in crosslinked polymer particles, using FRP and RAFT polymerization. They achieve a good performance for storage/release of thermal energy.
The lines of thought above described were also applied to tailor conductive polymers to be applied in dye synthesized solar cells.

Future Work

The following major issues are being addressed or define the future work in this research line:

- Improvement of the numerical efficiency to recover chain length distributions of non-linear polymers with controlled radical mechanisms (RAFT, NMRP, ATRP).
- Use of RAFT vulcanization of sulfur to improve the generated cathodes (functionalization of networks, etc).
- Use of RDRP mechanisms to design new architectures for encapsulation of animal/vegetal fats as PCMs.
- Tailoring conductive polymers for solar cells with RDRP.

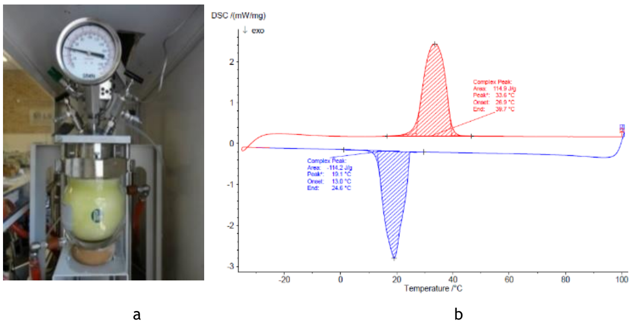


Figure 3. (a) Pressurized batch reactor used to encapsulate phase change materials (PCM) in crosslinked polymer particles through an aqueous suspension process. (b) DSC analysis of crosslinked poly(methyl methacrylate) particles with encapsulated n-octadecane showing the storage and release of thermal energy.

OUTPUTS

PhD Thesis

[1] Leandro Aguiar, Mathematical Modeling and Synthesis of Styrene/Divinylbenzene Copolymers using Classical Free Radical Polymerization and Nitroxide Mediated Radical Polymerization, PhD degree in Chemical and Biological Engineering (FEUP) and University of São Paulo (Brasil), 2013.

Master Dissertations

[1] Hugo Costa, Production of Materials with Electrochemical Applications through the Inverse-Vulcanization of Sulphur, MER, IPB, 2014.
[2] Paulo Tristão, Encapsulation of Phase Change Materials (PCM) for Applications in Energy Storage, MER, IPB, 2014.
[3] Carlos Almeida, Evaluation of the Electrochemical Properties of Sulfur Polymers using Cyclic Voltammetry, MER, IPB, 2015.
[4] Clementina Ribeiro, Synthesis and Characterization of Conductive Polymers with Applications in Solar Cells, MER, IPB, 2015.

Selected Publications

[1] M.A.D. Gonçalves, V.D. Pinto, R.C.S. Dias, M.R.P.F.N. Costa, L.G. Aguiar, R. Giudici, Macromolecular Reaction Engineering 7, 155 (2013)
[2] M.A.D. Gonçalves, V.D. Pinto, R.C.S. Dias, J.C. Hernándes-Ortiz, M.R.P.F.N. Costa, Macromolecular Symposia 333, 273 (2013)
[3] L.G. Aguiar, M.A.D. Gonçalves, V.D. Pinto, R.C.S. Dias, M.R.P.F.N. Costa, R. Giudici, Macromolecular Reaction Engineering 8, 282 (2014)
[4] L.G. Aguiar, M.A.D. Gonçalves, V.D. Pinto, R.C.S. Dias, M.R.P.F.N. Costa, R. Giudici, Macromolecular Reaction Engineering 8, 295 (2014)
[5] S. Lazzari, S. Hamzehlou, Y. Reyes, J.R. Leiza, M.R.P.F.N. Costa, R.C.S. Dias, G. Storti, Macromolecular Reaction Engineering 8, 678 (2014)
[6] C. Almeida, H. Costa, P. Kadhirvel, A.M. Queiroz, R.C.S. Dias, M.R.P.F.N. Costa Journal of Applied Polymer Science 133, 43993 (2016)

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FUNDING

LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017
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Polymer Reaction Engineering, NORTE-07-0124-FEDER-000014, 2013-2015
Development and Assessment of the Electrochemical Activity of Cathodes based in Sulfur Polymer Networks, 11/2015-08/2016

Processes and Product Engineering

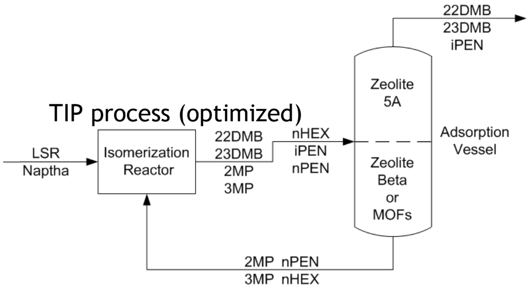
Separation of n/iso-Paraffins by Adsorption Process using MOFs and Zeolites

KEYWORDS: MOFs | Zeolites | Separation of Paraffin Isomers | TIP processes

Achievements:

1. Using layered and mixed adsorber bed configurations improves the separation of n/iso-paraffins resulting in high RONs;

2. Separation of hexane isomers using zeolite imidazolate framework (ZIF-8)



Introduction and Objectives

The combustion quality of gasoline is measured by the research octane number (RON). The light straight-run (LSR) naphtha fraction produced by fractional distillation is one of the feedstocks used to produce gasoline. Its major constituents include C5 and C6 normal paraffins which have relatively low RON compared to their branched isomers. Therefore, octane upgrading technologies commonly uses catalytic isomerization processes to rearrange the structure of the linear paraffins into branched components. Unfortunately, the catalytic conversion is an equilibrium-limited reaction and the isomer mixture produced still contains around 50% of mono-branched paraffins and at least 10% of linear paraffins. To obtain a better average C5/C6 RON, the unreacted paraffins are separated from its branched isomers and recycled to the isomerization reactor. Total isomerization Process (TIP) is a technology that performs this separation by adsorption employing zeolite 5A, which can completely sieve the linear paraffins from its branched isomers, after the isomerization stage. Typically, TIP process yields an 87 to 90 RON product. However, this material cannot separate mono-branched from di-branched isomers by a kinetic mechanism. The complete separation of mono-branched and di-branched isomers from a typical TIP process can reach an octane gain of 2 points. The increase of 1 RON in the final isomerate product corresponds to about US$ 900,000 per year for 300,000 tons per annum (tpa) hydroisomerization unit (50 tpa ≈ 1 barrel per stream day - BPSD). Thus, for a 10,000 BPSD hydroisomerization unit, a value around US$ 3,000,000 per year is achieved. So, it is crucial to find alternative adsorbents which can also separate branched isomers, showing an adsorption selectivity according to the branching degree to maximize the octane gain of this technology.

MOF's are porous metal-organic frameworks, which are new types of zeolite analogues, have been of major interest during the last decade because of their potential applications in gas storage, separation, heterogeneous catalysis, and sensing. Their framework consists of a network of transition metal ions linked by bridging organic ligands. MOFs are attractive for adsorptive applications because the pores can be systematically varied by the judicious choice of the metal ion and/or bridging organic linkers. This remarkable attribute is quite different from that of traditional zeolites whose pores are confined by rigid tetrahedral oxide skeleton that are usually difficult to alter. This superior feature is significantly important to develop novel functional materials for gas separation, which are based on their selective sorption behaviors and mainly determined by size exclusive effect in which smaller molecules can go through the microporous channels while larger molecules are blocked.

The goal of this work is the development of an adsorptive process to separate branched isomers and maximize the effectiveness of the actual TIP process in the petrochemical industry. For that, metal organic frameworks MOF´s and zeolites are been studied. The Figure in the graphical abstract is a schematic of a TIP process where zeolite beta or MOFs will be used together with zeolite 5A to completly separate iso and di-branched paraffins from the linear and monobranched molecules obtaining a high RON product.

Results (Zeolite)

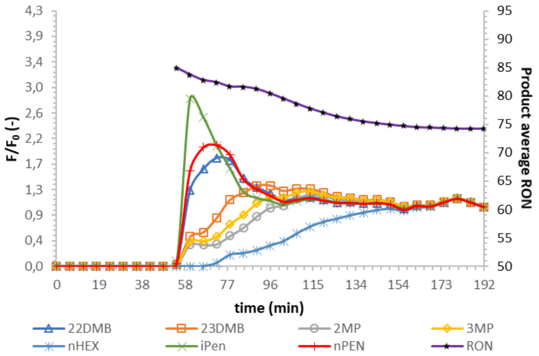


Figure 1. Breakthrough curves for an equimolar mixture of 22DMB, 23DMB, 2MP, 3MP, nHEX, iPEN, and nPEN at 423 K and total isomers pressure of 0.50 bars on a bed of zeolite beta.

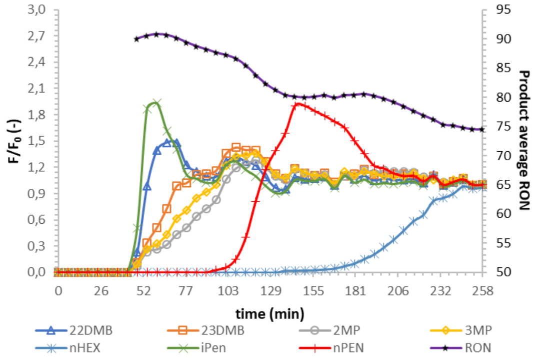


Figure 2. Breakthrough curves for an equimolar mixture of 22DMB, 23DMB, 2MP, 3MP, nHEX, iPEN, and nPEN at 423 K and total isomers pressure of 0.50 bars on a layered bed of zeolite 5A and zeolite beta.

Experiments were made with a mixture of hexane isomers: 2,2-dimethylbutane (22DMB), 2,3-dimethylbutane (23DMB), 2-methylpetane (2MP), 3-methylpentane (3MP), n-hexane (nHEX), as well as pentane isomers: iso-pentane (iPEN) and n-pentane (nPEN) to analyse the effect of the bed configuration on the adsorption process. Zeolite beta can separate mono-branched from di-branched molecules while zeolite 5A can sieve the linear parafins from the branched ones. Together, they can separate the di-branched molecules with high RON from the remaining isomers which will be recycled to the isomerization reactor. Figure 1 shows the breakthrough curves for an experiment with zeolite beta while figures 2 and 3 are the breaktrough curves for the experiments with a layered and mixed bed respectively. The presence of zeolite 5A on the adsorber bed displaces the breaktrough curves of nPEN and nHEX resulting in a higher RON value (purple line) obtained than in the experiment with only zeolite beta. Concerning the different absorber bed configurations studied, the layered bed and the mixed bed show a maximum RON of 91 by significantly increasing the separation time of branched isomers from the linear isomers in comparison to an adsorber bed with only zeolite beta.

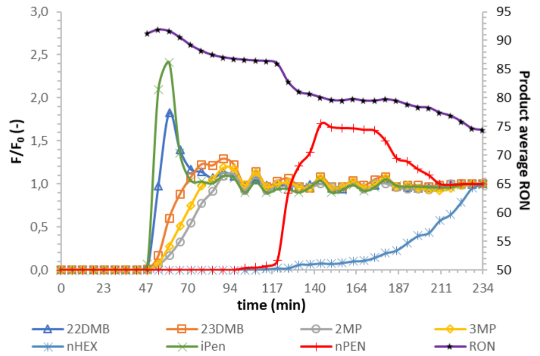


Figure 3. Breakthrough curves for an equimolar mixture of 22DMB, 23DMB, 2MP, 3MP, nHEX, iPEN, and nPEN at 423 K and total isomers pressure of 0.50 bars on a mixed bed of zeolite 5A and zeolite beta.

Results (MOFs)

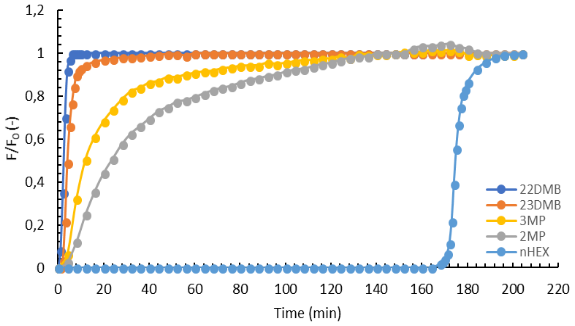


Figure 4. Breakthrough curve for an equimolar mixture of 22DMB, 23DMB, 2MP, 3MP, nHEX, at 373 K and total isomers pressure of 0.50 bars in ZIF-8.

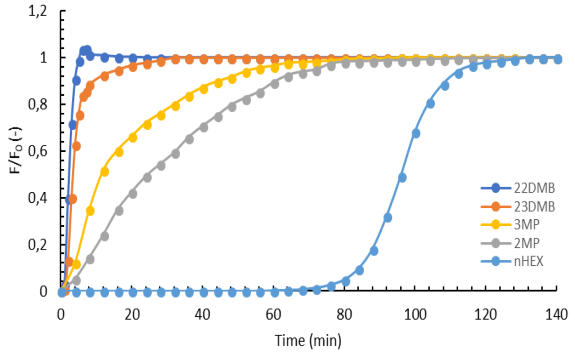


Figure 5. Breakthrough curve for an equimolar mixture of 22DMB, 23DMB, 2MP, 3MP, nHEX, at 423 K and total isomers pressure of 0.50 bars in ZIF-8.

The adsorption behavior of all five hexane isomers nHEX, 2MP, 3MP, 23DMB and 22DMB in ZIF-8 was investigated by performing a set of multicomponent equimolar breakthrough curves, analyzing the effect of temperature and hydrocarobn total pressure. The studied conditions were determined to ensure a contact time between the gas mixture and the adsorbent in the packed column of 45 s. Some of these experimental breakthroughs curves are illustrated in Figures 4, 5, and 6 for the hydrocarbon total pressure of 0.5 bar and 373 K, 423 K and 473 K temperature, respectively. Globally, the results shown in these figures indicate that the sorption hierarchy of hexane isomers in ZIF-8 is always nHEX > 2MP > 3MP > 23DMB > 22DMB, being similar to the order of the normal boiling point of the compounds. However, the shape of the breakthrough curve of nHEX is always different from the branched ones, eluting at a stoichiometric time different from the contact time of gas in column (around 45 s), which means that its sorption is essentially dominated by equilibrium. In contrast, the branched isomers start their elution practically at the contact time of the gas in the column due to strong diffusional limitations. But, the approach to feed concentration of the branched isomers is different since the di-branched isomers 22DMB and 23DMB rapidly saturate at the early stage of the experiments, in contrast with the mono-branched isomers 3MP and 2MP that due to less diffusion limitations produce a less sharp approach to inlet concentration. The effect of temperature on breakthrough curves is also very significative. At 423K the elution time becomes closer between nHEX and the mono-branched isomers 2MP and 3MP.

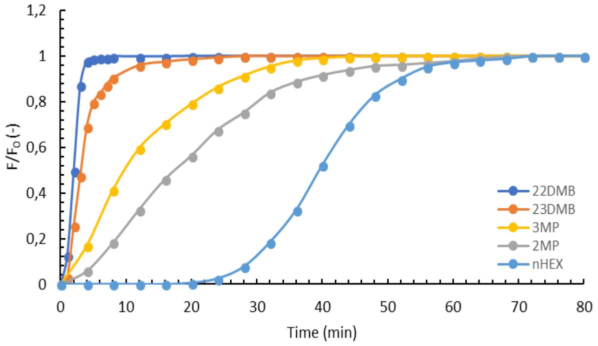


Figure 6. Breakthrough curve for an equimolar mixture of 22DMB, 23DMB, 2MP, 3MP, nHEX, at 473 K and total isomers pressure of 0.50 bars in ZIF-8.

As a final remark one can conclude that ZIF-8 has a significant working capacity (higher than zeolite 5A) for the linear hexane isomer and is able to separate all isomers by classes linear>mono-branched>dibranched, and can be an interesting substitute for zeolite 5A, which is an important result for petrochemical applications viewing the octane improvement of gasoline, such as the Total Isomerization Process.

Future Work

The next step is to test new MOF's to evaluate their separation potential as well as different configurations of adsorber beds using more than one MOF to achieve a complete separation between high RON paraffins from the remaining isomers. Then, the most appropriate material or combination of materials will be selected for testing in a TSA/PSA unit to analyse their performance in a cyclic industrial process.

OUTPUTS

PhD Thesis

[1] Patricia Mendes, Separation of Hexane Isomers in Metal Organic Frameworks, PDEQB/PDEA, FEUP, 2013.

Selected Publications

[1] Patricia Mendes et al., Micropor. Mesopor. Mater. 170, 251 (2013)

[2] Patricia Mendes et al., Adv. Funct. Mater. 24, 7666 (2014)

[3] Patricia Mendes et al., Micropor. Mesopor. Mater. 194, 146 (2014)

[4] Patricia Mendes et al., Adsorption Science and Tecnhology 32, 475 (2014)

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FUNDING

LSRE-LCM Strategic Project POCI-01-0145-FEDER-006984, 2015-2017

TIP_MOFs - Upgrading of Total Isomerization Processes with Metal-Organic Frameworks. Project POCI-01-0145-FEDER-016517, 2016-2019

Processes and Product Engineering

Separation of $CO_2/CH_4/N_2$ Mixtures for CO_2 iso-Paraffins for CO2 Capture/Biogas Upgrading Strategies

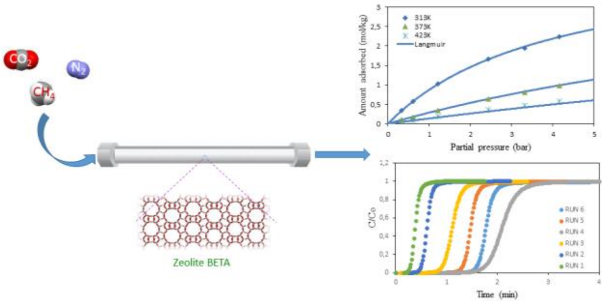
KEYWORDS: CO_2 Capture | Physical Adsorption | Zeolite | Activated Carbon | Biogas Upgrading | Global Warming

Achievements:

1. Equilibrium and kinetic study of zeolite BETA by considering the effects of SiO_2/Al_2O_3 ratio and cation type on adsorption equilibrium for biogas upgarding.

2. Introducing a novel modified activated carbon for CO_2 capture in post-combustion processes

3. Modelling and simulation of adsorption processes in all sorbent to describe the behaviour of adsorption process as well as detecting the interaction between main variables.



Objectives

Climate change has become one of the primary issues nowadays, which has attracted much attention in the recent years. One of the predominant greenhouse gases is carbon dioxide (CO_2) which its sharply increasing in the atmosphere and it has been the sources of dangerous effects on the environment. On the other hand, carbon dioxide (CO_2) has a bigger effect than other gases on energy fields to implement of successful separation and purification technology for biogas upgrading and natural gas sweeting. Thus, based on significant position of $CO_2/CH_4/N_2$ adsorption for biogas upgrading fields, kinetic and equilibrium adsorption of this components on zeolite BETA by considering different SiO_2/Al_2O_3 ratio and various cation types have been considered. In addition, a novel chemically and thermally modified activated carbons for CO_2 adsorption in the post-combustion conditions has been introduced.

Results

Zeolite BETA

At the first part of project, the adsorption equilibrium isotherms for single components, CO_2, CH_4 and N_2, on different zeolites including: H-BETA-25, Na-BETA-25 and H-BETA-150, were measured by breakthrough technique. Experimental data were collected at different temperatures, 313, 373 and 423 K, also various partial pressures in the range of 0.33 to 4.16 bar. Based on results, CO_2 has the highest adsorption capacity than CH_4 and N_2 for all sorbents and studied temperature and pressure, which it can be elucidated based on lower kinetic diameter and higher activation energy of carbon dioxide. A comparison between the uptakes capacities of studied zeolites for CO_2 at 313.15 (K) have been represented in Figure 1. Also selectivity of studied samples has a descending order, as $CO_2/N_2 > CO_2/CH_4 > CH_4/N_2$ for all three recorded temperatures. Among three samples, the highest selectivity was obtained from zeolite H-BETA-150 at the temperature of 313 K, with the values of 6.65, 3.24 and 2.05. At the same temperature, zeolite H-BETA-25 showed the lowest selectivity values (4.79, 2.56 and 1.87). It was observed, by increasing the temperature the selectivity values decrease and at 423 K, the selectivity for the CH_4/N_2 system is very low and almost close to 1.

In the next step, the ZLC studies were performed for CO_2 adsorption for all three zeolites, and before that, preliminary calculations were made regarding the diffusion mechanism of CO_2 in zeolite BETA. As zeolite BETA contains large pores, it was assumed initially that the mass transfer resistance occurs in macropores, then the performed desorption time of experiment for ZLC was measured to calculate the kinetics parameters. The Schematic breakthrough apparatus which has been employed in these experiments have been plotted in Figure 2.

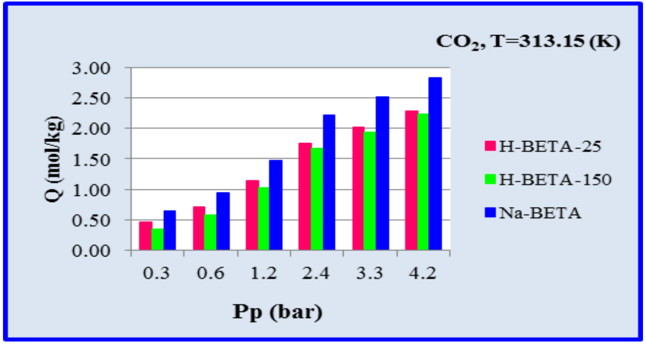


Figure 1. A comparison between CO2 uptake capacity (mol/kg) of studied zeolites at 313.15 (K) and different partial pressures.

Finally, it is concluded that the highest amount of adsorption is experienced for CO_2, followed by CH_4 and N_2. Variations in the zeolite BETA structure, such as the exchange of compensation cations (H+ by Na+) and the SiO_2/Al_2O_3 ratio contribute to the alterations in the adsorption properties. The cation exchange from H+ to Na+ enhances the uptake capacity, mainly for CO_2 and represents higher selectivity. SiO_2/Al_2O_3 exchange also brings important increment in CO_2 adsorption and selectivity. The breakthrtohg reults of Na-BETA-25, as best sample, for CO_2 adsorption at different temperature has been illustrated in Figure 3.

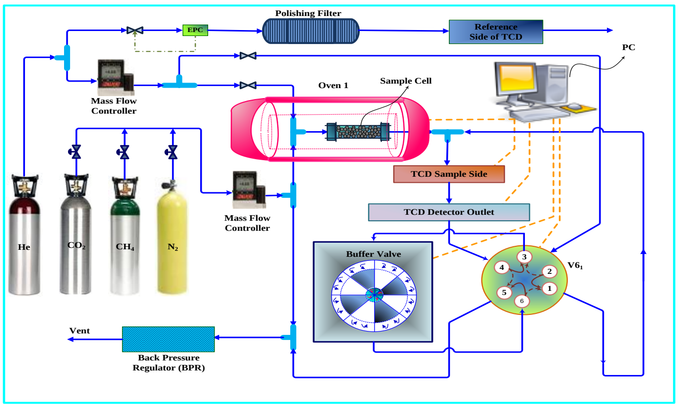


Figure 2. The breakthrough apparatus for breakthrough measurement of studied samples.

Though determination of the mass transfer mechanism for CO_2, regarding the diffusion studies, by using the ZLC technique is not feasible, but it is possible to conclude that there are no diffusional resistances. In sum, it should be noted that the zeolite BETA has the interesting characteristics in the relation to other materials for treatment of gas mixtures, such as biogas components, specially, since it's structure can be easily functionalized in terms of cation exchange and hydrophobicity index by SiO_2/Al_2O_3 ratio.

Modified Activated Carbons

The development of adsorption-based technologies for CO_2 capture in the post-combustion processes requires finding materials with high capacity of adsorption and low cost of preparation. In this study, the modification of a commercial activated carbon (Norit ROX 0.8 (PAC)), considered as a solid adsorbent for CO_2 capture, and the effects of different methods of activations, chemically (hydrogen peroxide, sulphuric acid, nitric acid and urea) and thermally (at 800°C) on the adsorption performance have been investigated. Then, CO_2 adsorption capacity was studied at different temperatures and pressures to evaluate the effects of various agents on sample performance.

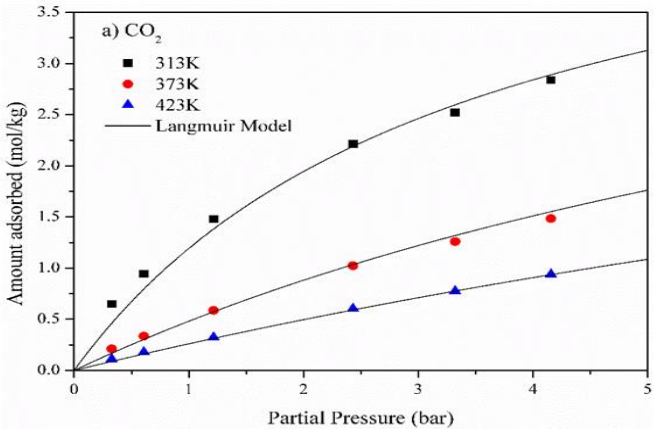


Figure 3. Adsorption equilibium isotherms of CO_2 at different temperatures on agglomerates of zeolite Na-BETA-25.

In Figure 4 a comparison between the ability of investigated sorbents for CO_2 capture at 313K and different partial pressures have been depicted. As can be seen, in all plots, by increasing the pressure of adsorption, uptake capacities of samples increase, also temperature enhancement has had a negative effect on the adsorption capacity, and this behavior is the same for all samples. About the uptake capacity of each one, PACSA which has been treated with sulfuric acid has had the worst performance and PACNAUT that has been chemically (with nitric acid and urea) and thermally treated is the best one.

Finally, Response Surface Methodology (RSM) was performed for investigation, analysis and verifying experimental results as well as determining the interaction effects of main parameters (temperature and CO_2 partial pressure) on CO_2 capture capacity and breakthrough time. The results indicated that partial pressure is the main one which its enhancement increases the CO_2 capture capacity and breakthrough time. In addition, it was observed that there is an interaction between temperature and partial pressure which its results can be significant at the high temperature.

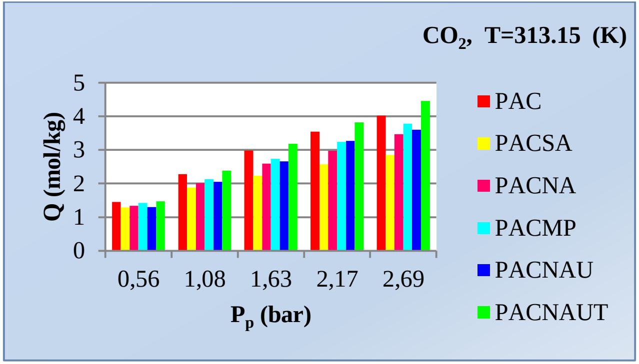


Figure 4. A comparison between CO_2 uptake capacity (mol/kg) of investigated adsorbents at) 40 °C.

For more clarification and justification of process behavior, three-dimensional response surface plots for the CO_2 capture capacity and the breakthrough time as a function of independent variables have been depicted in Figure 5.

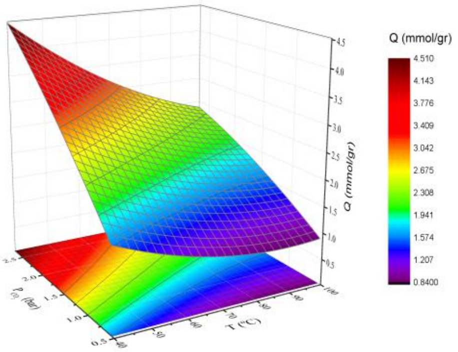


Figure 5. Response surface plots for CO_2 capture capacity (mol/kg) and breakthrough time (min), as a function of the independent variables,Pp (bar)and T (K).

Future Work

Currently the work has been focused on developing new sorbents for CO_2 capture in the post-combustion processes. In this way, based on urgent requirement to find a better solution for solid wastes in Europe, these materials have been considered as a good source of activated carbons. To this goal, at the first step these materials will be activated by chemically and thermally treatments then they will be employed as a source of biochar for physical adsorption. The elementary studies have been accomplished and we hope, by considering the importance of management of solid waste also the significance of CO_2 capture for global warming this project will be contributed to the interesting results.

OUTPUTS

Selected Publications

[1] M. Karimi et al., Ind. Eng. Chem. Res. under review.
[2] A. **Henrique** et al., Micro. Meso. Mater. J. under review.

TEAM

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FUNDING

LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017
LA LSRE-LCM Strategic Project, PEst-C/EQB/LA0020/2013, 2013-2014
AIProcMat@N2020, NORTE-01-0145-FEDER-000006, 2016-2019
FCT Scholarships: SFRH/BD/87084/2012

Processes and Product Engineering

Materials for Environmental Applications

KEYWORDS: Carbon-based Materials | Magnetic Nanoparticles | Upclycling Waste | Wastewater Treatment | Oily Wastewater

Achievements:

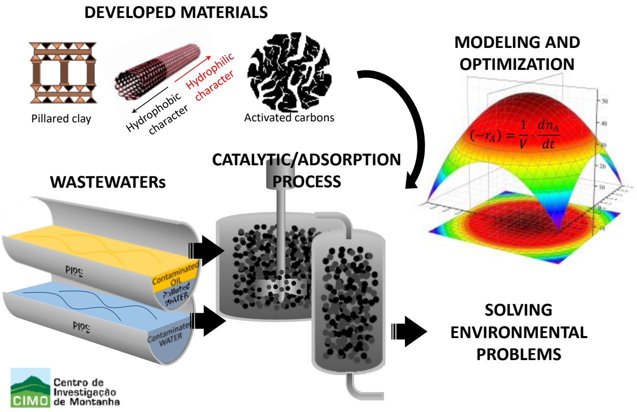
1. Synthesis of magnetic carbon-based nanostructured composites to serve as heterogeneous catalysts for catalytic wet peroxide oxidation.

2. Synthesis of low-cost useful materials (adsorbents and catalysts) for application in wastewater treatment and gas purification.

3. Tuning properties of materials (acidity, hydrophobicity, amphiphilicity, superficial area, among others) to optimize their performance in environmental applications.

4. Decontamination of wastewater effluents containing lipophilic pollutants (nitrophenols, dyes and pharmaceutical compounds) in aqueous or oily wastewater.

5. Kinetic and isotherm modeling of environmental applications and mechanistic studies for the design of suitable treatment processes.



Objectives

Development of carbon-based materials for environmental applications, including the development of magnetic nanostructures and the valorization of low cost sources, such as municipal solid waste derived residues (plastics and matured compost). The knowledge acquired, based on a detailed catalyst-adsorbent design with the most suitable physico-chemical properties for adsorption or catalytic wet peroxide oxidation (CWPO) process, allowed to move forward towards the treatment of oily wastewaters containing noxious lipophilic pollutants.

Results

The establishment of liquid phase and thermal treatment methodologies was achieved, in order to modify the properties of carbonaceous materials. These allowed the assessment of the effect of those properties on the treatment of oily wastewater by adsorption and CWPO processes (scarcely studied). Specifically, several activated carbon samples have been modified by liquid phase treatments with hydrogen peroxide (PACHP), sulphuric acid (PACSA) and nitric acid (PACNA), followed by hydrotreatment with urea (PACNAU) and thermal treatment at 800 °C under inert atmosphere (PACNAUT). Figure 1 show the BET surface of the prepared materials and the results obtained in the adsorption of a carcinogenic lipophilic dye (Sudan IV, S-IV).

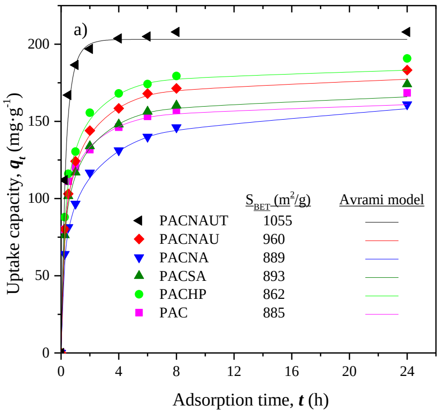


Figure 1. Effect of the activated carbon modifications on the adsorption capacity of S-IV.

As observed, the modification of the pristine material was successful, an increment of the specific surface area being achieved, leading to a higher uptake capacity when PACNAUT was used. The development of kinetic (Avrami, as shown in Figure 1) and isothermal models for the pollutant adsorption from the oily wastewater was done (to the best of our knowledge, there are no studies for the modeling of oily wastewater adsorption). The most relevant operating conditions in this treatment were studied. In this sense, the W/O ratio and pH were found to not affect significantly the process owing the hydrophobic character of both adsorbent and pollutant.

The operating conditions in the treatment of oily wastewater by CWPO have also been studied, in this case, with several amphiphilic magnetic carbon nanotubes with two well-defined structures as result of the synthesis perfomed in a fluidized reactor by feeding ethylene and acetylene as follows: 1) ethylene for 30 min (resulting in catalyst E30); 2) acetonitrile for 20 min, followed by ethylene for 20 min (A20E20); 3) acetonitrile for 20 min, followed by ethylene for 10 min (A20E10); 4) ethylene for 10 min, followed by acetonitrile for 20 min (E10A20); or 5) acetonitrile alone for 30 min (A30). The catalytic activity obtained in the treatment with these materials was found to be significantly affected by their amphiphilic properties and the capacity to stabilize the Pickering emulsion (Figure 2), which was achieved only with materials synthesized by feeding the two precursors (ethylene and acetonitrile), leading to the double structure with different chemical properties: hydrophilic-hydrophobic character.

Compost obtained from a mechanical biological treatment plant for municipal solid waste has been used in the preparation of new usefull low-cost materials. Two different materials were prepared by carbonization at different temperatures: 400 (C-400) and 800 °C (C-800). In addition, two further materials were prepared with H_2SO_4 before and after carbonization at 800 °C (C-S-800 and C-800-S, respectively). All materials were sieved and the particles with the lowest size (LSp) were used in the treatment of different lipophilic pollutants: 4-nitrophenol (4-NP), 2-nitrophenol (2-NP) and Sudan IV (S-IV). The results obtained are depicted in Figure 3.

As observed, the materials prepared from compost are catalytic active, the performance depending strongly on the lipophilic character of the pollutants (S-IV>2-NP>4-NP). The burn-off and catalytic properties were found to be affected by the treatment and the order of treatments (C-S-800 and C-800-S).

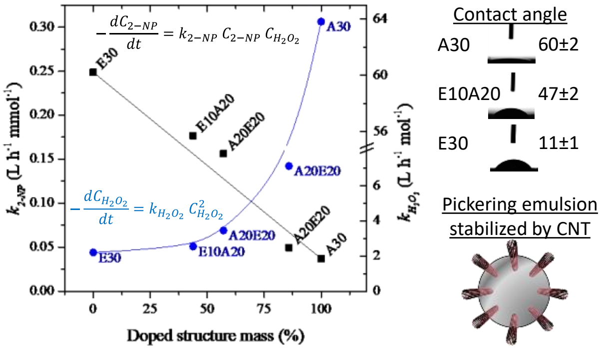
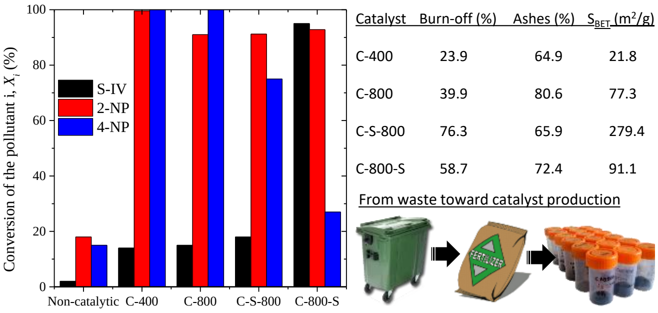


Figure 2. Kinetic constants in the removal of 2-NP by CWPO with amphiphilic carbon nanotubes and their main properties.



Catalyst	Burn-off (%)	Ashes (%)	S_{BET} (m^2/g)
C-400	23.9	64.9	21.8
C-800	39.9	80.6	77.3
C-S-800	76.3	65.9	279.4
C-800-S	58.7	72.4	91.1

Figure 3. Degradation of 4-NP, 2-NP and S-IV by using catalysts obtained from compost.

Future Work

The work done so far has been focused mainly on the modification of carbon materials and its application with different characteristics in order to deeply study their most suitable properties for the treatment of pollutants by adsorption and CWPO, specially in oily wastewaters, a field scarcely studied. On the other hand, the most relevant operating conditions in the treatment of this kind of wastewater and the development of analytical methods for the determination of pollutants in the oil phase have been achieved.

Nowadays, the development of low-cost materials for environmental applications is receiving great attention in order to decrease the associated operational costs. In this sense, new materials from compost and plastic solid waste are envisaged, under the framework of the running projects VALORCOMP and PLASTIC_TO_FUEL&MAT, for application in aqueous and oily wastewater treatments. On the one hand, it is expected the preparation of optimized materials from compost by applying different techiques to purify its organic fraction and novel activating methods. On the other hand, plastic solid wastes (PSWs) will be studied not only obtaining carbon materials, but also transforming the plastic into fuel. The manufacturing of two high quality products, coupled to the reduction of PSWs on landfills, make the solution an economic alternative for companies dealing with the Management of Wastes, which currently face PSW problematics. Furthermore, the alternative contributes to the objectives of the Directive 2008/98/CE, aimed to transform the waste management activity in a perspective of a circular economy. The composition of PSWs is a critical factor on the development of technical solutions for their treatment and valorisation. The properties depend closely on the region, *i.e.*, the sources (industrial, agricultural or urban) and the social conscience about separation and classification of solid wastes (as observed also in the valorization of compost, since it contain large quantities of glass and other elements as consequence of a social conscience in the filed that is needed to improve). The polymers which are typically found in PSWs are polyethylene, polypropylene, polystyrene, polyethylene, terephthalate and polyvinyl chloride, among others. For this reason, is it also important to develop versatile acidic catalysts that allow cracking different polymers and mixtures of PSWs. The high number of variables involved (composition of PSWs, catalysts and operational conditions, such as catalyst load, pressure, temperature and reaction time), coupled with the limited number of papers available in the field, make possible an elevated number of contributions from the research team.

The carbon-based materials prepared from compost and PSWs will be tested in the oxidative catalytic treatment of aqueous and oily wastewater contaminated with noxious pollutants and new emergent substances, namely pharmaceutical compounds. For this task, analytical methods will be developed and new wastewater treatment processes for these compounds, based on advanced oxidation processes, will be explored.

OUTPUTS

Master Dissertations

Rima Guliyeva, Amphiphilic Catalysts for the Treatment of Oily Wastewaters, MEQ, IPB, 2016.

Selected Congress Contributions

[1] J.L. Diaz de Tuesta et al., *Coupling HPLC and GC-FID for the Monitorization of Oxidized Intermediates from Wet Peroxide Biphasic Oxidation*, 10° Encontro Nacional de cromatografia, 2017 (Portugal)

[2] J.L. Diaz de Tuesta et al., *Development of Low-cost Materials from Compost obtained in Mechanical Biological Treatment Plants for Municipal Solid Waste: Application as Catalysts in H_2O_2 Decomposition*, XXIII Encontro Galego-Português de Química, 2017 (Spain)

[3] J.L. Diaz de Tuesta et al., *Adsorption of Lipophilic Pollutants from Biphasic Systems by using Modified Activated Carbon Materials*. XXV Encontro Nacional da SPQ, 2017 (Portugal)

[4] J.L. Diaz de Tuesta et al., *Carbon Nanotubes as Pickering Interfacial Catalysts for the CWPO of Oily Wastewater*. I Reunião do Grupo do Carbono, 2017 (Portugal)

Selected Publications

[1] R.S. Ribeiro et al. Catal Today 249, 204 (2015)
[2] M.T. Pinho et al. J Environ Chem Eng 3, 1243 (2015)
[3] M.T. Pinho et al. Appl Catal B: Environ 165, 706 (2015)
[4] S. Álvarez et al. Chem Eng Res Des 95, 229-238 (2015
[5] R.S. Ribeiro et al. Appl Catals B Environ 187, 428 (2016)
[6] R.S. Ribeiro et al. Appl Catals B Environ 199, 170 (2016)
[7] M. Martin-Martinez et al. Chem Cat Chem 8, 2068 (2016)
[8] S. Álvarez-Torrellas et al. Chem Eng J 296, 277 (2016)
[9] R.S. Ribeiro et al. Catal Today 296, 66 (2017)
[10] R.S. Ribeiro et al. Appl Catal B Environ 219, 645 (2017)
[11] M. Martin-Martinez et al. Appl Catals B Environ 219, 372 (2017)
[12] R.S. Ribeiro et al. Catal Today 280, 184 (2017)
[13] S. Álvarez-Torrellas et al., Appl Surf Sci 414, 424 (2017)
[14] J.L. Diaz de Tuesta et al., Appl Catals B Environ 209, 701 (2017)
[15] A. Outsiou et al., Water Res 124, 97 (2017)

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FUNDING

PLASTIC_TO_FUEL&MAT, POCI-01-0145-FEDER-031439, 2018-2021
VALORCOMP, POCTEP 0119_VALORCOMP_2_P, 2016-2020
LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017
LA LSRE/LCM Strategic Project, PEst-C/EQB/LA0020/2013, 2013-2014
AIProcMat@N2020, NORTE-01-0145-FEDER-000006, 2016-2019
Catalysis and Materials, NORTE-07-0124-FEDER-000015, 2014-2015
PTDC/AAC-AMB/110088/2009, 2011-14
FCT Scholarships: SFRH/BD/ 94177/ 2013

Processes and Product Engineering

Phase Equilibria for Separation Processes Involving Natural Products

KEYWORDS: Deep Eutectic Solvents | Melting properties | Solubilities | Solid-Liquid Equilibria | Extraction

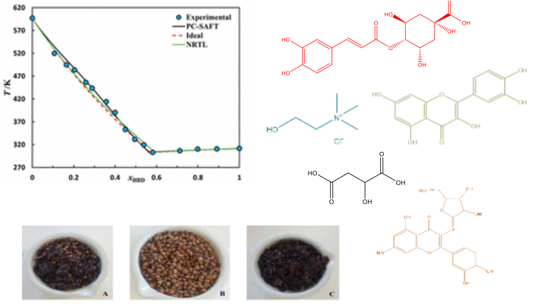
Achievements:

1. The study of the phase equilibria of biomolecules in reference and non-conventional solvents.

2. The experimental identification of new solvents based on deep eutectic systems and their thermodynamic modelling using the Statistical Associating Fluid Theory (SAFT).

3. Experimental and Molecular Dynamics studies for the interactions of biomolecules like sugars with ionic liquids.

4. Application of deep eutectic solvents in the extraction of bioactive compounds from plant material.



Objectives

Biomolecules such as phenolic acids, flavonoids, and sugars have wide application in the pharmaceutical, food, and cosmetic industries. However, there is still an enormous lack of experimental information regarding their phase equilibria and of robust thermodynamic modelling approaches to be used in the design of their extraction and purification processes. In a first approach, the phase equilibria of these biomolecules is being studied in water and volatile organic solvents such as methanol, ethanol, or ethyl acetate. After, the use of alternative solvents such as deep eutectic solvents (DES) or ionic liquids (IL) is also addressed aiming to find alternative solvents with better extraction and environmental performances.

Thus, the main objective in this topic is to built a body of knowledge that allows the establishment of procedures to screen compounds and mixtures that can be used to create new separation processes for a given target biocompound. Therefore, besides the use of quantum chemical model such as COSMO-RS, molecular dynamics, a predictive equation of state like PC-SAFT or the emprirical NRTL-SAC model, an important aspect is the development of methodologies to estimate fundamental properties of the natural products that cannot be assessed experimentally due to their degradation.

Results

1. The study of the solubility of biomolecules in water and organic solvents is a key reference parameter in the design of their separation processes. Previous work, carried out in our research group, was focused on the solubility of some natural phenolic compounds including phenolic acids and flavonoids in pure organic solvents and aqueous mixed solvents [1-3]. Solid phase studies were also carried out by Differential Scanning Calorimetry to obtain the enthalpies and temperatures of fusion of the solutes, and a selected set of solid samples were analysed by powder and single crystal X-ray diffraction allowing phase identification. These experimental studies were described by a semi-predictive tool, the non-random two-liquid segment activity coefficient (NRTL-SAC) model with satisfactory results as shown in Figure 1.

2. Deep eutectic systems are being proposed in the literature as alternative solvents in many applications [4]. However, the thermophysical properties of these mixtures are still poorly characterized. In this context, several studies have been carried out in this research topic. To estimate the melting properties of compounds that decompose upon melting, like choline chloride that is often used in the preparation of Deep Eutectic Solvents, experimental solid-liquid phase diagrams of ten quasi-ideal liquid eutectic mixtures composed by choline chloride and different ionic compounds were measured (Figure 2) [5].

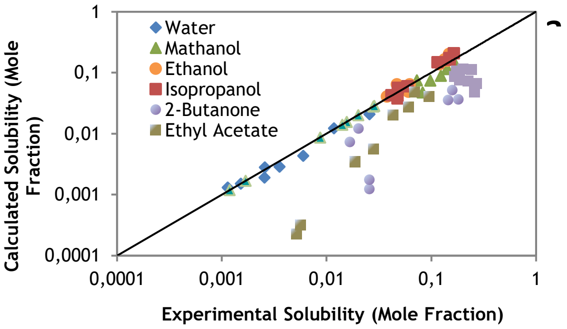


Figure 1. Comparison between experimental and calculated (NRTL-SAC model) solubility data of three phenolic acids in water and organic solvents.

Their ideality was evaluated through the activity coefficients calculated by COSMO-RS as well as by comparing the activity coefficients ratio of choline chloride in each pair of binary solutions. The robustness of the method was additionally verified using thermodynamic consistency tests and by the prediction of the solid-liquid curves with COSMO-RS model.

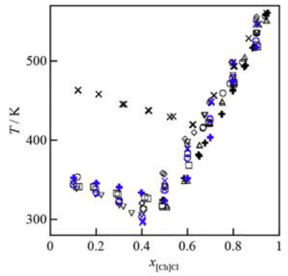


Figure 2. Solid-liquid phase diagrams for the ChCl + Ionic compounds systems studied. [Ch]Cl(1) + (◯) [Ch][Ac](2); (✚)[Ch][Prop](2); (◇)[Ch][Buta](2); (□)$[N_{4444}]$Cl(2); (△)$[P_{4444}]$Cl(2); (▽) [BzCh]Cl(2); (✖)$[C_4mpyr]$Cl(2); (✖) $[Ch][NTf_2]$(2); (○)$[C_2mim]$Cl(2); (✚)$[C_2OHmim]$Cl(2).

Concerning the identification of new DES, the SLE study of 15 binary mixtures composed of one of three different symmetrical quaternary ammonium chlorides and one of five different fatty acids [6], or one of 8 fatty acids and fatty alcohols with choline chloride were already measured [7]. The experimental data obtained showed extreme negative deviations to ideality causing large melting-temperature depressions (up to 300 K) that are characteristic of deep eutectic systems. The experimental data revealed the strengthening of the HBA-HBD complex with increase of the alkyl chain length of the salt and with increase of the chain length of the acids. The pronounced decrease of melting temperatures in these DES is mainly caused by strong hydrogen-bonding interactions, and thermodynamic modeling required an approach that takes hydrogen bonding into account. The PC-SAFT model has shown very good agreement with the experimental data (Figure 3) using a semi-predictive modeling approach, in which binary interaction parameters between the salts and and the organic compounds correlate well with the chain length of the components [6]. This supports the findings into the phase behavior and interactions present in these systems and allows estimating eutectic points of such highly non-ideal mixtures.

3. Regarding the studies involving sugars and ILs, experimental data of six monosaccharides, such as D-(+)-glucose, D-(+)-mannose, D-(-)-fructose, D-(+)-galactose, D-(+)-xylose and L-(+)-arabinose, were measured in four ILs, at temperatures ranging from 288.2 to 348.2 K. To ascertain the chemical features, which enhance the solubility of monosaccharides, ILs composed of dialkylimidazolium or tetraalkylphosphonium cations combined with the dicyanamide, dimethylphospate or chloride anions were investigated. It was found that the ranking on the solubility of monosaccharides depends on the IL. The results obtained show that both the IL cation and anion play a major role on the solubility of monosaccharides. In particular, molecular dynamic studies have additionally been performed over glucose and a series of cyano based ILs, calculating the number of hydrogen bonds, coordination numbers, energies of interaction and radial and spatial distribution functions. It was possible to explain the experimental results and to show that the ability to favorably interact with glucose is driven by the polarity of each IL anion.

4. To explore DES as extraction solvents, two natural matrices have been selected: the leaves of walnut trees (*Juglans regia* L.) and grape pomace. First, maceration (ME) and microwave assisted (MAE) extractions were studied using water + ethanol as reference solvent, aiming to maximize the extraction of phenolic compounds from *J. regia* leaves. An experimental design, assisted by response surface methodology, was developed to optimize the extraction parameters (time, temperature and ethanol-water proportion) [8]. After establishing the reference method, the extraction was carried out using deep eutectic solvents based on choline chloride and carboxylic acids. A preliminary solvent screening was performed using a selected group of carboxylic acids as hydrogen bond donors, as can be seen in Figure 3. Promissing results were obtained compared to the conventional methods [9].

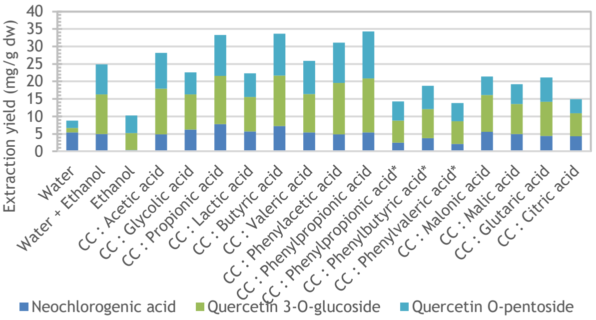


Figure 3. Extraction yield (mg/g dw) of 3-O-caffeoylquinic acid, quercetin 3-O-glucose and quercetin O-pentoside obtained in the initial screening of the choline chloride based DES, with the extraction conditions: 1 hr extraction time, 50 ºC, 600 rpm and 20 % (w/w) of water (solvents with * only contain 5 % (w/w) of water).

The bio-residues resulting from the wine industry, the grape pomace, are often undervalued but they are a potential source of bioactive phenolic compounds that can be applied in several industries. The biological properties (antioxidant, cytotoxic and antibacterial activities) of this plant material made up of skins, seeds and stems were evaluated, showing a strong correlation with the presence of phenolic compounds [10] and a great potential source of these biomolecules. This work is developed under strong collaboration with PATh, CICECO, University of Aveiro.

Future Work

Currently, the work has been focused on extending the phase equilibria studies to a higher number of DES, now based on sugars, amino acids and polyols. From the solute point of view, studies on the solubility of phenolic compounds in different type of solvents are also under progress. Moreover, it is envisaged to apply some of the proposed DES to perform further extractions over real matrices such as grape pomace or the leaves of walnut trees.

OUTPUTS

Master Dissertations

[1] Jocilene Furtado, Estudo de Propriedades Físicas de Solventes Eutécticos de Origem Natural, MTB, IPB, 2015.

[2] Juliana da Conceição Correia Dias, Novos Métodos de Extração de Compostos Bioativos de Verduras, MTB, IPB, 2015.

[3] Fátima Almeida, Extração de Compostos Bioativos de Fontes Vegetais Utilizando Solventes Eutécticos, MTB, IPB, 2016.

[4] Carla Patrícia Machado Peixoto, Extração e Quantificação de Compostos Fenólicos presentes no Bagaço da *Vitis vinifera L.* e Avaliação das suas Bioatividades, MEQ, IPB, 2017.

[5] Sérgio Antonio Mendes Vilas Boas, Studies on the Solubility of Phenolic Compounds, MEQ, IPB, 2017.

[6] Bruna de Paula Soares, Medições de Solubilidade de Compostos Pouco Solúveis em Água, MEQ, IPB, 2017.

Selected Publications

[1] O. Ferreira et al., Journal of Chemical Engineering Data 58, 2616 (2013)

[2] S. P. Pinho, J. A. P. Coutinho, Fluid Phase Equilibria 448, 1 (2017)

[3] L. Fernandez et al., Fluid Phase Equilibria 448, 9 (2017)

[4] P. V. A. Pontes et al., Fluid Phase Equilibria 448, 69 (2017)

[5] E. A. Crespo et al., Industrial Engineering Chemistry Research 56, 12192 (2017)

[6] V. Vieira et al., Industrial Crops and Products 107, 341 (2017)

[7] V. Vieira et al., Industrial Crops and Products 115, 261 (2018)

[8] C. M. Peixoto et al., Food Chemistry 253, 132 (2018)

[9] E. A. Crespo et al., Industrial Engineering Chemistry Research 57, 11195 (2018)

[10] C. P. Silva et al., ACS Sustainable Chemistry and Engineering 6, 10724 (2018)

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FUNDING

LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017
LA LSRE-LCM Strategic Project, PEst-C/EQB/LA0020/2013, 2013-2014
AIProcMat@N2020, NORTE-01-0145-FEDER-000006, 2016-2019
AllNat Project, POCI-01-0145-FEDER-030463, 2018-2021
FCT Scholarships: SFRH/BD/87084/2012

Processes and Product Engineering

Terpenes and Terpenoids: Phase Equilibria and Properties for Processs Design

KEYWORDS: Deep Eutectic Solvents | Activity Coefficients | Water Solubilities | Critical Properties | Environment

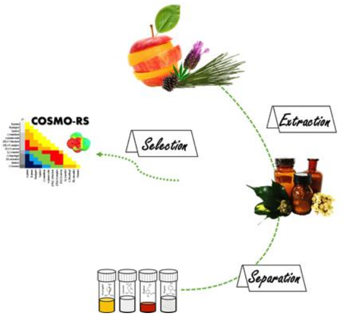
Achievements:

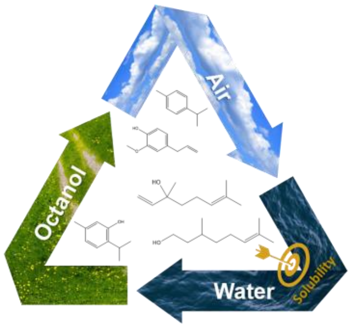
1. The proposal of indirect methodologies to estimate the critical properties of sensible compounds that decompose increasing the temperature.

2. Combining chromatographic measurements, with quantum chemical predicting tools, to select ionic liquids for fractionation of terpenes.

3. The experimental identification of new eutectic solvents based on terpenoids for different extraction types, and their accurate description by the Statistical Associating Fluid Theory (SAFT).

4. Description of the distribution of terpenes among different environmental compartmens.





Objectives

Terpenes belong to what is probably the largest and most diverse class of natural products with applications in several industries due to their flavor, and fragrance. Their high number, variety of stuctures and chemical complexity, make them a class of compounds for which there are still many studies to to be carried out and questions to be answered both concerning their thermophysical properties and phase equilibria, as well as the impact of their extraction and purification processes and environmental impact. Both are relevant for the development of the biorefinery where these compounds may play an important role due to their ubiquity, economic value and a variety of applications.

This work is related to terpenes extraction from natural sources and their subsequent separation and purification. Besides to the development of new experimental procedures for thermodynamic properties and equilibrium measurements, some theorethical approaches were also applied to this end. To develop new applications for this compounds, and taking advantage of their low solubility in water, as shown by our new and accurate experimental determinations, terpenes are firstly used to prepare sustainable and cheap hydrophobic solvents within the deep eutectic solvents framework. After, based on the activity coefficients at infinite dilution measurements and COSMO-RS predictions a selection of new ionic liquids was made with potential for terpenes fractionation. Finally, and targeting the development of accurate models for the fate of terpenes in the environment, a range of essential physicochemical properties of terpenes were measured and described by models.

Results

1. Recently some works reported claims that hydrophobic deep eutectic solvents (DES) could be prepared based on menthol and monocarboxylic acids. Despite of some promising potential applications these systems are poorly understood and we are addressing that issue. Here the characterization of eutectic solvents composed by the terpenes thymol or L(–)-menthol and monocarboxylic acids is studied aiming at the a priori design of these solvents [1].

The solid-liquid phase diagrams of the twelve mixtures were measured in the whole composition range using DSC, and showed a broader composition range in the liquid state, at room temperature than previously admitted. These systems exhibit a phase behavior characterized by a single eutectic point, for which the temperature and composition vary significantly with the alkyl chain of the monocarboxylic acid (Figure 1).

Generally, the systems exhibited small deviations to ideality and a eutectic point close to that predicted assuming ideality. Therefore, although often labeled as DES, these systems do not present negative deviations large enough to induce a significant melting point depression. However, it must be stressed that room temperature solvents can be obtained for many of these mixtures on a wide composition range and not fixed to any particular stoichiometric relationship between the hydrogen bond donor and acceptor, even at the eutectic point, what reinforces the tunable character of the liquid phase region of these mixtures.

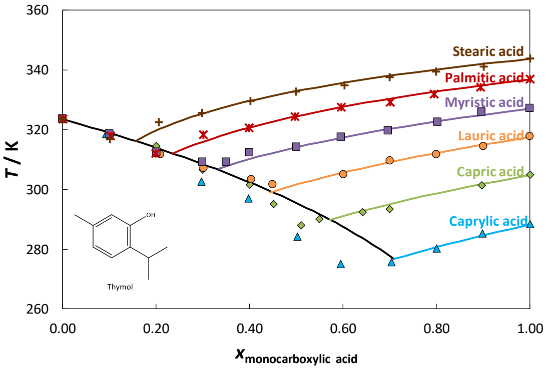


Figure 1. Solid-liquid phase diagrams of mixtures composed of monocarboxylic acids and thymol. Symbols represent the experimental data from this work while the solid lines represent the ideal solubility curves.

The experimental solid-liquid phase diagrams were successfully described using the PC-SAFT EoS, which provided reliable estimates of the eutectic points and of the solvents densities. The eutectic mixtures present densities lower than water and low viscosities (1.3 – 50.6 mPa·s) and in general eutectic mixtures containing thymol were less viscous but more dense than those with L(-)-menthol.

2. Searching for entrainers to enhance terpenes fractionation and purification, the gas-liquid chromatography (GLC) technique [2] has been applied, for the first time, to mixtures containing terpenes. The activity coefficients at infinite dilution of two terpenes, and fifteen terpenoids, at six different temperatures were measured in the ionic liquids (ILs) $[C_4mim]Cl$, $[C_4mim][CH_3SO_3]$, $[C_4mim][(CH_3)_2PO_4]$, and $[C_4mim][CF_3SO_3]$, using predicted critical properties [3]. These data were fundamental to identify ionic liquids for specific separations (Figure 2).

Additionally, COSMO-RS was evaluated for the description of the selectivities and capacities, and showed to be a useful tool for the screening of ionic liquids in order to find suitable candidates for terpenes and terpenoids extraction and separation, before extensive experimental measurements. COSMO-RS predictions on capacities and selectivities show that in order to achieve the maximum separation efficiency polar anions such as bis(2,4,4-trimethylpentyl)phosphinate or acetate should be used and combined with nonpolar cations, such as phosphonium, that would maximize the capacities of the solvents [4] For the specific α-pinene/β-pinene separation, the ionic liquids studied in this work present satisfactory selectivity values, but very low capacities, and a set of ILs were identified to have good potential for this difficult separation problem.

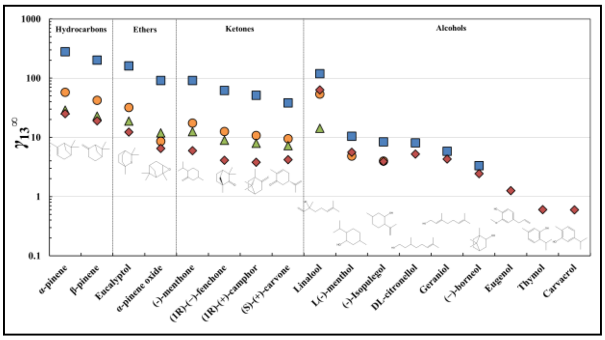


Figure 2. Activity coefficients at infinite dilution of terpenes and terpenoids in the ILs studied, at 408.15 K. □, $[C_4mim]Cl$; ○, $[C_4mim][CH_3SO_3]$; △, $[C_4mim][(CH_3)_2PO_4]$; ◇, $[C_4mim][CF_3SO_3]$.

3. On a global scale, the extensive anthropogenic use of terpenes and terpenoids associated with their natural emissions from coniferous forests are one of the principal sources of biogenic volatile organic compounds (BVOC). About half of global BVOC-emissions originate in isoprene, the terpenes building-block, and their role in aerosol formation became an important research topic on the chemistry of the atmosphere, with a renewed emphasis due to the ongoing climate change debate. Besides vapour pressure and octanol-water partition coefficients, aqueous solubility is an important parameter to allow an ample description of the distribution of a substance amongst the different environmental compartments.

This work reports their water solubility in the temperature range from (298.15 to 323.15) K using an experimental method recently adapted by us [5] for sparingly soluble solid compounds, and here applied for the first time to study the solubility of liquids in water. The new technique was validated against data for some well-studied aromatic compounds and the data reported is compared with literature values.

The thermodynamic properties of solution were calculated, indicating that the solubility of terpenes in water is an endothermic process, confirming the existence of UCST phase diagrams and, excepting carvacrol and eugenol, enthalpically driven. A two-dimensional chemical space diagram shows that, in general, terpenes partition into the three environmental compartments (Figure 3), while toluene and *p*-xylene partition exclusively into air due to their higher vapor pressure [6].

This work contributes and calls for increasing the availability of reliable experimental physico-chemical property data of terpenes, which are also of enormous importance to the improvement, development and test of new computational methods aiming for their prediction in such a vast family of compounds. Efforts must be focused in measuring or re-measuring basic important properties as mutual solubilities, vapor pressures, and octanol-water partition coefficients as they, when available, often present significant inconsistencies. This work is developed under strong collaboration with PATh, CICECO, University of Aveiro.

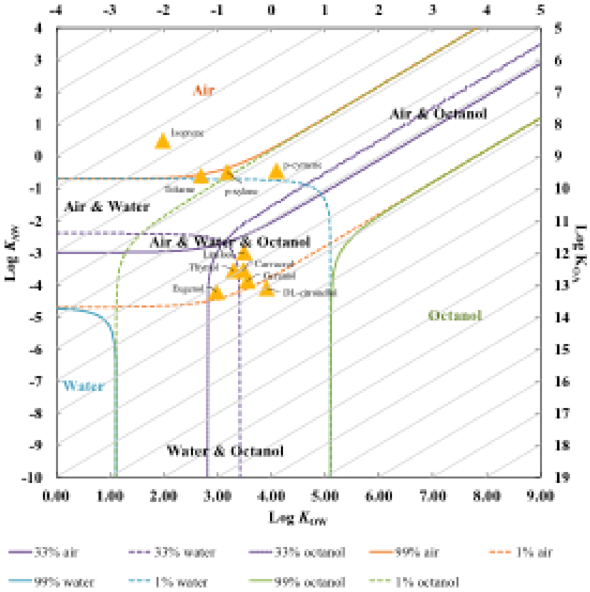


Figure 3. Chemical space diagram of the terpenes investigated and some other selected compounds, namely isoprene, toluene and *p*-xylene.

Future Work

Currently, the work has been focused on extending the phase equilibria studies to use hydrophobic DES to extract metals. Concerning terpenes distribution in the environment there are new water solubility and vapor pressure measurements, and also investigation on the prediction methods and empirical correlations to be applied to a large number of terpenes. It is also foreseen the identification of new ionic liquids that can be applied to separate terpenes. In this way infinite dilution activity coefficients of terpenes in different ionic liquids are going to measured.

OUTPUTS

PhD. Thesis

[1] Mónia Andreia Rodrigues Martins, Studies for the Development of New Separation Processes with Terpenes and Their Environmental Distribution, PDEQ, UA, 2017.

Master Dissertations

[1] Liliana Patrocínio da Silva, Studies on Terpenes Solubility in Water and Deep Eutectic Solvents Diagrams for Biomedical Applications, MTB, IPB, 2016.

Selected Publications

[1] M. A. R. Martins et al., Journal of Chemical Thermodynamics 91, 194 (2015)
[2] B. Schröder et al., Chemosphere 160, 45 (2016)
[3] M. A. R. Martins et al., ACS Sustainable Chemistry and Engineering 4, 548 (2016)
[4] M. A. R. Martins et al., Industrial Engineering Chemistry Research 56, 9895 (2017)
[5] M. A. R. Martins et al., Journal of Molecular Liquids 241, 996 (2017)
[6] M. A. R. Martins et al., ACS Sustainable Chemistry and Engineering 6, 8836 (2018)
[7] M. A. R. Martins et al., Journal of Solution Chemistry, Accepted (2018)

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LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017
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AIProcMat@N2020, NORTE-01-0145-FEDER-000006, 2016-2019
FCT Scholarships: SFRH/BD/87084/2012

Processes and Product Engineering

Thermodynamic of Systems containing Amino Acids, or Derivatives, with Electrolytes

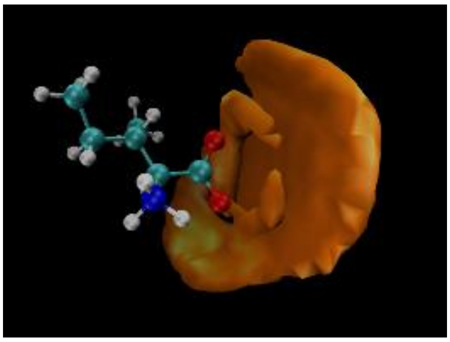
KEYWORDS: Amino Acids | Electrolytes | Solubility | Partial Molar Volumes | Water Activity | Molecular Dynamics

Achievements:

1. Thermodynamic description of aqueous mixtures containing amino acids or derivatives with electrolytes is explored contributing to understand many biochemical processes, and to support the design of biotechnological processes.

2. Comprehensive thermodynamic data (solubility, partial molar volumes and water activity) were experimentally measured for ternary mixtures contaning water, one amino acid or derivative, and one electrolyte.

3. Molecular dynamic studies were performed aiming to understand the molecular-level mechanisms governing the ion specific effects on the aqueous solubilities of the amino acids.



Objectives

The study of ion specific effects on the aqueous solubility of amino acids and proteins is crucial for the development of many areas of biochemistry and biotechnology. However, direct study of protein-electrolyte interactions is difficult and it is therefore useful to investigate the interaction of model compounds such as amino acids, peptides, and their derivatives. In this research topic, the effect of ions with biological relevance on the water activity, the aqueous solubility and the partial molar volumes of amino acids or derivatives is studied. Despite the importance of these systems, scarce experimental information is available in the literature and, therefore, an experimental plan was implemented, as described below.

Regarding thermodynamic modelling, there is still a lack of robust equations of state or activity coefficient models that can be used to describe different types of data, with a unique set of parameters, in a wide range of salt and amino acid concentrations. Therefore, different approaches were considered throughout this work.

Results

1. Following previous work, the aqueous solubility of three amino acids (alanine, valine and isoleucine), in the presence of the chloride and sulfate salts of K^+, Li^+, NH_4^+, Mg^{2+}, Ca^{2+} and Al^{3+} cations, was measured at 298.15 K. The salts were selected in order to cover a wide range of aqueous solubility effects, considering the Hofmeister series of ions.

Molecular dynamics simulations [1,2] were carried out in collaboration with researchers from the University of Aveiro. The evidence gathered indicates that the mechanism by which salting-in inducing polyvalent cations affect the solubility of amino acids in aqueous solutions is different from that of monovalent cations.

A consistent and refined molecular description of the effect of the cation on the solubility of amino acids based on specific interactions of the cations with the negatively charged moieties of the biomolecules was proposed. A snapshot of a simulation is exemplified in Figure 1.

The solubility studies were further extended to biomolecules presenting the peptide bound, by measuring the solubility of two compounds derived from glycine (diglycine and N-acetylglycine) in aqueous solutions of NaCl, KCl, NH_4Cl, $CaCl_2$ or $MgCl_2$. In general, by increasing the concentration of the salts, the solubility of N-acetylglycine decreases and the solubility of diglycine increases, as can be seen in Figure 2.

A computational model for classical molecular dynamics simulations is currently being considered to elucidate the molecular-level interactions in these complex solutions.

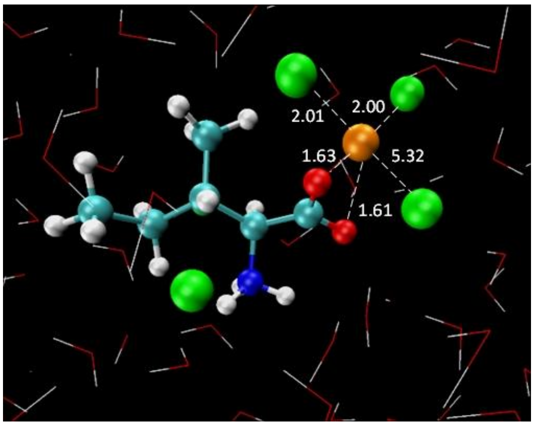


Figure 1. Snapshot from a simulation of (Ile+$AlCl_3$+water) mixtures, showing the distances (Å) between selected atoms.

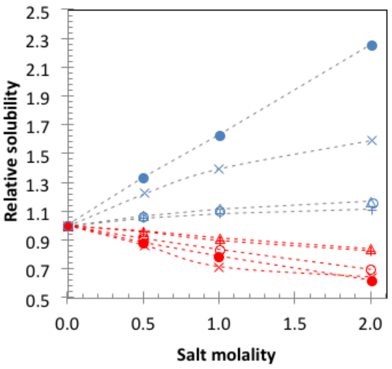


Figure 2. Relative solubility of: (a) diglycine (blue) and N-acetylglycine (red) in aqueous solutions of NaCl (○), KCl (+), NH_4Cl (Δ); $CaCl_2$ (●) and $MgCl_2$ (×), at 298.15 K. Lines are guides to the eyes.

2. The water activity in aqueous solutions of DL-alanine, glycine, or L-serine, with $(NH_4)_2SO_4$ (0.5 to 5.0 molal), was measured at 298.2 K [3].

Three different theoretical approaches such as Zdanovskii--Stokes-Robinson (ZSR), its extension, and the Clegg-Seinfeld-Brimblecombe (CSB) were applied to correlate the data, with global average absolute deviations in the calculation of the osmotic coefficient of 3.46 %, 0.93 % and 1.95 %, respectively. As an example, the results for L-serine are presented in Figure 3.

Unsymmetric molal activity coefficients of the electrolyte were predicted by the extended Zdanovskii-Stokes-Robinson method, in fair agreement with the experimental values found in literature. The measured experimental ternary data, in a wide salt concentration, constitute an important contribution for the extension of thermodynamic models to higher salt concentrations.

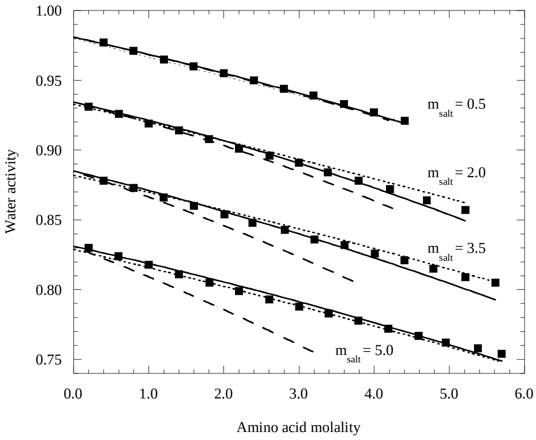


Figure 3. Water activity in aqueous $(NH_4)_2SO_4$ solutions containing L-serine at 298.2 K: calculated curves; (---) ZSR, (—) extended ZSR, (·······) CSB, and experimental data from this work (■)

3. The partial molar volumes of glycine, L-alanine, L-serine, and L-threonine were measured in aqueous solutions of magnesium chloride or ammonium sulfate at (0.0, 0.1, 0.3, 0.7, and 1.0) molal between 278.15 and 308.15 K [4-6].

For the magnesium chloride aqueous systems, the calculated volumes of transfer follow the rank sequence: serine > glycine ≈ threonine > alanine > valine. Compared to alanine, serine presents an –OH group, and that surpasses the increased hydrophobicity of the $–CH_2–$ group in alanine compared to glycine. In general, ammonium sulfate induces larger volumes of transfer than magnesium chloride. Figure 4 shows the results obtained for L-serine in aqueous ammonium sulfate solutions.

For both salts, dehydration of the amino acids occurs, either by rising temperature or salt molality. The decrease in the hydration number becomes progressively smaller as the hydrophobic part of amino acids increases. For all amino acids, the magnitude of the dehydration, represented as the difference (Δn_H) between the hydration numbers of the amino acid in pure water at 298.15 K to those in an aqueous solution containing an electrolyte with 1 molal concentration at the same temperature, is higher in the presence of ammonium sulfate.

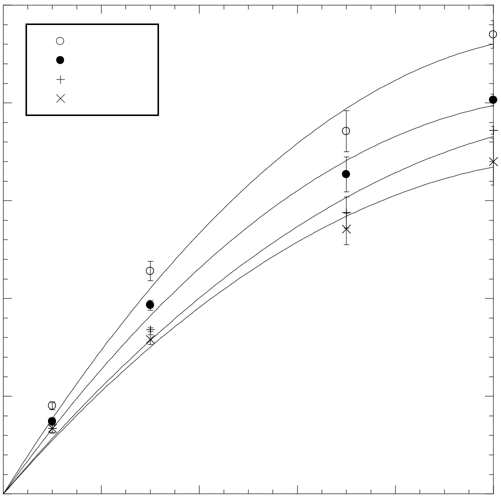


Figure 4. Partial molar volumes of transfer versus salt molality in glycine aqueous ammonium sulphate solutions at different temperatures.

Future Work

The thermodynamic modelling of these very complex mixtures, containing both associating compounds and electrolytes, is very challenging. In this respect, the Perturbed-Chain Statistical Associating Fluid Theory (PC-SAFT) is a very promising equation of state already applied to similar systems and will be, therefore, considered for the description of the thermodynamic data measured here. The final aim is to have an engineering tool capable of supporting the design of different separation processes envolving these biomolecules.

On the other hand, MD simulations also play a very important role to understand, at the molecular level, the experimentally observed behavior of peptides in aqueous saline solutions. Although models for amino acids are well established, the representation of the peptide bond requires a careful validation step. Hence, a computational model will be considered to elucidate the molecular-level interactions in these complex solutions.

Finally, the studies described here will be extended to larger oligopetides, aiming to have further insights on proteins behaviour.

OUTPUTS

Master Dissertations

[1] Catia Sofia Ribeiro de Sousa, The Cation Specific Effects on the Aqueous Solubility of Amino Acids: Experimental and Molecular Dynamics Simulations Contributions, MEQ, IPB, 2014.

[2] Ana Carolina Costa Mota, Partial Molar Volumes of Amino Acids in Aqueous MgSO4 Solutions between 278.15 and 308.15 K, MTB, IPB, 2014.

[3] Yoselyn Maria da Silva dos Santos, Efeitos de sais na solubilidade de diglicina e N-acetilglicina em água, MTB, IPB, 2016.

Selected Publications

[1] L.I.N. Tomé et al., Journal of Physical Chemistry B, 117, 6116 (2013)
[2] M.F. Vilarinho et al., Journal of Chemical Engineering Data 59, 1802 (2014)
[3] P.C. Mota et al., Journal of Solution Chemistry 43, 283 (2014)
[4] M.A.R. Martins et al., Journal of Solution Chemistry 43, 972 (2014)
[5] L.I.N. Tomé et al., RSC Advances 5, 15024 (2015)
[6] E. Javornik et al., Monatshefte fur Chemie 46, 1419 (2015)
[7] M. Arrad et al., Journal of Solution Chemistry 47, 774 (2018)

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FUNDING

LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017
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Processes and Product Engineering

Separation and Purification of High Added Value Products by Preparative and Simulated Moving Bed (SMB) Chromatographic Techniques

KEYWORDS: Chromatography | Simulated Moving Bed | High-added Value Products | Pharmaceutical and Natural Products

Achievements:

1. Optimization of solvent composition, using both polar and reversed phase modes and screening of chiral and achiral adsorbents for multicomponent chiral separations by simulated moving bed (SMB) and fixed-bed chromatography.

2. Development of a complete methodology concerning experimental, modeling and simulation tools was developed, including adsorption isotherm measurments, fixed-bed and SMB operations.

3. Different experimental preparative separations with several chiral (Chiralpak AD and IA) and achiral adsorbents (Waters C18) using a real SMB unit.

Objectives

Thirty years have passed since the international regulatory agencies for clinical safety started to realize the importance of the clear characterization of chiral compounds, as mixtures or single enantiomers, both pharmacologically and therapeutically. Since then, a large range of different and innovative techniques have been proposed and improved to obtain single enantiomers. Among these techniques, the use of preparative chiral chromatography has begun to increase, mainly due to the improvement of the technology involved, which allows, in a shorter period of time, the availability of all the single enantiomers present in a chiral mixture that are essential in the initial stages of the development of a drug. Chiral liquid chromatography, using simulated moving bed (SMB) technology, is an example. The development of new and more stable chiral adsorbents and the use of new and more efficient modes of operation again make SMB technology a topic of interest for industrial and academic groups. The chiral separation process is complex, governed by several different interactions between the chiral solutes, the solvent, and the chiral stationary phase. From a preparative point of view, a high selectivity of the enantiomers should not be the only goal, as it is frequently followed at analytical scale. In this choice, high solubility and low retention times should also be taken into account, in order to improve the preparative process performance, as has been extensively explicated for the separation of chiral nonsteroidal anti-inflammatory drugs. The choice of the proper combination between chiral molecules, the mobile phase composition and the chiral or achiral adsorbent is a critical and very important decision. In addition, at preparative scale (very high concentrations) the competitive behavior between all the stereoisomers will be expected.

Since 2013, several SMB and preparative Fixed-Bed strategies were identified, studied and implemented for the complete separation of all the four stereoisomers present in the nadolol pharmaceutical drug. Nadolol is a nonselective beta-adrenergic receptor antagonist (β-blocker) drug, widely used in the treatment of cardiovascular diseases. Using a laboratory-scale SMB unit (Fig. 1a, b) it´s possible to perform the complete separation by: (i) Three steps cascade SMB runs using a chiral adsorbent, with the more retained compound being collected in the extract stream; (ii) Three steps cascade runs using on the first step an achiral adsorbent for the separation of the nadolol racemates (racemate A: racemic mixture of enantiomers 2 and 3; and racemate B: racemic mixture of enantiomers 1 and 4), with the racemate A being collected in the extract stream and the racemate B being collected in the raffinate stream; (iii) Two steps cascade SMB runs using a chiral adsorbent. The first is the pseudo-binary separation of the more retained stereoisomer and the second step is a ternary separation using the JO (Japan Organo) advanced SMB mode of operation. This SMB mode of operation will need few modifications in the FlexSMB unit hardware. Several studies are also being tested for the complete separation of nadolol stereoisomers using the preparative liquid chromatography Fixed-Bed Azura unit (Fig. 1c). With this unit it is possible to perform the complete separation by: (i) only one step using a chiral adsorbent or (ii) Three steps, being the first a pseudo-binary separation of the two nadolol racemates using an achiral adsorbent and then two consecutive steps using a chiral adsorbent.

Results

Our research group reported the experimental pseudo-binary separation of nadolol by simulated moving bed (SMB) chromatography using both the coated Chiralpak AD and the Chiralpak IA immobilized chiral stationary phases (CSP). This technology is generally based on the use of chiral adsorbents which must have enough recognition for all the chiral species. The CHIRALPAK AD it is an amylose-based CSP and is produced by physical coating of the chiral polymer on a matrix. However, due to their coated nature, this CSP can only be used with a limited range of solvents such as the polar solvents (e.g. acetonitrile, alcohols) or non-polar solvents (e.g. alkanes). The immobilization of a polysaccharide-derivative on the support is an evolutionary strategy to make a CSP compatible with the whole range of organic solvents, which consequently extend its application scope. CHIRALPAK IA contains the amylose 3,5-dimethylphenylcarbamate immobilized onto silica gel.

The first SMB pseudo-binary separation (obtaining the more retained streoisomer from a mixture containing the four nadolol stereoisomers) was performed using a Chiralpak AD CSP. Using a feed solution of 2.0 g/L, the more retained RSR-nadolol was 100% recovered at the extract outlet stream, 100% pure, with a system productivity of 0.65 gRSR-nadolol/(Lbed.h) and a solvent consumption of 9.6 Lsolvent/gRSR-nadolol.

The second SMB pseudo-binary separation was performed using a Chiralpak IA CSP. For a feed concentration of 2 g/L, the more retained nadolol stereoisomer was recovered 100% pure in the extract outlet stream with a system productivity of 0.58 g L^{-1}hr^{-1} and a solvent consumption for 15.8 Lg^{-1}. This represents an improvement of the results obtained previously for the same separation using the coated Chiralpak AD CSP, and shows that the immobilized Chiralpak IA CSP is an interesting alternative for the separation of nadolol stereoisomers at both analytical and preparative scales.

A third experimental SMB pseudo-binary separation was performed using the Chiralpak IA adsorbent and a 25:75 methanol-acetonitrile solvent composition. This work stresses the fact that, a proper selection of the solvent composition can represent an important improvement on the performance of the preparative separation process. Comparing the obtained results, with the ones previously published for the same separation using a feed concentration of 2 g/L and a pure methanol solvent composition, it is observed an important increase in the performance parameters: productivity was increased to 0.77 $gL^{-1}hr^{-1}$ and the solvent consumption was decreased to 9.43 Lg^{-1}. Experimental and simulation results clearly show that, the selection of a solvent composition that allows a decrease in the non-linearity for the adsorption behavior, can promote an increase in the preparative separation performance, mainly, for high feed concentrations.

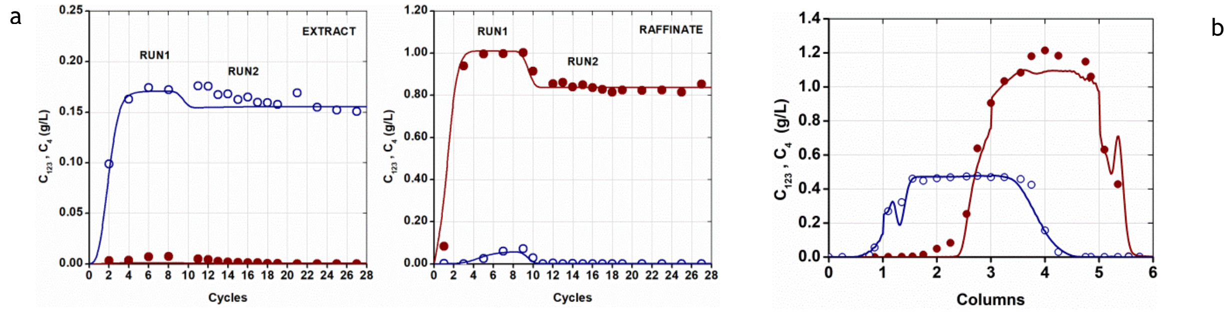

Fig 1. Experimental (points) and predicted (lines) results for the pseudo-binary separation of the nadolol stereoisomers using the FlexSMB unit, a Chiralpak IA CSP and a 25methanol:75acetonitrile mobile phase composition (a) Transient evolution of the nadolol stereoisomers concentration in both the extract and raffinate outlet streams for the two SMB runs: RUN1 and RUN2. (b) Internal concentration profiles at the cyclic steady state during the SMB operation (cycles 20, 22, 24 and 26). Closed circles and open circles for the three less retained and the more retained stereoisomer, respectively.

Future Work

The recent experimental SMB studies are targeted to explore an alternative strategy: the implementation of a first achiral separation step to be followed by two subsequent parallel chiral separation steps. In this first achiral step, C18 adsorbent is used to perform the separation of the two pairs of nadolol enantiomers (“racemate A” from “racemate B”) under reversed-phase mode. The C18 achiral adsorbent allows the separation of the two pairs of nadolol diastereomers, i.e., the first racemate (composed by the nadolol compounds 2 and 3) co-eluting in the raffinate, and the second racemate (composed by the nadolol compounds 1 and 4) to be obtained in the extract SMB stream. After this preliminary achiral separation step, two parallel SMB runs must be carried out using a chiral stationary phase to achieve the complete separation of all the four nadolol stereoisomers.

A fourth experimental SMB pseudo-binary separation was performed using XBridge C18 achiral columns under high pH reversed phase (30:70:0.1 ethanol-water-diethylamine) solvent composition. A 2 g/L nadolol feed solution was completely separated (purities and recoveries of 100%). However, performance parameters were not comparable with the SMB operation due to the high pressure drops and the hardware limitations of the SMB unit.

The most recent studies are concerned with the use of a preparative HPLC Azura system for the preparative separation of the stereoisomers of nadolol. Future work will be focus on the use of several alternative achiral C18 adsorbents, such as Waters XBridge, Shield and XSelect for the preparative separation of the nadolol racemates.

Future research objectives also involve the application of these separation chromatographic techniques and know-how to the refinement and purification of high added value products, such as pharmaceutical and natural products, to arise from the projects *Valor Natural* and the *MORE Collaborative Laboratory*.

Outputs

Master Dissertations

[1] Rami Sami Arafah, Multicomponent Separation of Nadolol Stereoisomers by Liquid Chromatography, MEQ, IPB, 2017.

Selected Publications

[1] A. E. Ribeiro et al., Chirality 25, 197 (2013)

[2] A. E. Rodrigues et al., Simulated Moving Bed Technology - Principles, Design and Process Applications”, 1st Edition, Butterwort-Heinermann, Elsevier (2015)

[3] R. S. Arafah et al., Chirality 28, 399 (2016)

[4] R. S. Arafah et al., Chirality *submitted* (2018)

Team

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Funding

LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017
AlProcMat@N2020, NORTE-01-0145-FEDER-000006, 2016-2019

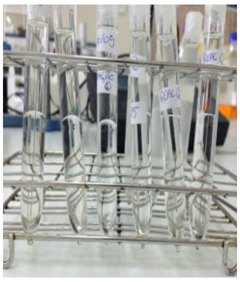
Processes and Product Engineering

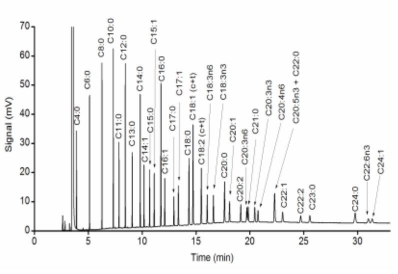
Biodiesel Production through Esterification and Transesterification Catalysed by Ionic Liquids

KEYWORDS: Biodiesel | Ionic Liquids | Catalysis | Product Characterisation

Achievements:

1. **Development of solutions for the recycling of waste oil feedstocks based on conversion processes catalysed by ionic liquids (ILs).**
2. **Characterization of the kinetics of esterification reactions for biodiesel production catalysed by ILs.**
3. **Optimisation of operational conditions for the synthesis of biodiesel (fatty acid methyl acids, FAME) by esterification from fatty acids and by transesterification from waste oil feedstocks using ILs as catalysts.**
4. **Development of standartized lab protocols for quantitative and qualitative characterization of biodiesel samples, based on the European Standard EN 14214.**





Objectives

Since the industrial revolution in the early 19th century, the world demand for energy has increased considerably. According to the International Energy Agency 2017 report, from 1971 to 2015, the total final consumption of energy doubled, with the transportation sector being one of the major responsible for this boost, with a consumption share that increased from 23% in 1971 to 29% in 2015. Additionally, the projection presented by the U.S. Energy Information Administration in the last report of 2016 sustains that, for the period of 2012-2040, the diesel fuel consumption will display the largest growth, relating to all other transportation fuels. The rising demand for fuels in the industrialised nations and emerging countries, mainly for the transportation sector, allied with an increasing concern for environmental issues, lead to a search for alternatives to petroleum-based energy sources. Hereafter, a new term has emerged: biofuels, liquid or gaseous fuels derived from biomass sources, such as wood, vegetation, organic residues, vegetable oils, amongst others. Biofuels have several advantages over traditional fuels obtained from other sources. They are obtained from renewable energy sources and have lower impact on the environment. Amongst the available biofuels, such as bioethanol, biogas and syngas, biodiesel is a good alternative as energy source and a promising replacement for petro diesel. It can be defined as a fuel suitable for compression ignition engines that is constituted by a mixture of fatty acids alkyl esters (FAME) derived from oils or fats. It is usually obtained industrially by a transesterification reaction of triglycerides, but it can also be synthetised by an esterification reaction of free fatty acids (FFA). Hence, it can be produced from a wide variety of raw materials. For these reasons, biodiesel has been greatly explored in the past decades, but the production process currently applied has several drawbacks related to cost issues, environmental concerns, food competition, amongst others. Hereof, searching for alternative ways to produce biodiesel is a contemporary concern, with most of the focus invested in finding new catalysts that allow overcoming the disadvantages of the traditional catalysts. In this way, ionic liquids appear as possible substitutes, due to its characteristics that promote a greener process.

Ionic Liquids (ILs) can be defined as organic salts that are consisted of ions (an organic cation and an organic or inorganic anion) and, opposed to inorganic salts, remain liquid at room temperatures. The melting temperatures of those salts lay usually below 100ºC due to the presence of a delocalized charge and large ions, causing packing to be difficult.

There are several advantages related to the use of ionic liquids. Due to the interactions between the anion and cation, they are non-flammable, have negligible vapor pressure, a good solubility in both organic and inorganic materials, a high catalytic activity and can be easily manipulated in order to achieve a specific property, by changing the anion/cation combination. The possible variations in the anion/cation combinations are advantageous in terms of variety, giving origin to at least 1 million binary ionic liquids and potentially 1018 ternary ionic liquids, comparing to only 600 organic solvents. Moreover, they are easily recyclable, can be used under mild conditions and produce less waste and can exhibit acidic, basic or neutral characteristics. On the other hand, ILs are typically more expensive than classic catalysts or solvents, although, this apparent disadvantage can be easily overcome due to the fact that ionic liquids can be easily recovered and reused. Usually, due to their low vapor pressure, distillation presents a suitable option for the recovery process, but when the system is sensitive to temperature or other non-volatile compounds are mixed, there are other options in terms of recovery, such as extraction with solvents, adsorption, separation applying membranes, etc.

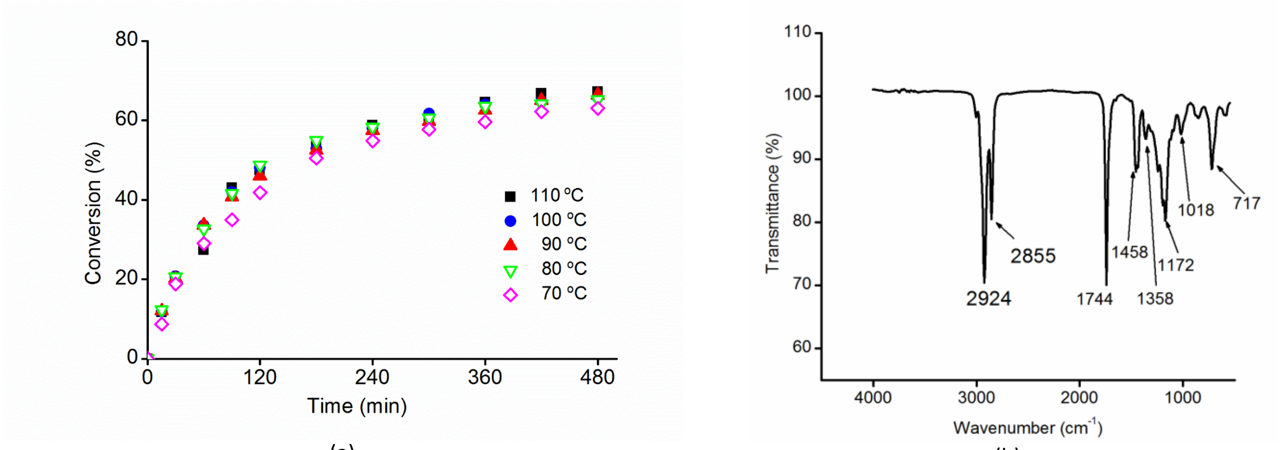
The use of ionic liquids in biodiesel production has attracted a great attention from the scientific community, since its use allows overcoming several issues related to the traditional processes of biodiesel synthesis, such as the possibility of exploiting low-cost feedstock, reducing environmental issues and the number of downstream steps, among others. In fact, in biodiesel production, ionic liquids allow the reduction of the number of reactions and purification steps, decreasing the production cost and energy consumption throughout the process. The majority of published studies focus on the application of Brønsted acidic ionic liquids to biodiesel production, since they usually exhibit high catalytic activity, although, a few publications report that basic ionic liquids require less reaction time and less temperature in biodiesel synthesis.

Therefore, the main focus of this project is the assessment of the potential replacement of classical catalysts by ionic liquids for biodiesel production processes. In this context it is intended the evaluation of the performance of different ionic liquids in the catalysis of esterification and transesterification reactions for biodiesel production, the otimization of the respective reaction conditions, the study of the kinetics of the referred reactions, and the development and testing of standartized lab protocols for quantitative and qualitative characterization of the produced biodiesel samples, based on the European Standard EN 14214 specifications.

Results

Our research group reported that ionic liquids seem to be a viable alternative to common acidic catalysts for biodiesel production by esterification. From the set of ionic liquids tested, 1-methylimidazolium hydrogensulfate [HMIM][HSO_4], 1-butyl-3-methylimidazolium hydrogensulfate [BMIM][HSO_4], and 1-butyl-3-methylimidazolium methylsulfate [BMIM][$MeSO_4$], displayed good catalytic activity and resulted in high conversions, reaching values over 90%, in relatively mild conditions.

The optimal esterification reaction conditions were assessed by statistical analysis through the construction of factorial designs for the test of relevant factors: time reation, temperature reaction, molar ratio between oleic acid and methanol and the catalyst dosage, and evaluating several responses, like reaction conversion based on acidity drop, and FAME content of produced biodiesel samples, measured by GC using the internal standard method. It was possible to set the optimum conditions that lead to the highest possible conversion and FAME content. For [HMIM][HSO_4] studies, the optimal reaction conditions for the conversion were estimated as 8h, 110°C, 15:1 molar ratio and 15wt% catalyst dosage, leading to a conversion of 95%. On the other hand, the optimum conditions that lead to 90% FAME content, were estimated as 8h, 110°C, 14:1 molar ratio and 13.5wt% catalyst dosage. The results indicate that [HMIM][HSO_4] has high potential in biodiesel production by esterification, because it not only allowed the achievement of high conversions, but it also lead to a product with high content of fatty acid methyl esters.



(a) (b)

Fig 1. (a) Conversion of an esterification versus reaction time for different temperatures. (b) FT-IR spectrum of a typical biodiesel (FAME) sample.

Other studies are in course in order to assess the performance of [HMIM][HSO_4] and [BMIM][HSO_4] for simultaneous esterification/transesterification reactions and for the evaluation of [BMIM][$MeSO_4$] potential for the biodiesel production processes.

Future Work

A complete evaluation of the suitability of imidazolium based ILs for the catalysis of biodiesel production implies the implementation of a wide range of studies, like:

- Multi-objective optimization of responses (conversion and FAME content) that would lead simultaneously to the highest conversion and FAME content in biodiesel samples.
- Evaluation of IL suitability for simultaneous promotion of the esterification reaction of FFA and the transesterification reaction of triglycerides for low quality waste oil feedstocks, or as a possible pre-treatment step for highly acidic oils.
- Recovery studies for IL, in order to assess the number of reaction cycles in which high reaction conversions and high FAME content in biodiesel samples could be attained.

OUTPUTS

Master Dissertations

[1] Irana Alimova, Production of Biodiesel through Esterification Catalysed by Ionic Liquids, MEQ, IPB, 2016

[2] Arevik Tadevosyan, Biodiesel Production through Ionic Liquid Catalysed Esterification, MEQ, IPB, 2017.

Selected Publications

[1] B. Meireles, Production of Biodiesel through Esterification Catalyzed by Ionic Liquids, XXI Encontro Galego-Portugués de Química, p. 57 (2015)

[2] S.T. Yordanova et al., Optimization of Biodiesel Production through Esterification Catalysed by an Acidic Ionic Liquid, XXII Encontro Luso-Galego de Química, 39 (2016)

[3] A. Tadevosyan et al., Biodiesel Production through Esterification using Ionic Liquids as Catalysts, 10ENC – 10.° Encontro Nacional de Cromatografia, 98 (2017)

[4] F.F. Roman et al., Ionic Liquid Catalyzed Reaction for Biodiesel Production, XXIII Encontro Galego-Portugues de Química, 71 (2017)

[5] I. Alimova et al., Study of Biodiesel Production through Esterification Catalysed by Imidazolium-based Ionic Liquids, CIEEMAT 2017 - III Congresso Ibero-Americano de Empreendedorismo, Energia, Ambiente e Tecnologia, 103-107 (2017)

[6] F.F. Roman et al., Optimization of Biodiesel Production through Esterification of Oleic Acid Applying Ionic Liquids as Catalysts, Fuel *submitted*

TEAM

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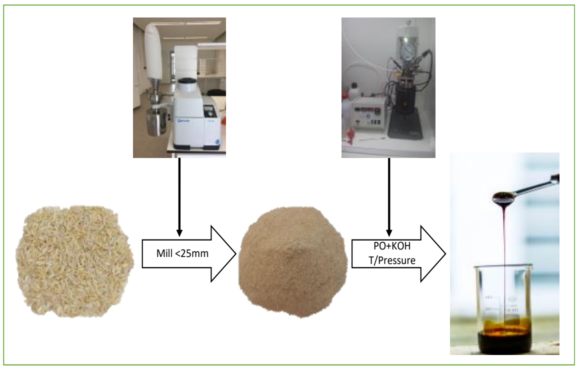
Processes and Product Engineering

Development of Sustainable Polymer Solutions

KEYWORDS: Biolpolyols | Oxypropylation | Polyurethanes | Biodegradablility | Composites

Achievements:

During the period 2013-2017 the work was mainly supported by two projects with industry (BIOBLOCKS and NEWALK) showing the impact and interest of the tested strategies. The use of lignin to produce biopolyols was demonstrated involving SOPORCEL/PORTUCEL (presently The Navigator Company) and its use as biodegradability ehancer proved in the production of shoesoles involving the Footwear Technological Centre of Portugal (CTCP) and PROCALÇADO (footwear components company). Following the successful results, two other projects with CTCP were started/approved and a profit international cooperation with UPV/Basque country (Spain) in the thematic of water based polyurethane dispersions (Group of Professor Arantxa Eceiza) was consolidadted.



Objectives

Scientific efforts towards the design, synthesis, and production of sustainable or green polymers have expanded tremendously in the last decades. This involves concerns related to feedstock origins, use of green and sustainable processes and material's biodegradability. The present topic follows 10 years of background experience in the field of sustainable polymer solutions development. Within the 2013-2017 period the following activities have been carried out: (i) Biobased polyols obtained through oxypropylation processes; (ii) Water Based Polyurethane Dispersions; and (iii) Biobased additives as biodegradability enhancers.

Results

Biobased polyols obtained through oxypropylation processes: the developed work comprised the synthesis of polyols through oxypropylation and their subsequent incorporation into rigid polyurethane (RPU) foam formulations. Disctinct biomasses were studied: lignin, almond shell and Brazilian pine-fruit shell. In what concerns lignin, a study conducted within the context of the BIOBLOCKS project, two *E. globulus* lignin streams were tested: (i) lignin isolated from industrial kraft liquor and (ii) depolymerized lignin resulting from an oxidation process developed at LSRE-LCM to produce vanillin and syringaldehyde. Polyols were firstly obtained using a 450-ml stainless steel PARR autoclave (setpoint temperature of 160 ºC), following a general oxypropylation procedure established in the group. The obtained polyols were characterized (homopolymer content (%, w/w), hydroxyl index (IOH) (mg KOH/g) and viscosity (20ºC, Pa.s) and incorporated into RPU foam formulations, alone or combined with 50% of a commercial polyol (Daltfoam TP 32015 polyol) at a NCO/OH ratio of 1.1. The following main conclusions were achieved: (i) among the resulting products, the integrated process for lignin valorisation developed at LSRE-LCM gives rise to a stream of depolymerized/oxidized lignin useful for polymeric applications; (ii) precipitated lignin (lignin directly obtained from the black liquor) and depolymerized lignin can be valorised to produce polyols by oxypropylation and polyurethanes; (iii) the generated polyols can be used at a content of 100% to produce RPU foams with properties quite similar to the ones of reference foams; and (iv) the introduction of polyols with higher lignin content favours the fire resistance properties of the obtained RPU foams.

Water Based Polyurethane Dispersions: Water-based poly(urethane-urea) dispersions (PUD) are becoming widely used in the field of adhesives and coatings, progressively substituting the solvent-based counterparts. Nevertheless, in the past recent years, PUD industry, and the prepolymer process in particular (the most representative process at industrial level), had to face important challenges due environmental restrictions imposed by EU legislation, implying in some cases, the substitution of some traditionally used raw materials. Moreover, emerging new markets are pushing this industry to search for new product solutions. In this context, the work carried comprised the development of a NMP-free process, and research on the development of antimicrobial and high performance PUDs.

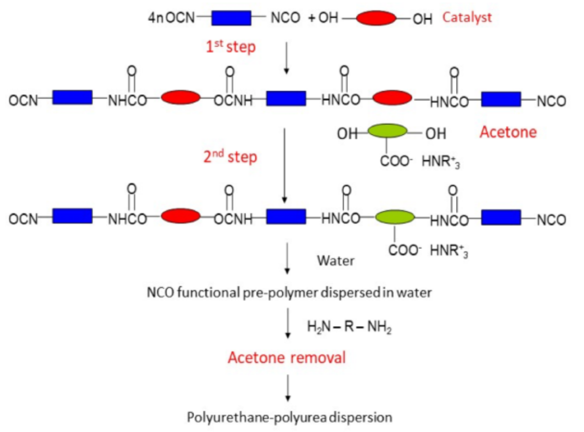


Fig 1. Schematic representation of the developed modified pre-polymer process (NMP-free process).

The following main developments/results were achieved: (i) a modified pre-polymer process was developed following the general process described in Figure 1. Several dispersions were synthesized using different DMPA contents (3.0, 4.0 and 5.0%, keeping the pre-neutralization degree at 100.0%), and different DMPA pre-neutralization degree (100.0 and 90.0%, using 5.0 % of DMPA). The effect of these variables in the particle size and dispersion stability was evaluated. Based on the obtained results, the developted modified pre-polymer process is a feasible alternative to obtain NMP-free PUD, thus fulfilling the restrictions imposed by the European Union; (ii) PUDs waterborne character was exploited to incorporate antimicrobial plant extracts: Melissa officinalis L. and Salvia officinalis L.. After 1 day of incubation all the samples (base

PUD and Salvia- and Melissa-based PUD series), showed bacteriostatic effect against the analyzed S. aureus, E. coli and P. aeruginosa bacteria. After 4 days of incubation, only samples containing Salvia or Melissa extract, presented bacteriostatic effect, being the magnitude of the effect dependent of the used content and incorporation route. This effect can be attributed, either to the extracts itself or to their distribution in the PUD system that is dependente on the used incorporation route (post-, in-situ and pre-methods); (iii) in a diferente prespective, PUDs waterborne character was also exploited to incorporate cellulose derived reinforcements Cellulose is the most abundant biopolymer which can be obtained from different sources like plants, agroindustrial residues or bacteria. It offers the possibility of being used in different forms such as fibers or crystals from the nano to macroscale. Based on a base-PUD, a series of nanocomposites loaded with different contentes of celulose nanocrystals (CNC) were prepared. The morphological analysis by AFM corroborated the effective dispersion of CNC in the matrix, which favored the creation of PUD-CNC ainteractions as observed by FTIR and DSC. In a general way, the effective CNC incorporation resulted in stiffer films, showin an increase in E and σy, maintaining high εb values, except for the highest CNC content.

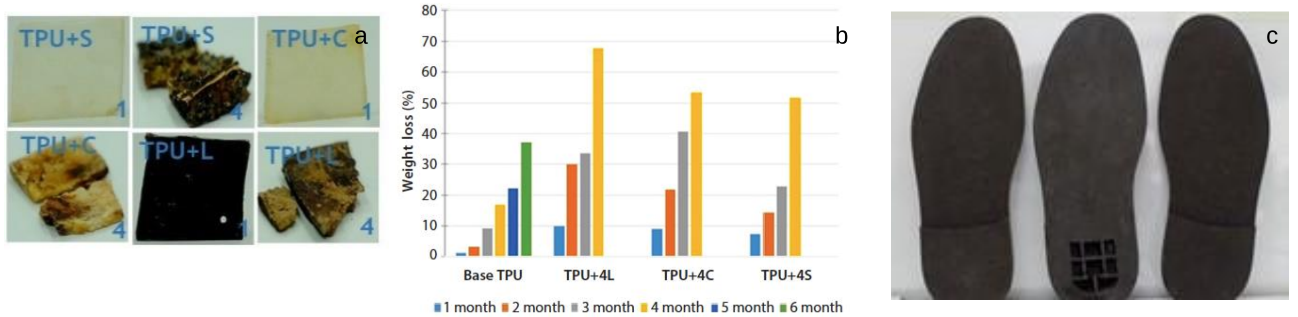


Fig 2. (a) TPU samples modified with starch (TPU+S), cellulose (TPU+C) and lignin (TPU+L), recovered after 1 and 4 months of the biodegradation test in soil at 58ºC. (b) Weight loss of TPU samples achieved in the soil biodegradation assay at 58 ºC (monthly sampling till 6 months). (c) Footwear soles prototypes produced with TPU modified with lignin.

Biobased additives as biodegradability enhancers: Presently, given the widespread use of polymeric materials and associated waste management problems, the development of more biodegradable and environmental compatible solutions assumes an increasing importance. For this purpose, the incorporation of biobased additives, even at a low content, in polymer formulations (e.g. thermoplastic polyurethanes (TPU)) can promote biodegradation. These additives, when dispersed into a polymeric matrix, can constitute preferential sites for microorganisms' attack thus favouring biodegradation initiation and progression. In addition, this strategy often leads to properties' improvement of the base material (e.g. tear strength, tensile strength, elongation at break and flex resistance). The preformed work, mostly in the context of the NEWALK project with the footwear sector, comprised the incorporation of biobased additives (lignin, cellulose and starch) as biodegradability enhancers of TPU-based components, and the application of lignin as reactive filler in PU flexible foams used for footwear insoles production, to improve resistance to fatigue and cushioning properties. The results obtained evidenced a positive effect of the tested biobased additives (lignin, cellulose and starch) in the improvement of the TPU biodegradability, the most favourable results being registered for the samples modified with 4% of Sarkanda lignin (67% weight loss after 4 months at 58 °C in soil). These results were corroborated by the structural modifications detected by the FTIR-ATR analysis. Moreover, the results of the mechanical properties evaluation proved the suitability of the TPU modified with 4% of lignin for footwear outsoles production.

Future Work

Considering the topic of biobased polyols obtained through oxypropylation processes, the work includes an ongoing PhD thesis (J.A. Pinto) where the cooperation with INP-Pagora (Grenoble, France) (co-supervision of Naceur Belgacem) was reestablished. Moreover, a cooperation with CUT (Cracow, Poland) (Group of Professor Aleksander Prociak) started in the last year under the same thematic. The work on PUDs will continue on the scope of functional materials with properties imparted by natural or natural derived additives (antimicrobials, natural colorants, etc.), also in cooperation with UPV. Finally, the topic of biobased additives as biodegradability enhancers will continue to be developed, mainly under industrial context (one ongoing project and one recently aproved, both with the footwear sector), and will be extended to other functionaties.

OUTPUTS

PhD Thesis

[1] I.P. Fernandes, Novel Materials based on Polyurethanes and Polyureas for Footwear Commponents, PDEQB/PDEA, FEUP, 2017.

Master Dissertations

[1] J.A. Pinto, Valorization of Almond Agro-industrial Residues: Production of Biopolyols from Almond Shell, MEQ/IPB, IPB, 2015.

[2] S. Rezende, Evaluation of the Pine-Fruit Shell, a Byproduct of the Araucaria Angustifolia Seed, for the Production of Polymeric Materials, MQSA/DD IPB-UTFPR, IPB, 2016.

Selected Publications

[1] C.A. Cateto, M.F. Barreiro, C. Ottati, M. Lopretti, A.E. Rodrigues, M.N. Belgacem. Journal of Cellular Plastics 50, 81 (2014)

[2] I.P. Fernandes, M.R.P.F.N. Costa, M.J. Ferreira, M.F. Barreiro. Polimery 60, 536 (2015)

[3] A. Santamaria-Echart, I.P. Fernandes, A. Saralegi, A., M.R.N. Costa, M.F. Barreiro, M.A. Corcuera, A. Eceiza. Journal of Colloid and Interface Science 476, 184 (2016)

[4] I.P. Fernandes, M. Barbosa, J.S. Amaral, V. Pinto, J.L. Rodrigues, M.J. Ferreira, M.F. Barreiro. Journal of Renewable Materials 4, 47 (2016)

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[6] A. Santamaria-Echart; I.P. Fernandes; L. Ugarte; M.F. Barreiro; A. Arbelaiza; M.A. Corcuera; A. Eceiza. Composites Part B: Engineering 137, 31 (2018)

TEAM

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FUNDING

LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017; LA LSRE/LCM Strategic Project, PEst-C/EQB/LA0020/2013, 2013-2014; AIProcMat@N2020, NORTE-01-0145-FEDER-000006, 2016-2019; Polymer Reaction Engineering, NORTE-07-0124-FEDER-000014,2013-2015; BIOBLOCKS, SI IDT - 33969/2012 F2, 2013-2015; NEWALK, QREN: Projectos Mobilizadores, 2011-2014; ExtraLightSafeShoe, POCI-01-0247-FEDER-017905, 2016-2019

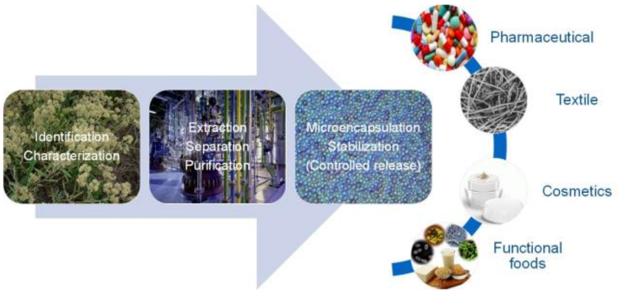
Processes and Product Engineering

Microencapsulation of Bioactives for Food Applications

KEYWORDS: Microencapsulation | Bioactives | Natural polymers | Food and Cosmetic Ingredients

Achievements:

The work developed during the period 2013-2017 highlights the cooperation between the two research topics of the Sustainable Processes and Products group (Processes and Product Engineering and Natural and Bio-based Ingredients). The cooperation resulted in 1 PhD and 7 MSc theses, 9 scientific articles and 1 book chapter. Moreover, this cooperation was important to establish the project "Valor Natural®", presently approved. This project of the typology "Mobilizadores", regarded as driven forces for the economy, involves a consortium including 13 partners (industrial and academic) covering complete agro-food value chains (valorization of bioactives and natural colorants).



Objectives

Bioactive ingredients are generally prone to degradation, both during storage and food processing, as many of them are physically, chemically and/or enzymatically unstable leading to their degradation or transformation with the consequent loss of bioactivity. In this context, microencapsulation emerges as a viable approach to overcome these problems and, additionally, to provide controlled or targeted delivery or release. Develop a microencapsulation process to be used with food ingredients presents some constrains and challenges, as the raw materials and processes must be properly selected to avoid toxicity, conform food contact legislation, and provide adequate performance to the produced microencapsulated systems. Also, choose technologies and processes able to reach high TRLs and perform final incorporation tests are of high relevance. The processes and chemical systems studied include: (i) Spray-drying using maltodextrin or maltodextrin crosslinked with citric acid; (ii) Spray-coagulation using alginate; and (iii) Complex coacervation with chitosan/whey protein.

Results

Spray-drying using maltodextrin or maltodextrin crosslinked with citric acid: Among different encapsulating materials used to protect bioactive ingredients and increase their bioaccessibility, maltodextrin is a competitive solution. It was tested with success with different bioactives, alone or in combination with several cross-linking molecules, showing the advantages of using this natural polymer. Two different studies have been conducted: (i); microencapsulation of two mushroom extracts (*Suillus luteus* (L.: Fries); Sl and *Cooprinopsis atramentaria* (Bull.); Ca) and study of their synergistic antioxidant effects. The produced microspheres were incorporated into cottage cheese samples and their antioxidant activity, nutritional value and colour were determined and compared with the counterparts, using extracts in the free form and a control (sample with no added extracts); and (ii) microencapsulation of *A. bisporus* extracts by spray-drying using maltodextrin cross-linked with citric acid, a novel material in the context of microencapsulation, as the carrier material. In order to guarantee an effective crosslinking reaction, a post thermal treatment was studied. The obtained microcapsules were used as functionalizing agents in yogurts. The main results pointed out for: (i) the combination Sl:Ca (1:1) was the best as it revealed synergistic effects. The microspheres with Sl:Ca (1:1) and with the free extract (in the same proportion) were incorporated into cottage cheese (Figure 1). The results showed that, in comparison with the free form, the encapsulated extracts became more effective since the antioxidant activity was preserved over time; and (ii) the incorporation of Agaricus bisporus extract, in free and microencapsulated forms, in yogurts resulted in products with promising bioactive properties, with the sample TME (loaded-extract microspheres subjected to post-thermal treatment) giving the best results in terms of bioactive properties preservation along storage.

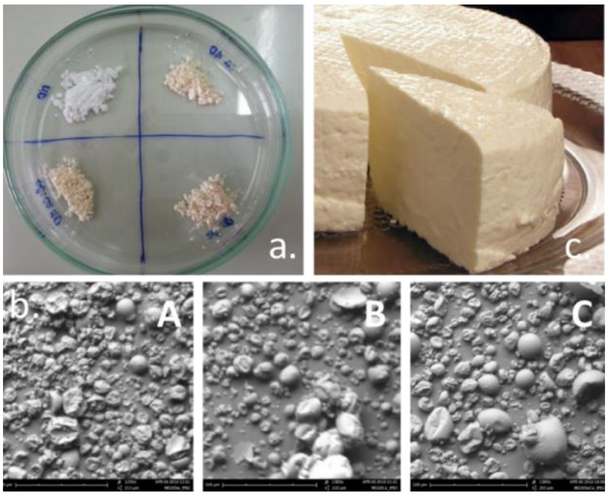


Fig 1. (a) Produced microspheres; (b) SEM images of microparticles containing Sl (A), Ca (B) and the mixture Sl:Ca (1:1) under a magnification of 1300x; (c) Cotage cheese functionalized with the produced food additives.

Spray-coagulation using alginate: The alginate, usually commercialized in the form of a sodium salt, is a polyanionic copolymer formed by α-L-guluronic acid and β-D-mannuronic acid units. It is derived from marine kelp, mainly the brown sea algae and has been extensively investigated due to its biocompatibility, low toxicity, relatively low cost, and mild gelation by addition of divalent cations such as Ca^{2+}. This polymer has received great attention since its behaviour varies with the pH, namely, it resists acidic media and solubilizes at basic pH. In the present context, several microencapsulation activities have been developed by using the spray coagulation technique. The studied examples include (Figure 2): (i) the microencapsulation of phenolic extracts of *Rubus ulmifolius* Schott flowers and incorporation in yogurts; (ii) microencapsulation *Fragaria vesca L.* extracts and application in k-carrageenan gelatin;

(iii) Microencapsulation of rosemary extracts and incorporation in cottage cheese; (iv) microencapsulation of *Ceratonia siliqua* L. hydroethanolic extract and functionalization of yogurts; (v) microencapsulation of fennel and chamomile extracts and incorporation in cottage cheeses; and (vi) microencapsulation of *Arenaria montana* L. hydroethanolic extract as a chemopreventive food ingredient. In a general way, the comparative studies dealing with the use of the free *versus* microencapsulated forms, in the tested food products, revelled the advantages of the later ones in preserving bioactivity.

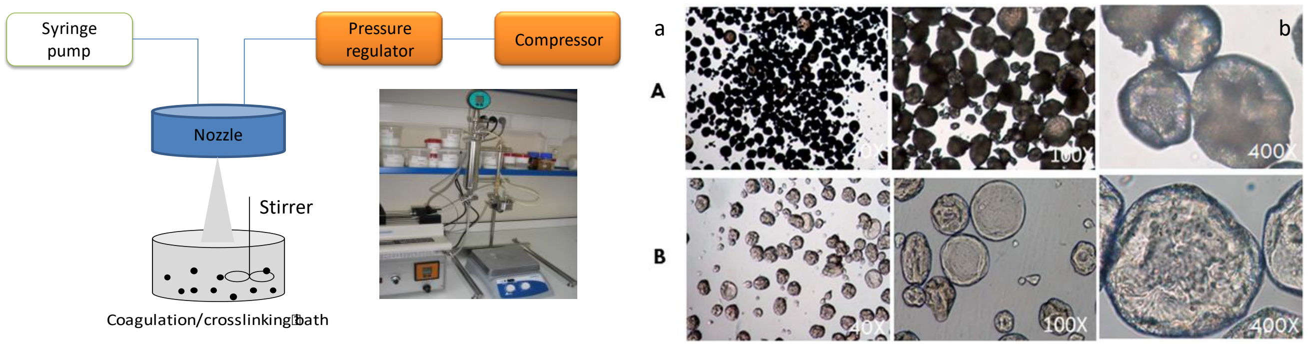


Fig 2. (a) Microencapsulation system for the application of spray-coagulation technique. (b) Alginate microspheres of phenolic extracts of Rubus ulmifolius Schott: morphology analysis by OM under magnifications of 40, 100 and 400X ((A) Lyophilized microspheres and (B) microspheres after 24 hours of rehydration).

Complex coacervation with chitosan/whey protein: Among the wall materials used in the complex coacervation, proteins and polysaccharides are the most common ones. In the present study chitosan and whey protein have been used to microencapsulate *A. bisporus* extracts. Chitosan is the second most abundant polysaccharide found in nature, obtained by deacetylation of chitin. Whey protein, a typical industrial waste material whose main representative is the β-lactoglobulin group, has also been extensively evaluated for microencapsulation purposes. The main objective of the work was the optimization of the process using the response surface methodology (RSM). The results pointed out for the advantage of using statistical tools in the design of the process.

Future Work

The use of microencapsulation was successfully applied to several bioactives and their use to functionalize various food products was found advantageous over the use of the free forms. Ongoing and future work involve 2 PhD and 3 MSC theses. Moreover, microencapsulation techniques start to be applied to natural colorants in order to viabilize their use at industrial level.

OUTPUTS

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Master Dissertations

[1] J. Viegas, Encapsulation of Phenolic Extracts of *Rosa micrantha* Borrer ex Sm for Food Purposes, MQSA, IPB, 2013.

[2] A.C. Martins, Phenolic Extracts of *Rubus ulmifolius* Schott: Chemical Characterization, Antioxidant Activity and Encapsulation for Nutraceutical Purposes, MQSA, IPB, 2013.

[3] A. Ribeiro, Phenolic Extracts of *Suillus luteus* and *Coprinopsis atramentaria*: Evaluation of Synergisms in their Antioxidant Potential and Encapsulation by Spray Drying for Nutraceutical Purposes, MTB, IPB, 2014.

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[7] H.H. Almeida, Stabilization of Natural Curcumin Dye and its Application in Yogurts, MQSA/DD IPB-UTFPR, IPB, 2017.

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[1] A. Martins, L. Barros, A.M. Carvalho, C. Santos-Buelga, I.P. Fernandes, F. Barreiro, I.C.F.R. Ferreira. Food and Function 5, 1091 (2014)

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[8] F.S. Oliveira, A. Ribeiro, L. Barros, R. Calhelha, J.C.M. Barreira, B.D. Junior, R.M.V. Abreu, M.F. Barreiro, I.C.F.R. Ferreira. Jornal of Functional Foods 38, 214 (2017)

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Andreia Ribeiro | PhD student
7 MSc students

FUNDING

LA LSRE-LCM Strategic Project, POCI-01-0145-FEDER-006984, 2015-2017; LA LSRE/LCM Strategic Project, PEst-C/EQB/LA0020/2013, 2013-2014; AIProcMat@N2020, NORTE-01-0145-FEDER-000006, 2016-2019; Polymer Reaction Engineering, NORTE-07-0124-FEDER-000014,2013-2015

Natural and Bio-based Ingredients

Irradiation as Foodstuff Preservation and Disinfestation Technology

KEYWORDS: Irradiation | Gamma Rays | Electron Beam | Chestnuts | Mushrooms | Medicinal and Aromatic Plants

Achievements:

1. Irradiation of different types of foodstuffs, namely chestnuts, mushrooms and, medicinal and aromatic plants.

2. Application and validation of both gamma and electron beam radiation as sources of decontamination and preservation.

3. Optimization of irradiation doses and time of exposition for traceability purposes.

Objectives

Preserving foodstuffs in all their dimensions (microbiological and pest load, appearance, texture, nutritional richness and bioactivities) throughout the food chain is a challenge for producers, shipping companies, retailers and consumers. Although many technologies of preservation and desinfestation exist, some tend to be far more researched than others, and likewise, have higher acceptance by the population, regardless of their efficacy. Food irradiation, although still looked upon with distrust, can be one of the cheapest manners of disinfecting and preserving foodstuffs, despite a very high initial investment for the instalation. Taking advantage of the rich and biodiverse region the Mountain Research Centre (CIMO) is based, three types of foodstuffs were irradiated (chestnuts, mushrooms and plants) to optimize and validade the irradiation process for each of them, aiming at an increase shelf-life, guarantee of their quality and safety, reduce or eliminate microbial and pest load, as an alternative to the mainstream preservation technologies, which have higher environmental impact.

Results

The Biochemcore research group focused their irradiation studies in three foodstuffs, chestnuts, mushrooms, and aromatic and medicinal plants. Many chemical, biochemical and physical analyses were caried out, from nutritional profiles, antioxidant activity to individual compounds like polyphenols, soluble sugars, tocopherols, organic acids and triacylglycerols. Color parameteres were also screened, as well as anti-toxin acitivty. Irradiation with gamma rays was carried out at the Portuguese Institute of Nuclear Technologies, while e-beam irradiation was carried out at an enterprise in Poland, both of which share a strong collaboration with the biochemcore group.

Regarding chestnuts (*Castanea sativa* Mill.), both irradiation types were used in several cultivars of Portuguese (judia, longal and cota) and Italian (palummina) origin. The application of irradiation to chestnuts is manily due to the ban on methyl bromide within the European Union since 2010, as a disinfestant, fostering reseach on viable alternatives [1] Regarding Portuguese cultivars, there seemed to be preservation of phenolic compounds in the irradiated chestnuts, linking the ionization of molecules with their preservation along storage time. Still, the ideal doses for gamma irradiation was defined at 3kGy, while electron-beam was 1 kGy [2]. Overall there seemed to be an interaction between storage time and irradiation in the chemical profile of chestnuts, meaning that irradiation could be used as a chemometric indicator of chestnut sanity [3, 4]. Overall, the changes on the chemical and antioxidant parameters was very low independently of all cultivars analysed, including the italian cultivar [4]. Triacylglycerols, analysed by high performance liquid chromatography coupled to electrospray ionization, seemed to be valuable indicator of irradiation and could be used as a fast and reliable source to avoid fraude or to certify types and intensity of radiation. Irradiation seemed to conserve the bioactivities of chestnuts without compromising their nutritional profile or colour, while also being interesting as a desinfestation alternative [1].

The inspiration for irradiation of mushrooms was quite different from the one to irradiate chestnuts. Mushrooms, contrary to chestnuts are not stored for large periods of time, in fact, their storage life is limited to a few days. Thus, their irradiation, beyond aiming at their decontamination also intended to stop their maturation process in order to improve shelf life and reduce their deterioration speed [5]. Dozen of species were irradiated both with electron beam and gamma rays, namely the *Boletus pinophilus* Pilát & Dermek, *Clitocybe subconnexa* Murrill, *Boletus edulis* Bull., *Macroleptiota procera* (Scop.) Singer, *Amanita* genera, among others. Overall, both commercial and wild samples were irradiated, as well as whole mushrooms and sliced samples. There seemed to be no organoleptic changes in *B. pinophilus* and *C. subconnexa* samples irradiated with 2 kGy, while the antioxidant activity actually increased [5]. Regarding sliced samples of mushrooms, the best dose of gamma rays was set at 6 kGy for *Boletus edulis*, which interestingly showed no signifacnt changes in the nurtitional profile, soluble sugars, fatty acids, tocopherols, organic acids, phenolic compounds and antioxidant activity, while extending their shelf life, acomplishing all the expected outcomes [6]. Concerning dried samples, doses of up to 10 kGy of electron beam irradiation seemed to conserve the antioxidant activity. The impact of irradiation on mushroom's dietary fibers, present in considerable amounts, and one of their best assets as food, was also researched. While at doses above 6 kGy, the soluble or insoluble fibers are reduced, lower doses seem to be less impacting, although the impact even at higher doses does not compromise the high quantity of these nutrients [7]. As for the chestnuts, triacylglycerols also seemed to be suitable chemometric indicator of irradiated mushrooms, given the effect of the ionizing radiation on the individual fatty acids on the glycerol chain [8]. Overall, irradiation was a viable method to halt ripenning mechnisms, to disinfest wild, commercial, sliced, fresh and dried mushrooms, being validated on various species and genera.

Finally, regarding irradiation of dried commercial plants, the main objective of using irradiation relate to the decontamination of the samples and preservation of their bioactice compounds. Throughout the years, many plants were irradiated by the biochemcore research group, namely *Aloysia citrodora* Paláu, *Mentha x piperita* L., *Melittis melissiphyllum* L., *Melissa officinalis* L., *Arenaria montana* Sieber ex Steud, *Ginkgo biloba* L., among others. Both gamma and electron beam irradiation were also employed for the plants. Gamma radiation applied to *A. citrodora* and *M. piperita* preserved the phenolic compounds over time when compared to control samples, while not displiying any changes in the antibacterial activity of the plants [9], on *Naturtium officinale* W.T. Aiton, the dose of 2 kGy was the most efficient, preserving monounsaturated and ployunsaturated fatty acids, tocopherols, flavonoids and the antioxidant activity [10]. For instance, higher doses of gamma radiation, namely 10 kGy increased the antioxidant activity on *Thymus vulgaris* L. [11], while also protecting the chemical profile and color of *Ginkgo biloba* L. [12]. Overall, gamma radiation seemed to have a protective function on many compounds in the dried plants, although there are variations of the preserved compounds, the doses applied, which depend on the plant species [13]. Electron beam radiation was also tested on these plants, and displayed similar preservation capabilities to gamma radiation in dried stored commercial plants. This type of radiation conferred the least changes in the nutritional profile and color, while attenuating the effect of storage time on the loss of activity of individual bioactive compounds [14]. Decontamination of dried plants was also tested, namely the decontamination of toxins, ochratoxin A and aflatoxin B1 (Figure 1). Although there was a reduction on their load in the irradiated plants, the results were not significative due to the very low water present in the plant extracts [15].

Figure 1. Preparation of the dried samples for analysis.

Overall, radiation was effective as a conservation technique in all studied matrices, whith variation on their efficacy dependent on the doses applied, type of radiation used and the water content of samples.

Future Work

In terms of future work, the group has taken a step further on the applicability of radiation, using it now as a converter of ergosterol, present in mushroom bioresidues, into vitamin D_2, with applicability in the panification industry, namely in flours. Beyond using gamma and electron beam irradiation, UV rays are also being tested. Beyond the scientific challenge, research on food irradiation intends to reduce the resistance of the general public has to the consumption of irradiated food, demystifying the diference between irradiated and radioactive.

OUTPUTS

PhD Thesis

[1] Amílcar L. Antonio, Ionizing Radiation Applications for Food Preservation: Effects of Gamma and Ebeam Irradiation of Physical and Chemical Parameters of Chestnut Fruits, University of Salamanca, 2014.

[2] Ângela Fernandes, Comprehensive Study of the Effects of γ-Rays and Electron Beam in Wild Mushrooms: Physico-Chemical, Nutritional and Bioactive Parameters, Universidade do Porto, 2016.

[3] Eliana Pereira, Evaluation of the Effects of Radiation on the Chemical Composition and Bioactivity of the Plants used in the Parmaceutical and/or Food Industries, University of Salamanca, 2017.

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[2] M. Carocho et al., Food Biopro. Technol. 7, 1917 (2014)
[3] Â. Fernandes et al., Food Chem. 159, 399 (2014)
[4] Â. Fernandes et al., LWT-Food Sci. Technol. 60, 855 (2015)
[5] J. Pinela et al., RSC Advances 5, 14756 (2015)
[6] E. Pereira et al., Food Anal. Method 8, 154 (2015)
[7] E. Pereira et al., Food Res. Int. 67, 338, (2015)
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[9] J. Pinela et al., Food Chem. 206, 50 (2016)
[10] Â. Fernandes et al., Food Control 72, 328 (2017)
[11] E. Pereira et al., Ind. Crops Prod. 97, 582 (2017)
[12] E. Pereira et al., LWT-Food Sci. zTechnol. 82, 386 (2017)
[13] E. Pereira et al., Molecules 22, 347 (2017)

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José Pinela | PhD Student

FUNDING

QREN I&DT Co-promotion No. 13198: "CHESTNUTSRAD" – Alternative treatment for chestnut preservation. 1/6/2010-31/05/2013

Eureka Project No. 7596: "CHESTNUTSRAD" – Ionizing radiation as an alternative treatment for preservation of Chestnut fruits. 01/2012-04/2014 (Portugal and Poland).

Research project: "Fostering e-beam food irradiation: Modelling and validation" – IAEA Coordinatd Research Project: D61024 – Development of electron beam and X Ray applications for food irradiation (DEXAFI).

Bilateral action FCT-CNP, Portugal/Brazil – Influence of the radiation process in bioactive compounds in edible flowers. 01/01/2014-31/12/2015.

PRODER project No. 53514: "AROMAP" – Preservation of aromatic condimentary plants. 05/2014-05/2016.

Natural and Bio-based Ingredients

Natural Colouring Agents as an Alternative to the Artificial Additives

KEYWORDS: Natural Colours | Anthocyanins | Betacyanins | Authenticity Studies | Incorporation in Food Matrices

Achievements:

1. Selection of different edible samples of plants, flowers and fruits containing in their chemical composition compounds with colouring ability.

2. Optimization of the extration processes of compounds with colouring capacity.

3. Characterization of the obtained compounds by chromatographic techniques such as HPLC-DAD-ESI/MSn.

4. Incorporation of the obtained extracts/compounds in different food matrices and their stabilization studies.

Objectives

The food industry seeks to satisfy the expectations of the consumer by presenting more and more attractive alternatives that increase the demand and the consumption of its products. Food colorings are recognized as one of the most important additives that attract consumers through visual perception. These are added to foods mainly to: i) make up for colour losses following exposure to light, air, moisture and variations in temperature; ii) enhance naturally occurring colours; or iii) add colour to foods that would otherwise be colourless or coloured differently (EFSA, Food colours). Given that some intolerance and allergic reactions have been associated with the consumption of artificial additives, in recent decades consumers have been choosing to ingest food containing natural alternatives [1,2]. Thus, the main goal of this topic is to try to offer a range of natural alternatives to the current synthetic colourants, obtained primarily from plants, flowers and fruits.

Results

In the recent years the research group has focused its research on this subject [1-4] and has already obtained some results, regarding extracts/compounds with colouring capacity. Among the studied colouring sources stand out the hibiscus flower (*Hibiscus sabdariffa* L.) [5] and the globe amaranth *(Gomphrena globosa* L.) [6,7].

Regarding hibiscus, an hydroethanolic extract and an infusion were prepared from this flower and were tested. Besides its nutritional characteristics, being a source of glucose and fructose, a source of vitamin E (mainly α-tocopherol) and having as main fatty acids palmitic and linoleic acids, hibiscus also revealed to be a good source of interesting pigments, namely anthocyanins (Figure 1).

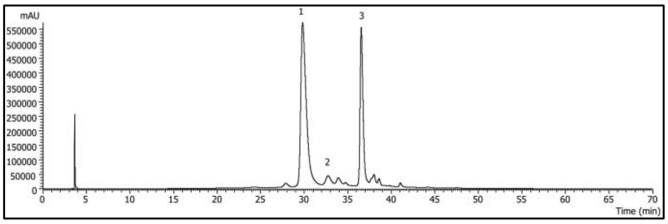

Figure 1. Anthocyanins profile of *Hibiscus sabdariffa* L. hydroethanolic extract, recorded at 520 nm. 1- Delphinidin-3-*O*-sambubioside; 2- Delphinidin-3-*O*-glucoside; 3- Cyanidin-3-*O*-sambubioside.

Regarding the HPLC-DAD-ESI/MSn analysis, for anthocyanin compounds, the solvents used were: (A) 0.1% trifluoroacetic acid in water and (B) acetonitrile. The gradient elution followed these parameters: 10% B for 3 min, from 10 to 15% B for 12 min, 15% B for 5 min, from 15 to 18% B for 5 min, from 18 to 30% B for 20 min, from 30 to 35% B for 5 min, and from 35 to 10% B for 10 min. Sixty minutes was the resulting total run time, followed by column reconditioning of 10 min, using a flow rate of 0.5 mL/min and with an injection volume of 10 μL. With a DAD, data were collected at 520 nm. In a mass spectrometer (MS) detection (Linear Ion Trap LTQ XL mass spectrometer, ThermoFinnigan, San Jose, CA, USA) positive mode was selected. Retention times, UV-VIS and mass spectra were compared with available standards and with literature data, in order to identify the compounds. Calibration curves of available phenolic standards were constructed based on the UV signal to perform quantitative analysis. In the case of unavailable commercial standards, the compounds were quantified via calibration curve of the most similar standard available. After this analysis, the authors concluded that Delphinidin-3-*O*-sambubioside was the major anthocyanin in both extracts.

It is important to highlight that, although being prepared from an edible flower, the cytotoxicity of both studied extracts was assessed in a porcine liver primary cell culture and none of them reveled toxicity. Overall, the studied plant species was considered interesting for colouring applications that can be used in food, cosmetic and pharmaceutical industries.

In another approach, globe amaranth was used with the purpose of extracting betacyanins, betalain pigments that display a red-violet colour that have been reported to be three times stronger than the red-purple-blue dye produced by anthocyanins. On a first attempt, a novel mechanical process for the pigmented flower parts separation was established [6]. This study aimed to optimize the conditions that maximize the extraction of betacyanins from *G. globose* as an alternative source of these pigments. Assisted by response surface methodology, an experimental design was developed for testing the extraction variables (time, temperature, ethanol-water proportion and solid-liquid ratio) and the responses used were betacyanins quantification (by HPLC-PDA-MS/ESI and spectrophotometric analysis) and the extraction-yield and the colour intensity of the produced powder.

The betacyanins identified were gomphrenin and isogomphrenin II and III. The highest betacyanins content was obtained by 165 min, 25 °C, 0% of ethanol and 5 g/L of solid-liquid ratio. The betacyanins content from the floral parts of *G. globosa* is higher than those normally found in other sources, highlighting its industrial application.

In a second phase, microwave (MAE) and ultrasound assisted extraction (UAE) were used to optimize the extraction of betacyanins from *G. globosa*, to replace conventional extraction techniques [7].

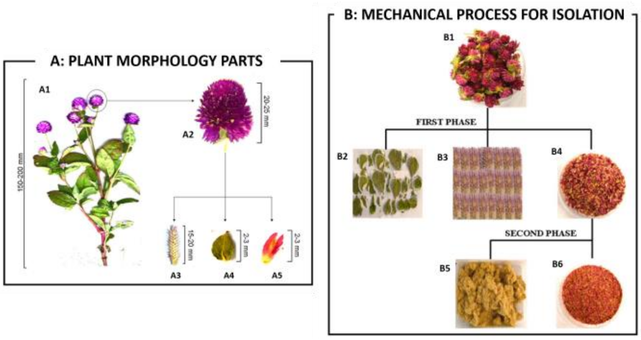

Figure 2. A: Relevant morphology parts of *G. globosa*: A1. *G. globosa* plant with several flower heads; A2. Flower head subtended by bract-like leaf-pair; A3. Head with many flowers shed (bracts persist on rachis); A4. Amplified bract-like leaf-pair; A5. Flower within bract and bracteoles seen from lateral side. B: Mechanical separation process for isolating the pigmented parts (bracts and bracelets) from the flower heads (B1). In a first phase of the separation process the pigmented parts of the flower within bract and bracteoles (B4, 52% of the flower head) are removed from the bract-like leaf-pair (B2, 12% of the flower head) and heads (B3, 36% of the flower head) in a rotary sieve device. Finally, in a second phase, the flowers within bract and bracteoles are blended and sieved (2 μm) to separate the fibrous inner part of the flower (B5, non-pigmented, 66% of fraction B4 and 34% of the flower head) from the pigmented parts (B6, 34% of fraction B4 and 18% of the flower head).

Both processes were optimized by the response surface methodology (RSM) to maximize the recovery of betacyanins from *G. globosa*. A five-level full factorial design of 24 combinations and 4 replicates was implemented for MAE and UAE. UAE shown the best conditions (t (time) = 22 min, P (power) = 500 W, Et (ethanol content) = 0%, and S/L (solid/liquid ratio) = 5 g/L), being in this case the optimal response value of 46.9 ± 4.8 mg/g, which validated UAE as an ideal extraction technique for obtaining the compounds of interest. Therefore, UAE can provide extracts rich in betacyanins and with high potential to be used as natural colourants [7].

After these optimization steps, some food incorporation studies were performed. Extracts from *G. globosa* were used as ice-cream colourants, and the products were compared with those enriched with beet-root extracts (already authorized by the regulatory entities) and commercial betalains [8]. For the assays, four batches were prepared: i) control (ice-cream without colouring agents); ii) ice-cream added with betalain standard (200 mg, *i.e.* ≈46 mg/100 g ice-cream); iii) ice-cream with *G. globosa* extract (670 mg, *i.e.* ≈154 mg/100 g ice-cream); iv) ice-cream with *Beta vulgaris* extract (670 mg, *i.e.* ≈154 mg/100 g ice-cream). The samples were analysed immediately after preparation and after: 15, 30, 45 and 60 days (maximum desired storage limit for this type of ice-cream) of storage at −22 °C. Besides evaluating the colour parameters L^*, a^* and b^*, the nutritional parameters, individual sugars and fatty acids profiles were also studied. These parameters were evaluated throughout time, up to a maximum of 60 days of storage (−22 °C). Betacyanins quantification of each formulation was also performed to determine its maintenance along the storage time. In general, ice-creams prepared with *G. globosa* were similar (considering nutritional, colour, individual sugars and fatty acids profiles) to those including beet-root extract. Additionally, the positive effects induced by the addition of this natural colourant were maintained throughout the storage time, thereby validating the suitability of this alternative plant as a source of food colourants, particularly as ice-cream colourants.

Future Work

In addition to the abovementioned work, the research group is also using some already authorized coloring agents such as curcumin (E100) [9], annatto (E160b) and paprika extract (E160c), in order to enhance their applicability.

Currently, under the scope of projects in partnership with companies in the bakery/pastry industry (*Valor Natural* and *DeCodE*), the group seeks the application of its research, in an attempt to offer a set of new products to the market. Within the scope of the project DeCodE, whose theme is “Bring natural colorants and preservers into a real alternative to artificial additives through a strategy of open data and experience based research”, the group is getting its first results. After optimization of the extraction processes and extract concentrations to be used of the different natural matrices (*i.e.*, elderberry, beet, blackberry and cherry or the petals of hibiscus, as well as the authorized natural colours E100, E160b and E160c), the extracts were incorporated into sugar pastes manufactured in a bakery/pastry company from the region, *TecPan*. The main objective of the addition of colours is also a challenge, since the loss of colour occurs easily due to physico-chemical factors induced by environmental conditions. Therefore, the team continues to do authenticity studies, selecting natural additives for food applications, maily for bakery/pastry mixtures (not only sugar pastes) performing the assays to ensure that the acquired natural additives contain the desired molecules. Moreover, the team is focus on determining the best conditions to ensure the stability of the natural additives, developing stabilization methods to maintain the colouring capacity.

OUTPUTS

PhD Thesis

[1] Custódio Lobo Roriz, Development of a New Colouring Agent from *Gomphrena globosa* L. : Optimization of the Extraction Process, Stability Studies and Proof-of-Concept in a Food Matrix, Universidade Complutense de Madrid/IPB, 2020.

Master Dissertations

[1] Heloísa Helena Scorsato de Almeida, Estudo de Formulações Hidrofílicas do Corante Natural Curcumina para Aplicação em Iogurtes, IPB, 2017.

[2] Filipa Alexandra Pires Fernandes, *Ocimum basilicum* var. *purpureum*: uma Fonte de Compostos Bioativos e Pigmentos Naturais de Interesse para a Indústria Alimentar, IPB, 2018.

[3] Liliana Primo da Silva, Caracterização Química e Bioativas de Frutos de *Rubus ulmifoilus* Schott: Otimização da Extração de um Corantes Natural com Aplicação na Industrial, IPB, 2018.

Selected Publications

[1] M. Carocho et al., Food Chem. Toxicol. 51, 15 (2014)
[2] M. Carocho et al., Trends Food Sci. Technol. 45, 284 (2015)
[3] N. Martins et al., Trends Food Sci. Technol. 52, 1 (2016)
[4] N. Martins et al., Food Funct. 8, 1357 (2017)
[5] I. Jabeur et al., Food Res. Int. 100, 717 (2017)
[6] C.L. Roriz et al., Food Chem. 229, 223 (2017)
[7] C.L. Roriz et al., Ind. Crops Prod. 105, 29 (2017)
[8] C.L. Roriz et al., LWT- Food Res. Technol. 92, 101 (2018)
[9] H.H.S. Almeida et al., Food Chem. 230, 224 (2018)

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FUNDING

Financial Program: PORTUGAL 2020 - SAICT, NORTE-01-0145-FEDER-023289: DeCodE, 2017-2019
Financial Program: PORTUGAL 2020-SI-IDT - Programas mobilizadores, VALOR NATURAL, 2017-2020
FCT Scholarships: SFRH/BD/117995/2016
DeCodE_BPD_032018

Natural and Bio-based Ingredients

Natural Food Preservatives and other Food Conservation Technologies

KEYWORDS: Modified Atmosphere Packaging | Natural Preservatives | Food Incorporation

Achievements:

1. Conservation of foodstuffs with modified atmosphere technology.

2. Conservation of foodstuffs by incorporation of natural ingredients and preservatives.

3. Extension of product shelf-life with alternative conservation.

4. Sustainable and clean technologies for food science.

Objectives

This topic has two lines of research, the development of decontamination/preservation techniques and the valorization of natural resources through the extraction of high value added compounds which act as preservatives and allow the increase of the shelf life of food products.

Regarding the first line, the research group bet on postharvest preservation treatments, including modified atmosphere packaging enriched with N_2 and Ar. Regarding the valorization of natural products as a source of compounds with preservative capacity, the research group seeks to obtain, natural extracts/compounds that can be incorporated in different food matrices in order to preserve their shelf life due to their antioxidant and/or antimicrobial capacity.

Therefore, the main objective in this topic is, through different approaches, to increase the shelf life of foods either by optimizing packaging technologies or by adding added value ingredients of natural additives with preservative capacity.

Results

The growing concerns among consumers about the freshness of the food they consume along with the requirement for minimum food processing has led to the development of new packaging technologies [1].

The use of modified atmosphere packaging (MAP) has greatly expanded during the last years and is now used to extend the shelf-life of a wide range of foods, including minimally processed and ready-to-eat fruits and vegetables. The effect of (MAP) on the postharvest quality of fresh-cut watercress (*Nasturtium officinale* R. Br.) stored at 4 ˚C for 7 days was studied [2]. A portion of watercress was immediately analyzed (non-stored control) and the remaining fresh material was stored packaged under four different atmospheres: i) atmospheric air (control in passive MAP); ii) vacuum; iii) N_2- enriched atmosphere; and iv) Ar-enriched atmosphere. After assessing the effect on individual quality parameters (nutritional and antioxidant activity of the samples), it was possible to conclude that the air was the less efficient atmosphere in preserving quality attributes of the non-stored control samples during cold storage. On the other hand, Ar-enriched MAP was the most suitable choice to preserve the overall postharvest quality. The Ar-enriched MAP was also the best packaging technique for preserving the postharvest quality attributes of buckler sorrel leaves, comparing with air-packaging, vacuum-packaging, and N_2-enriched atmospheres [3]. Considering the preservation of specific bioactive compounds, namely the phenolic profile, the Ar-enriched MAP as well as a simple refrigeration, were the packaging techniques which preserved the initial content of phenolic compounds in watercress stored suring 7 days at 4 ºC [4].

Regarding food additives, considerable improvements have been achieved since their first use back in the 1800's. However, there is some controversy in this area, a widespread distrust among consumers regarding the addition of molecules or extracts towards the food they eat. With this in mind, research and investment has shifted towards natural additives, which also seem to gather a higher appreciation from consumers [5,6]. This research line has also been developed over the last years, and the biochemcore research group has tested various aromatic and medicinal plants as ingredients and natural preservatives in an array of foodstuffs, namely cheese, cottage cheese, bakery products, and biscuits.

Traditional pastry from the northeastern region of Portugal (Bragança) called "económicos" were the incorporated with aqueous extracts of chestnut flowers, which had proved to have remarkable antioxidant and antimicrobial activity, to determine their preserving capacity in foodstuffs [7, 8]. After analysing the effects on the antioxidant profile of these cakes when stored for 15 and 30 days, it was concluded that their functionalization was achieved, and that the chestnut flowers had bennefitial properties, reducing the loss of antioxidant activity over storage time (15 and 30 days) [9]. Concomitantly, a study of the nutritional profile and some bioactive individual compounds like tocopherols and organic acids, in the cakes allowed the determination of interesting conclusions, namely that the chemical profile of the incorporated cakes was maintained over time, while the control samples showed changes in the fatty acids profile, which is an important indicator of spoilage and rancidity of food [10].

Regarding cheese, the Serra da Estrela cheese, made from ewe milk is one of the most famous Portuguese cheeses. It was selected to be incorporated with chestnut flowers, but also with another promising MAP's, like *Melissa officinalis* and *Ocimum basilicum*. A complete and thorough study of these two plants' bioactivities was carried out, namely antioxidant, antimicrobial and antitumor capacity, along with individual compounds [11, 12]. The functionalization of the cheese was better observed when the plants were added directly to the cheese, rather than with aqueous extraction, especially in the lipid peroxidation inhibition assays [13]. In terms of the preservation capabilities over time, the plants also achieved this goal, given that fatty acids and other nutritional aspects were conserved during the storage time of 6 months [14].

In terms of cottage chesse, other plants were studied and incorporated, although the main goal was the same; to functionalize and preserve the foodstuff. Fennel (*Foeniculum vulgare*) and chamomile (*Matricaria recutita*) where the plants used to be incorporated in this dairy delicacy. After 14 days of storage time, the incorporated samples seemed to preserve the nutritional profile much better than the control samples, thus creating a functional diary food that could have a longer shelf-life [15]. Microencapsulated forms of the extracts were also used and proved to have even higher functionalization capabilities, given the gradual release of the bioactive molecules over time, thus maintaining a constant ammount of preserving capacity for longer [16]. Cupcakes were another matrix functionalized with plants, namely with *M. officinalis*. The choice of this plant is related to the high amount of rosmarinic acid, a phenolic compound with high bioactivity, extracted from plants. It is the main component of the approved natural additive E392, rosemary extract. Thus, the addition of a pure extract of rosmarinic acid in one lot and the addition of it through *M. officinalis* in cupcakes, compared to a synthetic preservative (sorbic acid-E200) resulted in very similar preservation pattern. This proves that rosmarinic acid or aqueous extracts of *M. officinalis* can be natural alternatives for preservation, while also being a functionalizing agent [17].

Fig. 1 Cottage cheese incorporated with fennel and chamomile. A contamination is shown on the first picture, the control sample.

Fennel and chamomile extracts were once again used for incorportion in biscuits, to compare their preserving capabilities with butylated hydroxyanisole (BHA – E320) a known synthetic preservative. Interestingly, the results of the natural extracts were very similar to the synthetic one, proving that plant extracts can be a solution for consumers that prefer natural additives or ingredients in their foods [18].

Future Work

Plants, their extracts and their applicability in foodstuffs ad additives and ingredients are endless, thus making the pursuit for the best preservative endless, which gives hope to scientists to continue searching for new, safer , cheaper and more effective preservatives. While the pursuit for these additives is ongoing, other foodstuffs are expected to be tested in the near future with the ones already developed. Although this line of work is still instated in the research group, new methods of conservation are being tested, namely the development of bio-based food coatings, which reduce the processing of food and the dependance of plastic for preservation.

OUTPUTS

PhD Thesis

[1] Márcio Carocho, Developmet of New Food Products by Incorporation of Natural Food Additives. Nutritional, Bioactive and Antitumoral Assays in Foodstuffs, Universidade Complutense de Madrid, 2016.

[2] José Pinela, Recovery of the Sustainable use of Traditional Foods through Emerging Preservation Technologies, Universidade do Porto, 2017.

Master Dissertations

[1] Khira Amara, *In vitro* Production of *Ganoderma lucidum* mycelium from Northeast Portugal: The Antioxidant Potential of Tocopherols Extract in the Preservation of Natural Yogurt, IPB, 2017.

[2] Chaima Bouzgarrou, *Pleurotus* Species as a Source of Natural Preservatives: Mycelia Production to obtain Tocopherols used as Antioxidants in Yogurts, IPB, 2017.

[3] Takwa Sallawi, *Arbutus unedo* L. and *Ocimum basilicum* L. as sources of Natural Ingredients for Bread Functionalization, IPB, 2017.

[4] Cristhian Rafael Lopes Francisco, Micoesteróis como Agentes Funcionalizantes: Ensaios de Modificação Química do Ergosterol, Estabilização de Extratos de Micoesteróes e Desenvolvimento de Aplicações, IPB, 2017.

Selected Publications

[1] M. Carocho et al., Compr. Rev. Food Sci., Food Saf. 13, 377 (2014)
[2] M. Carocho et al., Ind. Crops. Prod. 62, 42 (2014)
[3] M. Carocho et al., Biomed Res. Int. 232956 (2014)
[4] M. Carocho et al., Food Funct. 5, 2989 (2014)
[5] M. Carocho et al., J. Food Comp. Anal. 44, 93 (2015)
[6] C. Caleja et al., J. Funct. Foods, 16, 114 (2015)
[7] M. Carocho et al., Trends Food Sci. Technol. 45, 284 (2015)
[8] M. Carocho et al., Food Funct. 6, 2240 (2015)
[9] M. Carocho et al., Eur. J. Lipid Sci. Technol. 117, 1607 (2015)
[10] M. Carocho et al., Food Chem. 204, 185 (2016)
[11] M. Carocho et al., Food Chem. 207, 51 (2016)
[12] J. Pinela et al., Postharvest Biol Technol. 112, 55 (2016)
[13] J. Pinela et al., J Food Sci Technol. 53, 2943 (2016)
[14] C. Caleja et al., Food Chem. 2016, 342 (2017)
[15] J. Pinela et al., Crit.Rev.Food Sci. Nutr. 57, 2095 (2017)
[16] J. Pnela et al., Food Chem. 254, 70 (2018)
[17] C. Caleja et al., Food Chem. 250, 67 (2018)

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FUNDING

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Natural and Bio-based Ingredients

Natural Matrices as Functional Foods and a Source of Pharmaceutical/Nutraceutical Compounds

KEYWORDS: Natural Matrices | Bioactive Compounds | Functional Foods | Nutraceuticals| Cosmeceutical Formulations

Achievements:

1. Natural matrices, mainly from the region of Bragança, chemically characterized in order to assess the interesting compounds present in these.

2. Optimization of compound extraction techniques with green solvents or solvent-free, such as ultrasound-assisted extraction.

3. Chromatographic techniques optimized to assess the main classes of nutritional and bioactive compounds (*e.g.*, UFLC- Ultra Fast Liquid Chromatography or LC/ESI/MS- Liquid chromatography-electrospray ionisation mass spectrometry).

4. Incorporation of extracts/compounds in food matrices and cosmetics.

Objectives

Natural products are a source of interesting compounds with numerous applications in the industry, such as sugars, vitamins, terpenes, carotenoids, sterols, or phenolic compounds. Over the years, the group has focused its research on obtaining extracts / compounds with biological activity, in order to value the resources of the mountain and to contribute to obtain new functional foods and nutraceutical or pharmacological formulations. Since CIMO is located in a region favored in natural resources, the research group has greatly contributed to the elaboration of in-depth databases on the identification and chemical and bioactive characterization of plant and mushroom species from the region (http://esa.ipb.pt/biochemcore). Moreover, the group has applied its knowledge in the elaboration of new food products and cosmetics. An example is the use of ergosterol and mycosterol extracts to fortify foods and beverages. Furthermore, studies aimed at increasing the incorporation of natural products into cosmetics have been carried out.

Results

Given the in-depth study of hundreds of plant and mushroom species in the region, and in an attempt to compile results for dissemination, the group has published several books/chapters and review articles containing all this information and possible knowledge applications [1-9].

The practical applications of the results obtained in the plant matrices have been applied at several levels, which will be detailed in the following sections. Regarding the matrices of mushrooms, the applications in daily beverages with the intention of fortification and in cosmetics stand out.

The consumption of beverages containing phytosterols has increased since these molecules have been pointed out as cholesterol reducing. Mycosterol extracts obtained from bio-residues from the widely consumed *Agaricus bisporus* (J. E. Lange) Imbach mushroom (champignon) have been explored as an alternative to phytosterol-based beverages [10]. Mycosterol extracts obtained by ultrasound-assisted extraction (UAE) and pure ergosterol were incorporated in daily beverages, at concentrations reproducing commercial phytosterol-added counterparts, and the nutritional and bioactive properties of the final products were evaluated. In addition to the use of residues from the mushroom cultivation and processing industries, returning added value products, in-line with the circular bioeconomy concept, the used extraction procedure is simple to apply, low-cost and avoid purification steps, providing functional ingredients suitable for food and/or beverage applications. The results obtained proved that *A. bisporus* extract seemed to preserve yogurt's protein and lipid fractions, probably due to its antioxidant potential. Besides, sucrose degradation over time also decreased and a lower toxic effect against normal cells was observed in comparison with pure ergosterol (although none of them are toxic). Moreover, yogurts incorporated with 2 g of the *A. bisporus* extract showed a stronger bioactivity than the samples incorporated with phytosterols, meaning that this extract presents high potential for application in the food industry. The authors concluded that mycosterols showed an efficacy very similar to phytosterols, highlighting their use and benefits associated with mushroom-derived sterols. It is also important to highlight that being the extract a mixture of molecules, the developed synergies evidenced beneficial effects over the ascribed bioactivities [10].

Regarding the cosmetics area, topical formulations using mushroom extracts have been developed. In a first approach, mushroom-based cosmeceutical formulations with anti-inflammatory, anti-tyrosinase, antioxidant, and antibacterial properties were developed [11]. The cosmeceutical potential of ethanolic extracts prepared from the mushrooms *A. bisporus*, *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm., and *Lentinula edodes* (Berk.) Pegler were analyzed in terms of anti-inflammatory, anti-tyrosinase, antioxidant, and antibacterial activities. The extracts were characterized in terms of phenolic acids and ergosterol composition, and further incorporated in a base cosmetic cream to achieve the same bioactive purposes. To obtain the extracts, 3.0 g of the dried powder from the mushrooms was mixed with 150 mL of ethanol and extracted in a Soxhlet apparatus for 4 h (12 cycles). Afterwards, the solvent was evaporated to obtain the dried ethanolic extracts. The mushroom extracts showed strong antioxidant activity suggesting that they can cope with oxidative stress, promoting ROS generation and acting as skin anti-aging. The extracts also revealed antibacterial activity against methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA), which are microorganisms found to colonize the skin during injury and inflammation. After incorporating the extracts into the base cosmetic cream, the mushroom cosmetic formulations still display antioxidant and anti-inflammatory activity by inhibition of nitric oxide (NO) production and melanin by suppression of tyrosinase activity. Furthermore, the final cosmeceutical formulations containing the mushroom extracts were found to inhibit important bacteria strains responsible for skin damage. These diverse functions displayed by the mushroom extracts, before and after incorporation in the base cream formulations, suggested that mushrooms contain sustainable bioactive compounds that can be the basis of cosmeceutical formulations capable to tackle skin aging, inflammation, and hyperpigmentation [11]. Still in this line, *Ganoderma lucidum* (Curtis) P. Karst, a very well-known medicinal mushroom was studied regarding its potential for incorporation is cosmetics [12]. *G. lucidum* was characterized in terms of nutritional value and chemical composition. Afterwards, ethanolic Soxhlet extracts were prepared and the antioxidant, anti-inflammatory, antityrosinase, antimicrobial and cytotoxic effects were assessed. Subsequently, the extracts were characterized in terms of phenolic acids, polysaccharides and triterpenoids. Finally, the obtained extracts were tested as cosmeceutical ingredients. The developed cosmeceutical formulation preserved the extract bioactivities, displaying a a light-yellow colour and a pH of 4.6, which is considered appropriate for cosmeceutical design. Behind the important nutritional/bioactive composition of *G. lucidum*, a potential towards its valorisation in the field of cosmeceuticals is foreseeable, as deduced from the bioactivities of its ethanolic extract and preservation in the tested formulation. The presence of different bioactive molecules in the classes of phenolic compounds, terpenes, and specially triterpenes can be correlated with the exhibited bioactivity [12].

Future Work

In the future it is intended to explore the potential of the bio-residues from the food industry, staying in line with the premise of returning added value products. The main goal will be the improvement of vitamin D_2 in mushrooms submitted to gamma and electron beam irradiation, and subsequent optimization and validation of the purification/separation processes to obtain enriched extracts and high purity ergocalciferol. In a second step, novel functionalized cookies will be developed, by direct incorporation of vitamin D_2 or after its stabilization/microencapsulation.

OUTPUTS

PhD Thesis

[1] Sandrina Alves Heleno, Important Human Metabolites of Phenolic Compounds from Diet with Wild Edible Mushrooms: Chemical Synthesis and Studies of their Antioxidant and Antitumor properties, Universidade do Minho, 2014.

[2] Maria José Alves, Potentialities of Wild Mushroom Extracts and Compounds Against Multiresistant Bacteria, Universidade Católica Portuguesa - Porto, 2014.

[3] Carla Pereira, Providing Scientific Evidence for the use of Dietary Supplements: A Case Study with Hepatoprotective Plants, Universidade de Salamanca, 2016.

[4] Filipa S. Reis, Chemical Characterization and Evaluation of the Bioactive Properties of Wild and Cultivated Edible Mushrooms, Universidade Complutense de Madrid, 2017.

[5] Maria Inês Dias, Cultura *in Vitro* e Técnicas de Microencapsulação: Aumento da Produção e da Estabilidade de Compostos Bioativos de Espécies Vegetais, Universidade do Porto, 2017.

Selected Publications

[1] M. Carocho et al., Anticancer Agents Med. Chem. 13, 1236 (2013)

[2] I.C.F.R. Ferreira et al., Special issue BioMed Res. Int. (2014)

[3] L. Barros et al., Springer, ISBN: 978-1-4939-3327-3 (2016)

[4] O. Taofiq et al., Ind. Crops Prod. 90, 38 (2016)

[5] O. Taofiq et al., Molecules 21, 1372 (2016)

[6] I.C.F.R. Ferreira et al. (Ed.), Wiley-Blackwell, ISBN: 978-1-118-94462-2 (2017)

[7] F. Reis et al., Trends Food Sci. Technol. 66, 48 (2017)

[8] O. Taofiq et al., Trends Food Sci. Technol. 70, 82 (2017)

[9] N. Martins et al., Trends Food Sci. Technol. 67, 12 (2017)

[10] R.C.G. Corrêa et al., Trends Food Sci. Technol. 67, 19 (2017)

[11] Sandrina A. Heleno et al., Food & Funct. 8, 103 (2017)

[12] O. Taofiq et al., Food Chem. Toxicol. 108, 139 (2017)

Master Dissertations

[1] Andreia Ribeiro, Extratos Fenólicos de *Suillus luteus* e *Coprinopsis atramentaria*: Avaliação de Sinergismos no seu Potencial Antioxidante e Encapsulação por Spray Drying para Fins Nutracêuticos, IPB, 2014.

[2] Vanessa Vieira, Caracterização Nutricional e Bioatividade de Cogumelos Silvestres: *Lactarius citriolens, Lactarius turpis, Leucopaxillus candidus* e *Morchella conica*, IPB, 2014.

[3] Custódio Miguel Lobo Roriz, *Pterospartum tridentatum* (L.) Willk, *Gomphrena globosa* L. e *Cymbopogon citratus* (DC) Stapf: Fitoquímica e Bioatividade das Espécies Individuais e Sinergismos Resultantes do uso Combinado, IPB, 2014.

[4] Tânia Cristina Pires, Comparação da Bioatividade do Entrecasco e Diferentes Formulações de Pau d'Arco (*Tabebuia impetiginosa* Martius ex DC), IPB, 2014.

[5] Maria Azucena Marques, Avaliação da Bioatividade de Quatro Lenhinas Técnicas: Alcell, Indulin-AT, Sarkanda e Curan 27-11P, IPB, 2014.

[6] Juliana Dias, Novos Métodos de Extração de Compostos Bioativos de Verduras, IPB, 2015.

[7] Oludemi Taofiq, The Contribution of Phenolic Acids to the Anti-inflammatory Activity of Mushrooms: Screening in Phenolic Extracts, Individual Parent Molecules and Synthesized Glucuronated and Methylated Derivatives, IPB, 2015.

[8] Inés Jabeur, The Broad Spectrum of Bioactive Properties of Phenolic Extracts: a Prospective Study in three Different Plants, IPB, 2016.

[9] Adenilson Rudke, Obtenção de Extratos de *Agaricus bisporus* com Ergosterol para Incorporação em Iogurtes e Procedimentos de Microencapsulação, IPB, 2016.

[10] Cristhian Rafael Lopes, Micoesteróis como Agentes Funcionalizantes: Ensaios de Modificação Química do Ergosterol, Estabilização de Extratos de Micoesterões e Desenvolvimento de Aplicações, IPB, 2017.

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FUNDING

FCT/COMPETE PTDC/AGR-ALI/110062/2009 - Portuguese wild mushrooms: chemical characterization and functional study of antiproliferative and proapoptotic properties in cancer cell lines, 2010-2013.

FEDER Interreg Spain-Portugal “IBERPHENOL” - Red Ibérica de investigación en polifenoles, 2014-2020.

FCT Scholarships: SFRH/BPD/72802/2010
SFRH/BPD/68344/2010
SFRH/BPD/101413/2014
SFRH/BPD/114650/2016
SFRH/BPD/114753/2016
SFRH/BPD/122650/2016
SFRH/BD/70304/2010
SFRH/BD/78307/ 2011
SFRH/BD/87658/2012
SFRH/BD/84485/2012
SFRH/BD/93007/2013
SFRH/BD/92994/2013
SFRH/BD/111753/ 2015
SFRH/BD/108487/2015
SFRH/BD/129551/2017

Natural and Bio-based Ingredients

Beekeeping Technology: Valorization of Honey Bee Products and Development of Sustainable Innovative Productions

KEYWORDS: *Apis mellifera iberiensis* | Honey Bee Products | Authenticity | Bioactive Properties | Production Mode and Tools

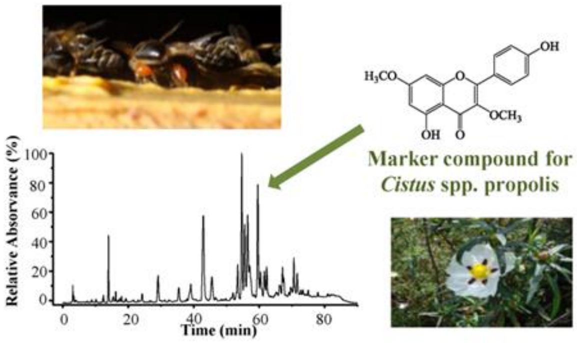
Achievements:

1. The characterization of honey bee products towards its quality evaluation, botanical origin and authenticity

2. The study of the phenolic and volatile profile of Portuguese honey bee products through chromatography and mass spectrometry. Discovery of markers compounds, and definition of national standards to establish geographical authenticity

3. The evaluation of the bioactive properties of the different honey bee products

4. The improvement and development of management tools and equipment's to enhance bee products production



Objectives

Bees have been in existence for over 125 millions of years and their evolutionary success has allowed them to become perennial species that can exploit virtually all habitats in the world. This success in the animal kingdom is largely because of the chemical ecology and functionality of its bee products: honey, beeswax, venom, propolis, pollen and royal jelly. For this reason, bee products became the subject of intense pharmacological and chemical studies over the last years. Thus, one of the main objective in this topic is to perform extensive chemical characterization of honey bee products, creating quality standards, promoting its potential applications and thus adding commercial value. At the same time, there is a need to develop the production process with the introductions of innovative tools and technics, able to follow the demands on quality and sustainable products and to deal with the constant modifications on honey bee management due to climate changes.

Results

Propolis is a resinous substance collected by honey bees *Apis mellifera* from several plant sources and used in the hive to seal the walls, to strengthen the ends of the honey comb or embalm dead invaders. After an extensive characterization of propolis from six different Portuguese regions one of the most important results was to define quality and biological activity criteria, Table 1, allowing the association of this beehive product with its phytogeographic origin and thus contributing for its standardization and commercial valorization. The evaluation of the physicochemical parameters, total phenolic composition and bioactive properties enabled the definition of two distinct types of propolis in Portugal, which are linked to its geographical origin, Table 1. The first, typical of temperate zones from poplar, included the majority of the north, central coast and Azores archipelago samples, and reveal high phenolic content and bioactivity. The other, comprising the majority of the central interior, south and Madeira Island samples, presented a different colour, a higher amount of wax, lower phenolic content and less bioactivity. The phenolic profile identification, performed through electrospray ionization coupled to mass spectrometry (ESI-MS), electrospray ionization coupled to tandem mass spectrometry (ESI-MS^n) and liquid chromatography with diode-array detection coupled to electrospray ionization tandem mass spectrometry (LC/DAD/ESI-MS^n), allowed the identification of seventy six phenolic compounds within the propolis samples and the discrimination between two groups of propolis types: the common temperate propolis, which contained the typical poplar phenolic compounds such as flavonoids and their methylated/esterified forms, phenylpropanoid acids and their esters and an uncommon propolis type with an unusual composition in quercetin and kaempferol glycosides some of them never described in propolis. The phenolic composition of the bud exudates of *Populus x canadensis* and the resins of *Cistus ladanifer*, two potential propolis sources in Portugal, were also evaluated and used as an indicator for propolis botanical origin. The phenolic profile of Poplar was comparable to the one observed in Portuguese common temperate propolis type earlier described through the analysis of the exudates of *Cistus ladanifer*, only flavonoids were detected, mainly kaempferol derivatives. These rich composition was also present in some central interior and south samples, standing out the compound kaempferol-3,7-dimethyl-ether which was absent in the other propolis samples with a poplar type composition and therefore could be considered as a marker compound for *Cistus ladanifer* propolis.

Table 1. Standard proposal for Portuguese propolis

	Propolis Type I	Propolis Type II
Ash content (%)	Max. 2	Max. 4
Wax content (%)	Max 25	Max. 31
Water Content (%)	Max. 5	Max 5
L^*	Min. 40	Min.23
a^*	Min. 2	Min. 1
b^*	Min. 23	Min. 4
C^*ab	Min. 23	Min 4
Balsam content (%)	Min. 65	Min. 45
Total phenolics (% in raw propolis)	Min. 18	Min. 6
Flavones/flavonols (% in raw propolis)	Min. 3	Min. 2
Flavanones/dihydrofavonols (% in raw propolis)	Min. 5	Min. 3
EC_{50} DPPH· (mg/mL)	Max. 0.02	Max. 0.06
Reducing power (mg/g extract)	Min. 600	Min. 96

Concerning honey, a natural product produced by *Apis mellifera* bees from the nectar or secretions of plants, a volatile profile, as a quality parameter of honey, was established for the two most representative Portuguese honeys, the *Lavender* spp. and *Erica* spp. honeys. Volatiles may arise from the nectar source, from the transformation of plant compounds by the honeybee, directly generated by honeybee, from heating or handling during honey processing and storage or from microbial or environmental contamination. For that, eighteen samples from both lavender and heather honey, were analyzed. Volatiles were sampled by headspace solid phase microextraction (HS-SPME) using a 65 μm polydimethylsiloxane divinylbenzene (PDMS/DVB) fiber. The chemical identification was performed by gas chromatography-mass spectrometry (GC-MS). A complex total ion chromatogram was obtained, with nearly seventy compounds identified and quantified. Aldehydes and terpenic derivatives were the most likely to relate honey to its floral origin, being phenylacetaldehyde and nonanal the most representative in lavender honey while hotrienol was the most abundant in the heather honeys, Figure 1. The above methodology was suitable for the isolation of low-molecular-weight aroma compounds, particularly for the short-chain aliphatic compounds that are important for authentication of lavender and heather honey.

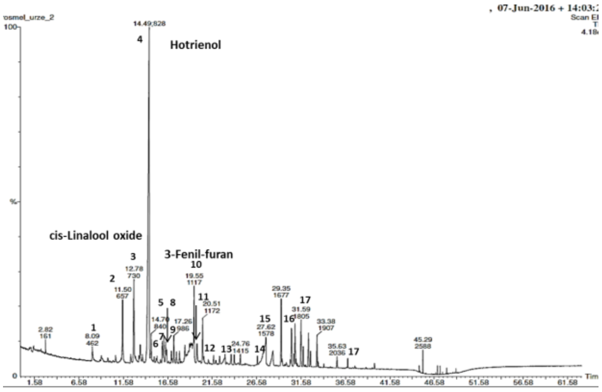


Figure 1. GC-MS volatile profile of *Erica* spp. honey.

Beebread is a fermented mixture of plant pollen, honey, and bee saliva that worker bees use as food for larvae, and for young bees to produce royal jelly. The potential use of beebread as a food supplement and nutraceutical greatly depends on its chemical richness, which varies upon the flora diversity of the region and the season of pollen collection by the bees. The evaluation of this bee product as a food supplement and source of nutraceuticals, was achieved on beebread produced in the northeast region of Portugal. The analysis focused on pollen composition, nutritional parameters (moisture, ash, fats, proteins and sugars), the presence of vitamin E, phenolic composition, and its bioactivity. Beebread proved to be a hive product with a rich composition in proteins, carbohydrates and tocopherols and thus with high nutritional potentialities for the consumption as food supplement. The sugar composition of this bee product is rich in fructose and glucose, resembling the honey sugar profile. Moreover, its bioactivity, due to the rich composition in phenolic compounds, makes this product a good complement in human diet as a functional food.

Bee venom or apitoxin is a complex mixture of substances with reported biological activity. Through the chemical characterization, using LC/DAD/ESI-MS^n analysis, of bee venom samples obtained from *Apis mellifera iberiensis* from the Northeast Portugal it was possible to observe that melittin peptide was the most abundant compound, followed by phospholipase A2 and apamin. All the samples revealed antioxidant and anti-inflammatory activity but without a direct relation with any of the individual chemical components identified. The results highlight that there are specific concentrations in which these compounds are more active. The BV samples showed similar cytotoxicity for all the tested tumour cell lines, nevertheless, it seem to be suitable to treat breast, hepatocellular and cervical carcinoma since the samples were not toxic for non-tumour cells (PLP2) at the active concentrations. Regarding the non-small cell lung carcinoma, BV should be used under the toxic concentration for non-tumour cells. Overall, the present study corroborates the enormous bioactive potential of BV being the first report on samples from Portugal. have good potential for this difficult separation problem.

Beekeeping activity faces today several threats associated with massification of agriculture activities and constant changes on climate. Those factors impact negatively on the honey bee performance through the introduction of external stressors such as new pest and diseases, environmental contaminants, weather constrictions and food starvation periods, among others.

The use of essential oils from Portuguese native flora was explored as a potential source of acaricides against *varroa* mite. Several bioassays with oils of *thymus, lavandula, lemon grass* and *rue* revealed promising potential with acaricide activity and limited toxicity on bees. This is also an opportunity to enhance the available acaricides in accordance with organic production principles.

Future Work

In the last decade, beekeeping in Portugal began an important professionalization process, thus reducing the recreational aspect of the activity. Consequently, economic exchanges with the outside increased significantly, but still confined to honey. This handicap is even stronger to the reduced scientific information on the quality of other national bee products, in contrast to the amount of international information. Currently, the work has been focused on diversification and characterization of honey bee products towards its quality evaluation, botanical origin and authenticity with the final goal of improve its commercial value. Also, the improvement and development of management tools and equipment's to enhance bee products production in Portugal, which is at an early stage of development, it is an important aspect to obtain high quality products and thus high added value in the business markets.

OUTPUTS

PhD Thesis

[1] Soraia Isabel Domingues Marcos Falcão, Chemical Composition of Portuguese Propolis. Bioactive Properties, PDQ, FCUP, UP, 2013.

Master Dissertations

[1] Andreia Vanessa Ferreira Tomás, Beebread from the Northeast of Portugal: Chemical and Nutritional Composition, and Antioxidant Activity, MFQPN, IPB, 2013.

[2] Joana Patrícia Mendes Coelho, Identification and Quantification of Phenolic Compounds in Propolis from South of Brazil. Evaluation of Antioxidant Activity by Electrochemical and Spectroscopic Technics, MFQPN, IPB, 2013.

[3] Melissa Andrea Lopes, Quality of Bee Products from Guinea-Bissau: Honey and Propolis, MFQPN, IPB, 2014.

[4] Gomes da Silva Silveira Cahango, Impact of the Geographical Origin of Honey Bees in the Quality of Propolis, MQSA, IPB, 2017.

[5] Elsa Marisa Silva Caveiro, Caracterization of Commercial Honeys Labelled as Heather Honey, MQSA, IPB, 2017.

Selected Publications

[1] S. I. Falcão et al., J. Am. Oil Chem. Soc. 90, 1729 (2013)
[2] S. I. Falcão et al., Ind. Crops Prod. 49, 805 (2013)
[3] S. I. Falcão et al., Phytother. Res. 28, 437 (2013)
[4] S. I. Falcão et al., Phytochem Anal. 24, 309 (2013)
[5] R. C. Calhelha et al., Biomed. Res. Int. DOI: 10.1155/2014/897361 (2014)
[6] S. I. Falcão et al., Rec. Nat. Prod. 10, 176 (2015)
[7] F. Sobral et al., Food Chem. Toxicol. 94, 72 (2016)
[8] S. I. Falcão et al., Eur. Food Res. Technol. 242, 1393 (2016)
[9] A. Tomás et al., J. Apic. Res. 56, 219 (2017)
[10] J. Coelho et al., J. Apic. Res. 56, 21 (2017)
[11] V. Bankova et al., J. Apic. Res. 59, 1 (2017)
[12] M. Lopes et al., J. Apic. Res. 57, 406, (2018)

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