Aluno: Alexandra Sofia Moreira Amendolia da Costa Maia

Orientador: Prof.ª Doutora Paula Maria Lima Castro

Co-Orientador: Prof.ª Doutora Maria Elizabeth Tiritan; Prof.ª Doutora Quezia Bezerra Cass

Tema da Tese: Fluoroquinolone antibiotics in environment: biodegradation studies and presence in several environmental matrices

Sumário (máx. 3000 carateres, incluindo espaços)

Fluoroquinolones (FQ) are a class of antibiotics widely prescribed in both human and veterinary medicine. Due to its halogenated characteristics, ecotoxicity and persistence, a special importance is given to their occurrence in the environment. FQ presence has been described in many environmental matrices, but few studies were done in Portugal regarding environmental fate and occurrence. This project aims to develop liquid chromatography methods hyphenated to fluorescence (LC-FD) and mass analyzer (LC-MS/MS) able to quantify FQ residues in different environmental matrices, such as influents and effluents of waste water treatment plants (WWTP) in the north region of Portugal, and to allow further research in biodegradation and environmental fate. The identification of metabolites/transformation products is often neglected in monitoring studies. As the metabolites/transformation products may present higher ecotoxicity than the parent compound, the identification of metabolites produced during the degradation processes is of this project.

While following degradation study of four fluoroquinolone antibiotics, namely Ofloxacin (OFL), Norfloxacin (NOR), Ciprofloxacin (CPF) and Moxifloxacin (MOX), by a mixed bacterial culture, the formation and accumulation of several metabolites/transformation products was observed, suggesting an incomplete mineralization. Some of these intermediate compounds were identified by LC-MS/MS and quadrupole time of flight mass analyzer (QqTOF MS). Most of the intermediates had been reported as biodegradation and/or photodegradation products in different conditions; however unknown metabolites were also identified.

A LC-FD method is being developed in order to determine the enantiomeric fraction of a chiral fluoroquinolone, OFL, during biodegradation by activated sludge. The chromatographic separation is being optimized using a chiral column Chirobiotic ™ R, 5 µm (15 cm x 2.1 mm). This column uses macrocyclic glycopeptides as chiral selectors, namely Ristocetin A that has been covalently bonded to the silica support. The optimization is currently ongoing using reversed phase mode with a mobile phase consisting in 0.45% triethylamine in water (pH = 3.5, adjusted with acetic acid) as the aqueous eluent, and ethanol as the organic modifier. This method will be thereafter adapted to the quantification of ofloxacin in environmental matrices.

Future work will include the study of the enantioselective degradation of OFL. For that the biodegradation assays with racemic form OFL and the pure enantiomeric form levofloxacin, (*S*)-enantiomer, will be performed using activated sludge from a WWTP and using pure cultures isolated in the lab. The enantioselective toxicity of ofloxacin and degradation products will also be evaluated using sensitivity tests and aquatic organisms.

Aluno: Ana Isabel Teixeira Carvalheira

Orientador: Doutora Paula Teixeira

Tema da Tese: *Acinetobacter* and public health: risks posed by strains isolated from community and hospital

Sumário (máx. 3000 carateres, incluindo espaços)

Acinetobacter spp. are ubiquitously distributed in nature. In the past, these microorganisms were considered saprophytes of little clinical importance. However the occurrence of nosocomial infections caused by *Acinetobacter* spp., mainly *A. baumannii*, has increased rapidly and many outbreaks have been reported worldwide representing an important public health issue. There have been reports of panresistant *A. baumannii*, which are resistant to every marketed antibiotic. Several studies have been published on the occurrence of *Acinetobacter* spp. in clinical specimens and hospital environments, with the main focus on a restricted group of species within this genus (namely *A. calcoaceticus – A. baumannii* complex*)*. However, information about environmental and food sources of the different species of this organism is scarce, and the mechanisms of transmission and spread between community and hospital have not been explored.

The aim of this study is to identify and characterize *Acinetobacter* spp. from food both community and hospital origin, specifically: assess the most prevalent species; genotypic characterization of isolates from different origins; and evaluation of resistance to antibiotics.

The presence of *Acinetobacter spp.* was evaluated in 42 samples of lettuce; 93% were contaminated with this organism. A total of 146 isolates of *Acinetobacter* spp. were recovered and subtyped by PFGE (*Pulsed Field Gel Electrophoresis*). One isolate representing each PFGE pattern obtained for each sample was selected for species identification. The predominant species recovered were *A. calcoaceticus* (37.8%), *A. johnsonii* (28%) and *A. guillouiae* (13.4%). Other species such as *A. pittii* (4.9) and *A. baumannii* (3.7%), with clinical significance, were also found. The disc diffusion method was used to evaluate the resistance patterns of the recovered isolates to the following antibiotics: imipenem (10 µg), meropenem (10 µg), amikacin (30 µg), tobramycin (10 µg), tetracycline (30 µg), minocycline (30 µg), piperacillin (100 µg), piperacillin-tazobactam (100/10 µg), ampicillin-sulbactam (10/10 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/ 23.75µg). It was observed higher resistances to piperacillin (93.9%), piperacillin-tazobactam (83%), ceftazidime (53.1%), imipenem (23%) and meropenem (10.2%).

Fifty-five clinical isolates (2 *A. pittii* and 53 *A. baumannii*) were also subtyped by PFGE and 20 different PFGE patterns were observed. The resistance of these clinical isolates to the same set of antibiotics were also tested and it was observed the resistance to all the antibiotics tested, with higher resistances to piperacillin (100%), piperacillin-tazobactam (95%), ciprofloxacin (85%), trimethoprim-sulfamethoxazole (75%), imipenem (65%) meropenem (65%) and ceftazidime (65%).

Aluno: Ana Lúcia da Silva Oliveira.

Orientador: Professora Doutora Maria Manuela Pintado.

Tema da Tese: Preservation of nutritional and functional properties of fresh and processed fruit with emphasis on phytochemicals.

Sumário (máx. 3000 carateres, incluindo espaços)

Fruit are an essential part of a healthy diet and vehicle of a number of unique health promoting phytochemicals. Being very perishable, fruit require preservation, both as fresh or processed foods. From nutritional and functional point of view is important to understand the consequences of processing in food composition in order to select the best technological conditions required for the preservation of fruit health benefits. Processing of fruits may entail different phases that naturally affect the nutritional and functional quality of the final product.

The general objective of this Ph.D. programme is understand the effect of factors related to storage and with fruit processing on the nutritive and functional properties of fruits — strawberry and peach. This information will be integrated to optimize processing conditions that maximize the content of beneficial antioxidants compounds aiming the development of functional food with specific properties.

Experiments developed until now have involved the study of pasteurization, matrix pH modification, modified atmosphere, freezing during strawberry and peach storage. Additionally, the interactions established between fruit compounds and food matrix (yoghurt) throughout shelf-life was also studied.

The studies performed during the last year were focused on protein-polyphenol-polysaccharide interactions. Fruit polyphenols when incorporated in yoghurt matrix can interact with milk proteins namely β-lactoglobulin and with polysaccharides typically added to fruit formulations as stabilisers. In order to better understand these interactions, determinations by dynamic laser light scattering (DLS), dynamic interfacial tension, surface dilatational properties and HPLC-DAD were made in order to evaluate the development of potential complexes between (+)-catechin/cyanidin-3-glucoside with β-lactoglobulin and pectin/chitosan.

These mixtures can limit polyphenol bioavailability and their potential as antioxidant compounds. However, there are some evidences revealing the protective effect of proteins and polysaccharides, on polyphenol degradation. To determine that, the mixtures of polyphenol:protein:polysaccharide were subjected to simulated gastrointestinal system and polyphenol bioaccessibility determined.

Based on these results the industries can predict and improve the impact of these factors on the functional properties of processed strawberries and peaches.

Aluno: Ana Rita Boura Varela

Orientador: Dr.ª Célia Manaia (ESB-UCP), Dr.ª Olga Nunes (FEUP)

Tema da Tese: Tracking antibiotic resistance from hospital effluents to the surrounding environment.

Sumário (máx. 3000 carateres, incluindo espaços)

Thesis chapter: Genetic characterization of fluoroquinolone resistant *Escherichia coli* from urban streams and municipal and hospital effluents

Escherichia coli isolates with reduced ciprofloxacin susceptibility, collected from urban streams, wastewater treatment plants and untreated hospital effluent, between 2004 and 2012, were characterized based on multilocus sequence typing (MLST), plasmid replicon typing and detection of quinolones and beta-lactam resistance genes. Isolates from all water types and sampling periods clustered together, suggesting that some *E.coli* lineages are stable in the in the environment and can propagate through distinct water compartments. The most prevalent lineages belonged to sequence type (ST) ST10 complex (n=13) and ST131 (n=7), reported frequently in clinical and/or veterinary context. Almost all isolates (98 %) carried mutations in the genes *gyrA* and/or *parC* in the quinolone resistance determining region (QRDR), and 10 % possessed plasmid mediated quinolone resistance (PMQR), specifically *qepA*, *aac(6')-Ib-cr* and/or *qnrS1.* PMQR genes were only observed in isolated recovered under selective pressure. The most prevalent beta-lactamase encoding genes were bla_{TEM} followed by $bla_{CTX\text{-}M\text{-}15}$, co-existing with plasmid mediated quinolone resistance. Over 80% of the isolates were resistant to more than three antibiotic classes and harbored more than one plasmid replicon type. Plasmid replicon types integrated in the group incF were the most prevalent and distributed by different MSLT groups, although were not observed to be co-transferred with PMQR. Indeed, the genes *aac(6')-Ib-cr* and/or *qnrS1* could be transferred by conjugation in combination with the genes bla_{TEM} or $bla_{SHV\text{-}12}$ and the plasmid replicon types I1-Iγ, K and/or HI2. This study demonstrates that determinants of antibiotic resistance from the clinical environment have successfully spread to the environment and can persist over time. The risks of transmission back to humans are not known, but should not be neglected.

Aluno: Carla Sofia Sancho dos Santos

Orientador: Doutora Marta Vasconcelos / Prof. Doutor António Osmaro S. S. Rangel, Doutora Susana Carvalho

Tema da Tese: IDC: Unravelling the Molecular and Physiological Components that Contribute to Iron Deficiency Chlorosis

Sumário (máx. 3000 carateres, incluindo espaços)

Iron Deficiency Chlorosis (IDC) is a serious environmental problem affecting the growth of several crops in the world. Legumes, such as soybean (*Glycine max* L.), are particularly susceptible to IDC when grown in alkaline soils. Several strategies have been attempted in order to prevent and correct iron (Fe) deficiency in plants, but these are not economically feasible. Soil application of synthetic Fe(III)-chelates remain the most practical measure for avoiding IDC and the search for alternative and more effective chelators should be considered. Chelators of the 3-hydroxy-4-pyridinone (3,4-HPO) type are nontoxic ligands with high affinity for iron and are utilized in biomedical applications. To the best of our knowledge, these chelators have not yet been used to provide Fe complexes to plants. Herein, we analysed the applicability of two (3,4)-HPO Fe complexes as novel IDC correctors and compared them to the commercial fertilizer Fe(III)EDDHA. Plants treated with the (3,4)-HPO Fe complexes were significantly greener and healthier, with 29% higher chlorophyll content, 22% and 27% bigger shoots and roots, respectively, and a 29% increase in total leaf area. Total Fe content was measured using ICP-OES and whilst plants with (3,4)-HPO iron complexes accumulated about 187 µM, plants treated with the commercial chelator accumulated 124 µM. These results point to use of (3,4)-HPO Fe chelates as promising Fe fertilizers, and research is being conducted to understand how these products interfere with plant Fe metabolism.
Future work will focus on analyzing plants IDC responses at a metabolomic level.

Aluno: Dália Rosa Alves Carvalho

Orientador: Susana Carvalho / Marta Vasconcelos, Ep Heuvelink

Tema da Tese: Understanding and enhancing potential vase life of Rosa hybrida: from preharvest conditions to post-harvest longevity

Sumário (máx. 3000 carateres, incluindo espaços)

Water stress reduces longevity of cut flowers especially in plants grown at high relative air humidity (i.e. RH > 85%) mainly due to a poor stomatal functioning associated to lower abscisic acid (ABA) concentrations. However, the reasons behind this reduced longevity and possible ways to solve it are not yet identified. The main objective of this thesis is to understand the physiological and genetic mechanisms involved in the reduced longevity of plants grown under high RH.

So far, two strategies have been tested to improve stomatal functionality: (1) increased air movement (MOV) and (2) increased salinity (EC) in the irrigation solution. It was concluded that MOV increases stomatal functionality by enhancing stomatal sensitivity to ABA rather than increasing leaflet ABA concentration (presented in 2012), and that increased EC within the studied range can partly counteract the negative effect of high RH on stomatal functioning (presented in 2013).

This year, the focus will be on the most recent work aiming at understanding whether rose cultivars with contrasting tolerance to RH show different gene expression mainly related to ABA metabolism, and to identify genomic loci (QTLs) that may be related to the higher tolerance trait. Four genotypes from a cut rose segregating tetraploid population, with contrasting stomatal function in response to leaf desiccation (K023, P867, P540, K099, by increasing order of tolerance) were grown at moderate (60%) and high (90%) RH. RNA was isolated from fully developed leaves, harvested 10 and 180 min after the beginning of the dark and light periods and the relative expression was evaluated in genes involved in: (i) biosynthesis (*AAO3* and *NCED1*), oxidation (*CYP707A1* and *CYP707A3*) and conjugation (*UGT75B2*) of ABA; (ii) mechanism between ABA perception and reactive oxygen species (*OST1*); (iii) ABA responsive genes (*ABF3*); and (iv) tolerance to water stress (*DREB1B* and *Rh-APX1*). At 180 min after the beginning of the light period, six out of the nine studied genes (*AAO3*, *CYP707A3*, *UGT75B2*, *OST1*, *ABF3*, *Rh-APX1*) were significantly affected by the high RH depending on the genotype revealing a contrasting genotypic sensitivity to the high RH during growth. Since *DREB1B* was neither affected by the RH or the genotype we believe that its expression dynamics does not explain contrasting genotypic tolerance to high RH. We expect that after transferring the plants to the postharvest conditions, inducing water stress, a more contrasting gene expression among genotypes and RH levels will appear. Moreover, QTLs related to water loss tolerance were identified and mapped in both parents on linkage groups (LG) six and four, and several gene sequences were identified in these loci that may be associated with tolerance to high RH.

Aluno: Helena Maria Gomes Moreira

Orientador: Paula Maria Lima Castro

Co-Orientadores: António Osmaro Santos Silva Rangel e Ana Paula G. C. Marques

Tema da Tese: Investigations into the role of soil microorganisms in the re-vegetation of environmentally disturbed sites as a tool for land use restoration and biomass production

Sumário (máx. 3000 carateres, incluindo espaços)

Soil has been continuously degraded worldwide mainly as a result of anthropogenic activities such mining and other industrial activities, contributing for the increasing of hazardous pollutants rendering extensive land areas useless for e.g agriculture purposes. Among the pollutants, metals pose serious risks to living organisms, including humans, because of their non-degradable nature, tending therefore to accumulate in the environment. Moreover, without a vegetation cover, erosion and off-site pollution can be significantly enhanced inducing regional and global contamination. Phytomanagement appears as a feasible solution to lighten the risks posed by the presence of heavy metals in soils using plants envisaging both pollution attenuation and land valorization. Regarding this, and particularly in non-severely contaminated land, biomass based products can bring important incomes for the lands owners. A combination of different biotechnology approaches such as the use of energy crops and plant growth promoting rhizobacteria (PGPR) can improve the success of remediation strategies. Energy maize gathers the characteristics that should be present in an ideal energy crop, such as high yield, low cost and low energy input and can withstand high levels of heavy metals, including Cd and Zn. The PGPR represent efficient plant allies in metal contaminated land, improving their health and their establishment in those areas. These rhizobacteria produce substances that alleviate plant stress such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, promote plant growth due to the production of phytohormones and facilitating the nutrient uptake. Also, they play an important role on the increment of the metal bioavailability in the rhizosphere which also may increase metal uptake by the plants. Levels of inoculated PGPR may affect their efficiency in field conditions, especially when concerning metal-polluted soils. Therefore, an appropriate formulation has to be taken into account to increase plant´s productivity. The objective of the work was to assess the ability of five metal resistant PGPR to promote growth and metal accumulation in maize plants growing in a metal-contaminated environmental soil collected from a mine, using two different quantities of inoculums (10 and 20 ml). Generally bacteria increased shoot and root biomass, especially strain P. reactans EDP 28 for both inoculum volumes applied. Bacterial inoculation decreased Zn concentration in roots but generally increased Zn shoot accumulation. However, a different trend was observed especially when the volume applied was 20 ml both in roots and shoots with an increase of Cd accumulation in plant tissues. Soil bioavailable Zn was also increased by bacterial inoculation but especially for a volume of 10 ml of bacteria. Generally results showed that bacterial maize inoculation can increase the effectiveness of remediation techniques approaches. However, the volume of inoculums applied seem to have a more pronounced effect on Cd accumulation.

Aluno: Joana de Freitas Salgado do Fundo

Orientador: Cristina L.M. Silva / Mafalda A. C. Quintas

Tema da Tese: A systematic approach to the study of physical properties and stability in food systems – the relationship with molecular mobility.

Sumário (máx. 3000 carateres, incluindo espaços)

Food physical properties are critical for product and process design, safety and sensorial attributes. Food scientists often collide with difficulties in comparing data obtained by different experimental methods, and few studies compare different food systems data. The main objective of this project was to contribute to clarify the influence of molecular mobility on the physical properties of food systems. For that, chitosan/ glycerol films (with different chitosan/ glycerol and water concentrations) and fresh-cut fruits (melon and pear) were used as samples. Micro and macroscopic behaviours were analysed, by means of assessing texture, dynamic linear viscoelastic behaviour and thermodynamic transitions. Molecular mobility was evaluated by means of Nuclear Magnetic Resonance.

Another important goal of this project was to identify the "baseline" mobility for stability in high water content food products. Therefore, the molecular mobility and quality factors of fresh-cut melon and pear, along shelf-life, were compared. Due to fruits cellular structure, water can be present in both intra and extra cellular spaces, and this influences the behaviour of water mobility.

In relation to chitosan / glycerol films experiments, NMR measurements showed two different behaviours for the two analysed components, water and glycerol: while glycerol is mainly bounded to the chitosan chain network, the water present in the system is predominantly free from the polymeric chain. Water content and a_w measurements allowed concluding that not only the water content affects the water mobility, but also structural differences in the film may influence the water relaxation times. Also, at room temperature, molecular mobility decreases while glass transition temperature increases, according to classic polymer theory.

Differences along storage life are observed for fresh-cut melon and pear. However, for both products, NMR demonstrated that the distribution function for T_2 presents one peak corresponding to cells total water content. The peak position (T_2) decreased in the first storage day. This indicates an increase in biochemical reactions and water-solutes bonds. Analysed quality parameters showed a close relationship with the value of T_2, where the distribution function is maxima. However, the two fruits presented different behaviours. These relationships are explained by several phenomena, such as loss of membrane integrity, disruption of cellular structures, leakage of cellular osmotic solutes into the apoplastic space, and alterations enhanced by processing-related wounding. The differences found, between melon and pear, are explained by differences in the biochemical mechanisms responsible for alterations in colour and firmness and also by structural characteristics, as confirmed by microscope images.

As future work is the thesis conclusion.

Aluno: Joana Inês Bastos Barbosa

Orientador: Paula Cristina Maia Teixeira

Tema da Tese: Production of orange juice powder, with a probiotic culture incorporated, by spray-drying

Objective

The use of probiotics as food supplements is increasing, because of their health benefits, as well as the increased diversity in food choices they provide. Producing an orange juice with prebiotics and probiotic bacteria can be an innovative way to increase this diversity, especially among consumers who preferred functional non-dairy based foods.
The objective of this study involves the production of an orange juice powder containing a probiotic culture, using three different drying techniques: spray drying, freeze drying and hot air drying, in order to evaluate the drying process for which the survival of the probiotic was the best during drying and storage (at room temperature and 4 ºC).

Results

The technique of hot air drying was the only one that showed a reduction in the number of cells of the probiotic during the drying process.
During storage differences were observed between the cultures used in the experiment (*Lactobacillus plantarum* and *Pediococcus acidilactici*), being *P. acidilactici* more resistant, since for all three techniques showed the smallest logarithmic reductions.
With the results obtained, it was possible to prove that spray-drying is a technique that allows the creation of a new functional dried product with both pre and probiotic characteristics.

Future Work

The identification and quantification of proteins, in order to obtain an intracellular proteins profile from the cells exposed to various stress conditions, as the drying process itself. Also the sensorial analysis of this new product is a goal to achieve.

Aluno: Maria Manuela Faria Amorim

Orientador/ Co-orientador: Manuela Pintado/Hélder Pinheiro

Tema da Tese: Development and Characterization of functional ingredients through valorization of spent brewer yeast: peptide concentrates and ß-glucans with biological activities.

Sumário (máx. 3000 carateres, incluindo espaços)

Spent brewer's yeast (BY) (*Saccharomyces cerevisiae*), it's a by-product of the brewing process, and is sold as animal feed at very low price or disposed as waste. However, this by-product contains valuable components, e.g. structural cell wall polymer β-glucans and proteins that, besides its nutritional value, may present several bioactive properties: antihypertensive, antioxidant, immunostimulating, and prebiotic activities. Thus, the main objective of this work was to obtain new functional ingredients (peptide concentrates and β-glucans), and characterize its composition and biological activities, to assess its potential in the development of new functional foods.

In order to obtain peptide and polysaccharide enriched extracts, the BY (provided by Unicer) was initially submitted to autolysis. Afterwards, the mixture was concentrated by ultrafiltration with a 10 kDa cut-off membrane and hydrolyzed with proteases from extract of *Cynara cardunculus*. Retentate and filtrate were nanofiltrated with 3 kDa cut-off membrane, and resulting extracts concentrated by reverse osmosis and dried by freeze drying. Herein, only results related with beta-glucans fractions will be presented.

In order to study the structural features of BY glucans, total fraction and higher molecular weight retentate (>10kDa) were submitted to sequential extraction with hot water and alkali/acid solutions. All fractions were analyzed concerning sugar, which showed 98% mol of glucose. Glycosidic-linkage composition was determined by gas chromatography-quadrupole mass spectrometry (GC-MS) and (1→3)- and (1→6)-linked glucose branched with (1→4,6)- and (1→3,6)-Glc were obtained as result. Since β-glucans from BY are composed by linked glucose residues, these results allowed to verify that the fractions have mainly glucose and does not have any other unwanted sugars.

Furthermore, the biological potential – prebiotic and immunostimulating activities - of the polysaccharide fractions was also evaluated. Prebiotic potential was tested using two different protocols: as carbon sources for supporting the growth of single probiotic strains viz *Lactobacillus acidophillus* Ki and *Bifidobacterium lactis* Bb12 and testing *in vitro* fermentation by fecal inoculum from healthy human volunteers. The results show the ability of these substrates as carbon sources for increasing mainly the *Bifidobacterium* population, leading principally to the production of acetic, propionic and butyric acids. The impact of the extracts on different target microbial groups present in feces is currently ongoing.

The immunostimulating activity was tested using *in vitro* cultures of spleen cells and subsequent assay by flow cytometry analysis. The results show that for low concentrations ~250 µg/mL of polysaccharide extracts, there is a significant activation (50%) of B lymphocytes (the major antibody-producing cells).

Results confirm the potential of tested extracts, both as activators of the immune system and as excellent candidates for prebiotic β-glucans, thus showing their potential as new functional ingredients for further application in foods and animal feeding.

Aluno: Rui Miguel Barros de Sousa Magalhães

Orientador: Gonçalo Almeida e Paula Teixeira

Tema da Tese: Characteristics of specific strains of *Listeria monocytogenes* potentiating persistence in food and food processing environments and relevance to food safety.

Sumário (máx. 3000 carateres, incluindo espaços)

Listeriosis is an uncommon but severe foodborne illness, caused by the bacterium *Listeria monocytogenes*, that affects mainly individuals with recognized underlying conditions: the elderly, immunocompromised individuals, and pregnant women and their foetuses. The ubiquitous nature and ability of *L. monocytogenes* to grow in harsh conditions makes this pathogen of special concern, as incidence of listeriosis is not decreasing despite worldwide efforts. *Listeria monocytogenes* has been isolated from raw materials, final products, equipment and general food-processing environment (FPE). Some strains have been observed to cause food plant FPE contamination over long periods of time (up to several years). The reason behind the recurrent isolation *L. monocytogenes* strains presenting identical molecular subtypes, while others are recovered only sporadically, is still not well understood. Some authors reported that persistent strains are better adapted to the FPE and possess specific characteristics that enhance survival to stresses in the FPE, namely: resistance to cleaning and sanitising agents, capacity to form a biofilm and resistance to different stress conditions (e.g. temperature, pH, and NaCl)

The aims of this study are (i) to identified the potential characteristics that individually or in combination confer advantages to particular persistent *L. monocytogenes* strains isolated from cheese processing-plants environment, and (ii) to obtain epidemiological data on cases of listeriosis occurring in Portugal collected on health care units on a voluntary basis, and characterize the clinical isolates by molecular subtyping techniques (i.e. pulsed-filed gel electrophoresis (PFGE), molecular serotyping) and determine the minimal inhibitory concentration (MIC, µg/ml) of twelve antibiotics.

Forty-nine persistent and non-persistent strains of *L. monocytogenes* are being characterized in terms of: growth behavior, biofilm formation on different surface materials, and susceptibility to disinfectants (at different exposure times). For the conditions investigated, it was demonstrated that these are strain dependent but no significant differences have been observed between persistent and non-persistent strains.

Between 2008 and 2012, the annual incidence rate observed ranged from 0.19 to 0.65 cases per 100,000 inhabitants. Nineteen cases (9.5%) corresponded to maternal/neonatal (MN) infections. The majority of listeriosis cases were caused by geno-serogroup IVb isolates and PFGE analysis revealed a high molecular diversity. The incidence of antibiotic resistant isolates of *L. monocytogenes* was low but significantly higher than in previous years (2003 to 2007). The implementation of a national surveillance system monitoring the incidence of listeriosis and antimicrobial resistance of strains would be most valuable, allowing identification of sporadic and outbreak cases, to detect general trends in antibiotic susceptibilities, and potentially identify food sources of clinical strains.

Aluno: Sara Nunes da Costa e Silva

Orientador/Coorientadores: Doutora Manuela Pintado Pintado/ Doutor Rui Morais e Doutora Conceição Calhau

Tema da Tese: Study of *Vaccinium corymbosum* berries and leaves toward incorporation in functional foods — characterization of extracts**.**

Sumário (máx. 3000 carateres, incluindo espaços)

There is a wide recognition of the biological potential of phenolic compounds, with their potential as antimicrobials gaining a particular interest when taking into account the emergence of microbial resistance and the need for alternative sources of antimicrobial compounds. Blueberries, the fruits of *Vaccinium corymbosum,* are among the fruits with the highest anthocyanin contents, while their leaves are reported as rich in phenolics acids. So it is important to understand the role and potential of both anthocyanins and phenolic acids, as antimicrobial agents. Taking all the previous arguments into account, the present work is split in two different sections, one for each tissue.

Leaves

The collection of green leaves is, from production standpoint, unthinkable as it compromises fruit production, therefore commercially available, dried, senescent leaves were used. However, as phenolic acids are not as known for their biological potential as other phenolic families, a screening was made to determine the interest of these extracts. As the antimicrobial activity stands as a focal point for this project, the activity of an array of phenolic acids against *Escherichia coli*, *Pseudomonas aeruginosa* and Methicillin Resistant *Staphlylococcus aureus* (MRSA) was assessed. While no minimum inhibitory concentration was found, some antibiofilm activity was detected against biofilm formation; biofilm propagation and mature biofilms. MRSA proved to be the most susceptible microorganism, followed by *P. aeruginosa* while for *E. coli* reduced inhibitions were observed. However, it was possible to conclude that the compounds present in leaves may be of interest, particularly if possible synergies are taken into account.

Fruits

As commercial blueberries not only come from varying sources (Spain, Chile, Holland) but aren't always in the best condition, the use of Portuguese blueberries was preferred particularly as we could have some control over the selection and quality of the fruit. The first stage was then to characterize four different blueberry cultivars (Ozarkblue, Goldtraube, Bluecrop and Duke) in different ripening stages to support the selection process. The results allowed us to conclude that Goldtraube blueberries possess both higher amounts and a wider range of compounds (fact that stands in line with previous works) and that the amount of compounds, particularly anthocyanins, tends to be higher in later stages of ripening.

Additionally, four different solvents were compared for their extraction capabilities (water, methanol, ethanol and acetone) with results showing that methanol and ethanol were preferable when aiming to extract anthocyanins.

As another approach, a traditional solid phase extraction was used to purify an extract. The resulting powder was chemically characterized and used as an antimicrobial agent against several clinical isolates and collection *strains*. Though further assays and more efficient purification methods are underway, the results hint at an interesting antimicrobial and antibiofilm activity.

Aluno: Tânia Cristina Ferreira Ribas Vaz Pedro

Orientador: António Osmaro Santos Silva Rangel, Ildikó Vargáné Tóth

Tema da Tese: Flow-based methods for water monitoring

Sumário (máx. 3000 carateres, incluindo espaços)

High throughput microplate assays for the spectrophotometric study of metal complexes involving Zincon and PAR

Com o aumento da poluição nos últimos anos, devido essencialmente à ação do Homem, as análises ambientais são de momento uma rotina. Neste contexto torna-se extremamente importante o desenvolvimento de novas técnicas com o intuito de determinar a presença de metais em variadas amostras ambientais. Adicionalmente, é desejável proceder a estudos de especiação, pois o conhecimento do estado em que o metal se encontra na amostra é uma mais-valia para perceber como este poderá afetar o ecossistema.

O trabalho realizado durante o primeiro ano do doutoramento teve como objetivo o desenvolvimento de técnicas para o estudo preliminar de reações envolvendo metais com complexantes, estudo esse realizado em microplacas. Os protocolos desenvolvidos tiveram como base o estudo da utilização de dois complexantes, **2-Carboxy-2′-hydroxy-5′-sulfoformazyl-benzene (**Zincon) e 4-(2-Pyridylazo)resorcinol (PAR), que formam um complexo azul e laranja respetivamente.

Os estudos realizados em microplacas demonstram ser uma ferramenta bastante útil para a investigação de processos de equilíbrio de complexos de metais. Ao comparar a ação dos dois complexantes, o PAR demonstra providenciar maior sensibilidade e menores limites de deteção.

Os resultados obtidos nesta fase inicial serão usados num estudo futuro para o desenvolvimento de um método em fluxo para a determinação de diferentes metais em amostras ambientais. Os sistemas de análise em fluxo, especialmente em análise de águas, demonstram ser muito adequados, uma vez que são cada vez mais precisos, têm elevada reprodutibilidade, baixo custo de equipamento, elevado ritmo de amostragem, o manuseamento de amostras é relativamente simples, apresentam baixo risco de contaminação, elevada possibilidade de automatização e baixo consumo de reagentes/amostras e baixa produção de efluentes (1).

Tabela 1: Limites de deteção (□M) para cada metal em estudo (Me) com Zincon (pH=9) e PAR (pH=10)

Complexos	Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn
Me:2Zincon	-------	0.7	0.8	-------	-------	0.6	-------	0.5
Me:2PAR	0.1	0.1	0.2	0.2	0.1	0.2	0.4	0.1

Referência:

(1) M.A. Segundo, A.O.S.S. Rangel, J. Flow Injection Anal. 19 (2002) 3-8.

Agradecimentos: T.C.F.Ribas agradece a Fundação para a Ciência e Tecnologia (FCT, Portugal) e Fundo Social Europeu (FSE) pela bolsa SFRH/BD/91820/2012. I.V. Tóth agradece a FSE e Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) pelo suporte financeiro do programa POPH-QREN. Este trabalho recebeu suporte financeiro da União Europeia (fundos da FEDER pela COMPETE) e fundos nacionais (FCT) pelos projetos PEst-C/EQB/LA0006/2013 e PEst-OE/EQB/LA0016/2013.

Aluno: Teresa Maria Martins Deuchande

Orientador: Marta Vasconcelos / Christian Larrigaudière, Fernanda Fidalgo, Susana Carvalho

Tema da Tese: *Internal browning disorders in 'Rocha' pear during long term storage*

Sumário (máx. 3000 carateres, incluindo espaços)

'Rocha' pear is one of the few export-oriented products of Portuguese agriculture with a market value at farm gate estimated in about 140 million euro. These pears could be stored under controlled atmosphere (CA) for up to nine months. Internal browning disorders (IBD) are a major cause of postharvest losses in 'Rocha' pear during long term storage under CA.

This Ph.D. program aims to: 1) characterize the IBD in 'Rocha' pear and determine their etiology; 2) elucidate the physiological and biochemical bases of IBD in 'Rocha' pear, with emphasis on the antioxidant defense system and fermentative metabolism; and 3) develop and test postharvest handling recommendations aiming at reducing the incidence of IBD.

During the last year four experiments were performed. In a fist experiment, focused on defining markers of fruit sensitivity to CO_2-related IBD, fruits from 6 orchards, three harvested at an optimal maturity stage and three in late maturity stage were stored under cold for 45 days, after which they were switched to high CO_2. At harvest and during storage, ascorbate (AsA), fermentative metabolites (FM), ethanol and acetaldehyde, and the activity of PPO and POX were analysed. Higher incidence of IBD was related to delayed harvest and higher activity of PPO and POX at harvest and increased levels of FM and decreased levels of AsA during storage. In a second experiment aiming to study the physiological basis of IBD, fruits from one orchard in a late maturity stage were stored under regular CA and high CO_2. At harvest and during storage the levels of AsA and FM as well as the activity of antioxidant enzymes were analysed. It was concluded that IBD may be the result of both fermentation and oxidative stress.
In a third experiment two strategies to control IBD were tested: delayed CA and 1-MCP treatment. The two strategies were not effective in reducing IBD. On the contrary, both treatments combined with high CO_2 enhanced the incidence and severity of the disorder in late harvested fruits, suggesting that these strategies combined with high CO_2 storage of susceptible fruits should be avoided.
In the fourth experiment fruits were stored under static CA and dynamic CA monitored by an ethanol sensor (DCA_{EtOH}) and a chlorophyll fluorescence sensor (DCA_{CF}). The sensors detect fruit responses to low O_2 stress allowing the adjustment of optimal storage conditions. The aim was to evaluate the potential of these methodologies on IBD prevention. The results suggest that DCA_{CF} is an effective strategy on IBD reduction in contrast to the DCA_{EtOH} which did not avoid fermentation.

In future work, various strategies to reduce IBD incidence in 'Rocha' pear will be tested under high CO_2. An experiment focused on the effect of storage conditions on the antioxidant system and fermentative metabolism will be conducted to better understand the biochemical pathways and gene expression of the principal enzymes involved on the development IBD in 'Rocha' pear.