Nutraceuticals and Delivery Lab

Proposals for MSc Research Projects 2012/13

TITLE: Development of a "green process" for the isolation of natural functional extracts with anti-cancer activity - Application of high-pressure technology

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This Master Plan is part of a major project whose main goal is to develop adequate clean and mild methodologies for the isolation or fractionation of terpenes-containing extracts (in particular perillyl alcohol (POH)-rich extracts from cherries, citrus, mint and Lavender), alternatively to conventional harmful solvents extraction. Either specific processes or integrated methodologies are being explored for tuning the chemical compositions and targeted characteristics of end-products. SFE (Supercritical Fluid Extraction and PLE (Pressurized Liquid Extraction) will be explored as reliable tools to extract POH from different natural sources, namely cherries, citrus, mint and lavender.

These technologies are safe and environmentally friendly (all the solvents employed are non toxic) and thus the final product is regarded as natural, being allowed for food and pharmaceutical applications.
The student enrolling in this project will have opportunity to acquire expertise in high-pressure technology (handling high pressure apparatus) and in diverse analytical techniques for the bioactive-rich extracts phytochemical characterization.
The student will perform diverse analytical techniques for the bioactives quantification and also evaluate the efficacy of the extracts as chemotherapeutic candidates through in vitro cell bioassays.

TITLE: Preparation of functional microsystems with application in cellular expansion and differentiation - Application of supercritical fluid technology

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Recently, pharmacologically active microcarriers (PAM) has been developed to overcome certain problems encountered in cell therapy, particularly cell survival, lack of cell differentiation and integration in the host tissue. Clean processes such as supercritical fluid technology has been applied as alternative to conventional precipitation methods to produce controlled-release microparticulate systems.

The aim of this project is to exploit and optimize a (SCF)-based precipitation technology (as alternative to conventional processes) to produce pharmacologically active microcarriers (Microsystems) for a sustained and controlled-release of a growth-factor that promotes cellular expansion and differentiation. The materials to be explored as carriers are polymers (or mixtures of) such as poly(D,L-lactic-coglycolic acid), chitosan, polyethylene glycol (PEG).
These (SCF)-based precipitation technologies are safe and environmentally friendly (all the solvents employed are non toxic) and thus the final product is regarded as natural, being allowed for pharmaceutical applications.
The student enrolling in this project will have opportunity to acquire expertise in high-pressure technology (handling supercritical fluid precipitation apparatus) namely in supercritical fluid precipitation techniques.
The student will learn how to perform physico-chemical characterization of the Microsystems prepared that involves diverse methods of solid-state analysis (e.g. morphology, structure organization, particle size

distribution) and evaluation of the process efficiency (through growth-factor release profile and functionalization efficiency).

TITLE: Nutraceuticals from *Brassicaceae*: Solubility measurements of bioactive compounds from crucifeous vegetables in supercritical carbon dioxide.

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Cruciferous vegetables (Brassicaceae family) are recognized as an important source of biologically active compounds with a large spectrum of biological actions. In fact, several studies linked the presence of glucosinolates and its hydrolysis products, isothiocyanates (ITCs) with the observed health-promoting effects, particularly, chemoprotective activity. Particularly, ITCs are pointed as the phytochemicals responsible for the beneficial properties of cruciferous but their intake in daily diet may not be sufficient to achieve those claimed effects mainly due to heat treatment during cooking.

The work to be developed is part of a major project whose main goal is to design, optimize and develop high pressure methodologies (such as supercritical fluid extraction or pressurized liquid extraction with CO2) to obtain high value extracts with health-promoting activities from cruciferous vegetables by-products.
The work planned for this proposal is essential for the design, development and optimization of the high-pressure extraction procedures since the results obtained will strongly influence the selection of processing conditions to be adopted. The student will perform solubilities measurements of targeted bioactive molecules present in cruciferous vegetables in supercritical CO2 [x bioactive compound + (1-x) CO2] and evaluate the impact of the use of diverse co-solvents.
The student enrolling in this project will have opportunity to acquire expertise in high-pressure technology (handling high pressure apparatus), namely supercritical fluid technology and perform diverse analytical techniques for the bioactives quantification.

TITLE: Antioxidant activity evaluation of commercial fruit juices using cell-free and cell-based assays

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Food science has progressively evolved and now there are wide evidences that foods have biological activities that are beyond their classical nutritional value. In this field, the antioxidant activity of foods has been extensively studied and several chemical and cell-based assays have been developed.

The main aim of this project is to evaluate the biological activity of several commercial fruit juices claiming antioxidant properties. This will be achieved by combining different chemical and cell-based assays in order to evaluate the i) radical scavenging capacity, ii) radical prevention ability and iii) cellular antioxidant effect of products. In particular, two *in vitro* fluorimetric assays will be used, namely ORAC (antioxidant activity against peroxyl, radicals) and HORAC (metal chelating radical prevention ability). Additionally, cellular assays will be performed on Caco2 (human colon) cell line, submitted to oxidative stress induced by chemical agents (AAPH or H2O2). The cellular antioxidant potential of products will be assessed by measuring several relevant oxidative stress-biomarkers, namely intracellular reactive oxygen species (ROS) formation, glutathione homeostasis, carbonyl proteins content, and DNA oxidation using adequate analytical methodologies (DCFH-DA assay, HPLC and ELISA). Results obtained will be correlated with phytochemical composition in order to identify which compounds are responsible for the antioxidant

effect of fruit juices. Moreover, the influence of *in vitro* gastro-intestinal simulated digestion (pH, temperature and enzymes) on antioxidant properties of products will be evaluated. Permeability studies through the intestinal epithelia using the Caco2 cell model will be also performed aiming to determine the bioavailability of products' antioxidant compounds.

TITLE: Development of 3D cell-based bioassays for antioxidant activity evaluation of natural compounds

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The antioxidant effect of many compounds present in natural sources has received an increased interest in food and pharmaceutical industries. Today many foods and supplements claiming health benefits are available in the market. For the development of these functional products, more robust and physiological relevant cell based assays are required prior to the antioxidant research in human clinical trials.

The main aim of this project is to develop 3D cell-based bioassays for the evaluation of the antioxidant properties of functional foods and supplements. In particular, the work will be focused on the design of human central nervous system (CNS) model of oxidative stress. This will be achieved by cultivating NT2 cells as aggregates in environmentally controlled stirred tank bioreactors operating at physiological conditions. These cells will be further differentiated into neurons and astrocytes that better mimic the human CNS structure. Oxidative stress will be induced by chemical stressors (e.g. H2O2), and oxidative damage in both neurons and astrocytes will be evaluated by measuring cell viability (measured by MTT, alamarBlue and/or intracellular LDH release assays) and functionality at the level of oxidative-reduction status (ROS production, glutathione level and antioxidant enzyme system: SOD and catalase) and oxidative damage (proteins, lipids and DNA oxidation). After the establishment of an oxidative stress model of neuronal cells, the neuroprotective effect of standard antioxidant compounds, natural extracts and functional food samples will be evaluated. The suppression of amyloid β formation in neuronal cells will be also measured aiming to evaluate the protective effect of antioxidants against neurodegenerative disorders, namely Alzheimer disease.

TITLE: Characterization of antioxidant capacity of food and beverages - Evaluation and establishment of structure- activity relationships.

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Polyphenols are the most abundant antioxidants in our diet and are widespread constituents of fruits, vegetables, cereals, olive, dry legumes, chocolate and beverages, such as tea, coffee, wine and fruit juices. Total phenolic content (TPC) determined using Folin-Ciocalteau method and other fluorimetric assays (like ORAC e HORAC) have been used to evaluate and compare phenolic composition and antioxidant capacity of foods, related products or extracts. However it is known that sample matrix can interfere in this measurements, as the chemical reaction occurring is not specific from phenolic compounds and ascorbic acid, proteins, sugars may interfere in the determination. Also phenolic compounds from different families may not give the same kind of response.

The aim of this work will be:

(i) selection of a standard mixture representative of samples (food and related samples) understudy and simulation of the contribution of different interfering compounds. In parallel results will be controlled by LC-DAD;

(ii) to evaluate and conclude about the response of different standards from different families of phenolic compounds to different antioxidant capacity assays;

(iii) compare results obtained with Folin Ciocalteau reagent and detection at 280 nm using LC-DAD for the different samples.

TITLE: Exploring wild olive seeds as source of bioactive compounds with health promoting effects

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The olive tree belongs to the family of *Oleaceae* that have characteristic chemical compositions with emphasis on secoiridoid compounds. The structures of these compounds contain elenolic acid and its derivatives as building blocks. Some phenolic compounds have been identified as 11-methyl oleoside and nüzhenide [1]. Recently, the olive trees are being used for ornamental purposes in gardens and the fruits that are produced every year are not used.

The aim of this project is to evaluate the biological activity of an extract of wild olive seeds that has been characterized in terms of phenolic compounds, in an attempt to evaluate the possibility to use it as a raw material to the production of a bioactive extract to be used in food or cosmetic industry.