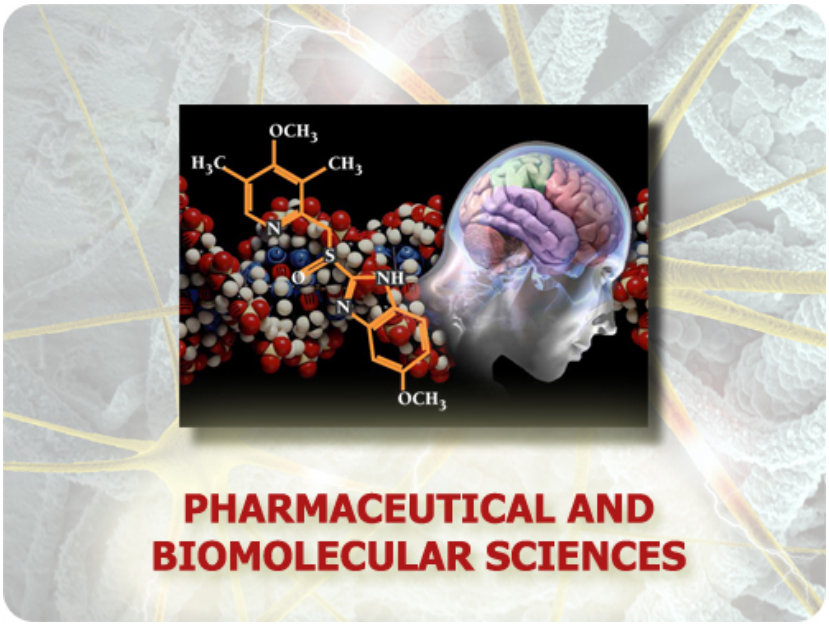
Scuola di Dottorato dell'Università degli Studi di Torino

Dottorato in Scienze Farmaceutiche e Biomolecolari



Advances in

at the University of Torino

Presentazione dei risultati dei progetti di ricerca
dei Dottorandi e delle Dottorande del XXXII ciclo

Aula Leonardo

Dipartimento di Biotecnologie Molecolari e Scienze per la Salute
Via Nizza 52 - Torino

4 ottobre 2019

Le Dottorande ed i Dottorandi del 32° ciclo del Dottorato in Scienze Farmaceutiche e Biomolecolari presentano le loro ricerche

Il Dottorato in Scienze Farmaceutiche e Biomolecolari si caratterizza nell'ambito della Scuola di Dottorato dell'Università degli Studi di Torino per lo spiccato carattere multidisciplinare, che risulta dal coinvolgimento dei Dipartimenti di Biotecnologie Molecolari e Scienze della Salute, Chimica, Scienza e Tecnologia del Farmaco, Scienze della sanità Pubblica e Pediatriche, Scienze della Vita e Biologia dei Sistemi. Tale carattere, proprio di una proposta innovativa di formazione alla ricerca, è stato riconosciuto a questo Dottorato anche per il 2019 dall'Agenzia Nazionale di Valutazione del Sistema Universitario della Ricerca (ANVUR), che ne ha parimenti riconosciuto i caratteri di internazionalità ed intersettorialità. Nelle concretizzazioni operative di quest'ultima, in termini di connessione tra sistema universitario e sistema delle imprese, si pone anche l'edizione 2019 della giornata "Advances in Pharmaceutical and Biomolecular Sciences @ UniTO", appuntamento annuale in cui le Dottorande ed i Dottorandi che hanno concluso i loro progetti di ricerca presentano i risultati raggiunti. Quest'anno giunge a compimento il 32° ciclo di Dottorato, e nelle pagine seguenti sono raccolti i riassunti dei 16 progetti di Dottorato svolti nel triennio. I destinatari non sono solamente i componenti della comunità scientifica del Dottorato (nella totalità dei suoi docenti afferenti e dei suoi studenti), ma anche rappresentanti di aziende e operano nell'ambito del territorio regionale, e dei Poli di innovazione.

L'Università di Torino è ben consapevole dell'elevato valore aggiunto connesso al percorso di formazione dottorale, che vede protagonisti giovani preparati e motivati, che affrontano con impegno le sfide dei loro progetti di ricerca, consapevoli dell'investimento che stanno facendo su loro stessi e della necessità di cogliere al meglio le opportunità di questo percorso. La nostra Università ha messo in campo una Scuola di Dottorato e programmi di Dottorato che cercano di attuare al meglio i percorsi formativi che offrano le migliori possibilità alle nostre Dottorande ed ai nostri Dottorandi, e a questo proposito ringraziamo sinceramente il Rettore eletto, Prof. Stefano Geuna, per la sua disponibilità a venire ad aprire la nostra giornata, nonostante i molti impegni dei primi giorni del suo insediamento.

Concludo, porgendo, a nome di tutta la comunità del nostro Dottorato, un sincero benvenuto a quanti hanno potuto rispondere all'invito a partecipare alla giornata, che ci auguriamo ricca di spunti e occasioni di interazione.

Il coordinatore del Dottorato
in Scienze Farmaceutiche e Biomolecolari

Prof. Gianmario Martra

SOMMARIO

PROGRAMMA

I riassunti in formato elettronico saranno resi disponibili in un'apposita sezione della home page del sito web del Dottorato:
http://dott-sfb.campusnet.unito.it

ORARIO	*DOTTORANDA/O*	*PRESENTAZIONE*	*SETTORE*
8.45-9.00		***Apertura dei lavori***	
9.00- 9.30	Stefano Acquadro	Isolation and characterization of plant extracts of pharmaceutical, cosmetic and food interest by bioassay guided studies	Health (pharma)/Food
9.30-10.00	Federica Bessone	Design and development of novel nanoformulation-based delivery systems for the prevention and the treatment of challenging pathological conditions	Health (pharma)
10.00-10.30	Davide Bonanni	Computational strategies for structure-based lead optimization and analysis of SAR transfer	Health (pharma)
10.30-11.00	Cristina Campobenedetto	Identification of new products with biostimulant action and evaluation of their effects on plant growth and development by using genomic and metabolomic approaches	Agri/Bio
11.00-11.20		***coffee break***	
11.20-11.50	Federico Capuana	Design and testing of novel in vivo imaging probes for SPCCT and MRI	Health (diagnostics)
11.50-12.20	Federica Galati	Structural and functional characterization of Mycobacterium tuberculosis Suf machinery	Health (biochemistry)
12.20-12.50	Xinyu Ge	Process development for the adsorption/desorption of pharmaceuticals and other organic pollutants from industrial wastewater	Sustainability
12.50-13.20	Giorgio Grillo	Design of non-conventional chemical processes for biomass valorization	Sustainability
13.20-14.30		***Pranzo***	
14.30-15.00	Monirul Islam	Plant magnetoreception: ionomics and lipidomics of plant responses to reduced magnetic fields	Agri/Bio
15.00-15.30	Francesca La Cava	Improving the diagnostic efficacy in pathological models with novel high relaxivity gadolinium chelates	Health (diagnostics)
15.30-16.00	Maria Jesus Moran Plata	Combining ultrasound and microwaves in chemical processes	Sustainbility
16.00-16.30	Stefano Nebbia	Effect of thermal processing on food protein digestibility and allergenicity	Food
16.30-16.50		***coffee break***	
16.50-17.20	Marta Cialié Rosso	Advanced analytical approaches for "omic" investigations of high quality food matrices of vegetable origin	Food
17.20-17.50	Ana Luisa Soutelo Maria	Ultrasound-assisted C=C bond activation	Sustainability
17.50-18.20	Ivano Vigliante	Molecular and chemical fingerprinting of high oleic sunflower hybrids. A systems biology approach	Food
18.20-18.30		***Chiusura dei lavori***	

Abstract
dei progetti di ricerca
delle Dottorande e dei Dottorandi
del 32° ciclo

(2017-2019)

Isolation and characterization of plant extracts of pharmaceutical, cosmetic and food interest by bioassay guided studies

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The scientific area in which this doctoral project is located is the wide and compiste filed of natural products. Specialised metabolites from plants can serve as defence against herbivores, microbes, viruses or competing plants (1). Since ancient times natural products have played an important role all over the world in the treatment and prevention of human diseases and were successfully used for the discovery of new lead compounds (2). In this context, the main goal of this thesis is the chemical and biological investigation of unexploited parts of food plants with potential healthy properties: pomegranate (*Punica granatum* L.) and grapevine (*Vitis vinifera* L.) **(FIG.1)**. Regarding pomegranate, preliminary experiments were carried out on leaves, bark and peel ethanolic extracts which resulted active towards two enzimes involved in HIV-1 replication and were therefore phytochemically characterized. Subsequently, the attention was focused more deeply on the poorly investigated leaves for which the phytochemical pattern was evaluated for plants of different origin, harvesting season and year. Beside the whole ethanol extracts, the attention was also focused on phenolic and triterpenoidic enriched fractions, obtained by fractionation with chromatographic techniques. To evaluate the constancy of the extracts, phytochemical analysis were performed using HPLC-PDA-ESI-MS/MS and GC-MS systems combined with unsupervised multivariate data analyses. Biological assays were performed on pomegranate leaves' extracts, fractions and pure compounds against HIV-1 and also Zika virus since its infection has recently attracted the attention of the medical community (3). At the same time, *in vitro* studies were also carried out on their anticancer activity against acute lymphoblastic leukemia and multiple myeloma cell lines, considering the urgent need of cytotoxic agents that may overcome the ever increasing drug resistance. The second investigated matrix was the residues generated by the spring pruning of the grapevine plants (GPRs), an abundant but uninvestigated by-product of the wine supply chain. The study was therefore focused on the polyphenols characterization of GPRs from 16 red and white *V. vinifera* cultivars from Piedmont (Italy). The results were compared with those obtained for the leaves, in literature reported as a good source of bioactive nutraceutical compounds (5). *In vitro* antioxidant assays were also carried out on the leaves and GPRs, to compare their potential activity. In conclusion, the investigations carried out on the two investigated plants showed that these unexploited parts of plants can be a promising source of active compounds to be used in several fields.

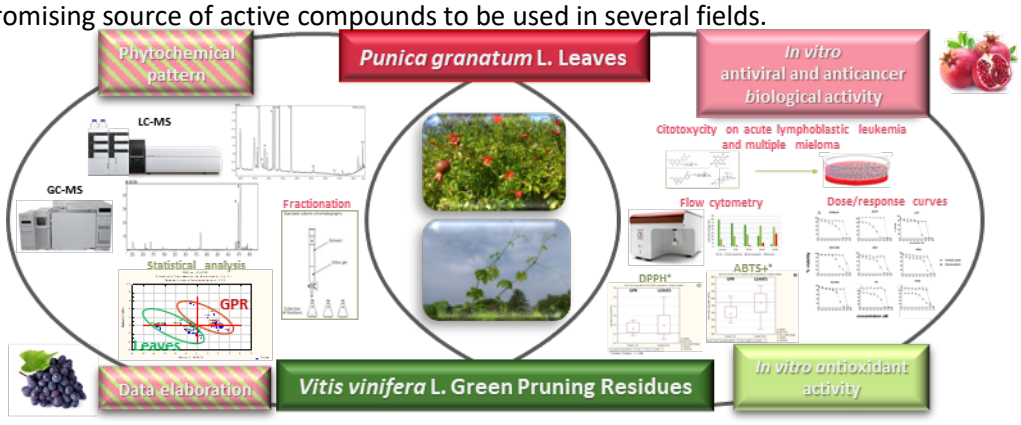

Figure 1: Visual summary of the strategy applied for the investigation of pomegranate leaves and GPRs.

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Design and development of novel nanoformulation-based delivery systems for the prevention and the treatment of challenging pathological conditions

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In the last decades, nanoformulation-based tools started to draw attention as novel entities in diagnostic and therapeutic fields. Nanomedicine has become an important key to overcome and solve intricate medical limitations (1). The challenge to design and develop new nanodelivery systems was faced in this doctoral project. In particular, two main topics were investigated: tissue hypoxia and drug resistance. **THE AIM OF THE FIRST TOPIC WAS TO DEVELOP NANOFORMULATIONS SUITABLE FOR THE DELIVERY OF OXYGEN IN A CONTROLLED MANNER.** Notably, hypoxic tumors are more resistant to chemotherapy and radiation. In particular, the pancreatic tumor is an aggressive type of cancer in which the microenviroment is extremely hypoxic and the cells become easily resistant to drugs. **FOR THIS PURPOSE, CORE-SHELL NANOBUBBLES WERE DESIGNED FOR** the supply of oxygen, drugs and adjuvant agents to the tumor site. Novel nanobubble formulations were developed to combine oxygen, Gemcitabine as chemotherapy and Curcumin as a modulator of overexpressed efflux pump proteins (P-glycoprotein) in resistant cancer cells. The synergistic effect of Oxygen-loaded nanobubbles carrying Gemcitabine and Curcumin may represent an interesting platform to overcome current drawbacks with the present treatment of pancreatic cancer (2). In addition, for preventing metastatic spreading in prostate cancer cells, **CURCUMINOID-LOADED IN DEXTRAN-SHELLED NANOBUBBLES WERE PREPARED AS** adjuvant theranostic tools. Indeed, Curcuminoids were released with a prolonged *in vitro* kinetics and the nanobubbles prevented their degradation. Additionally, nanobubble echogenic properties can be visualized by ultrasound imaging **(3).** On the other hand, resistance to chemotherapy is a major problem that limits the effectiveness of successful treatment of cancer. Albumin-based Doxorubicin-loaded nanoparticles were prepared to provide controlled release of the drug, to reduce the toxicity due to side effects and to overcome the resistance. They showed a significant viability and proliferation inhibition on cancer cell lines resistant for Doxorubicin, representing a promising platform for increasing the efficacy of chemotherapy (4). Finally, hypoxia-sensitive liposomes were studied for co-delivery of siRNA and Paclitaxel specifically targeting hypoxic areas. The synergic effect of the silencing of P-glycoprotein with the siRNA **AND CHEMOTHERAPY** could potentially offer a novel and effective treatment for patients who have established multidrug resistance (5). In conclusion, the study of several nanoplatforms was conducted with the aim to overcome therapeutic limitations and enhancing the successful of the treatment of different pathological conditions.

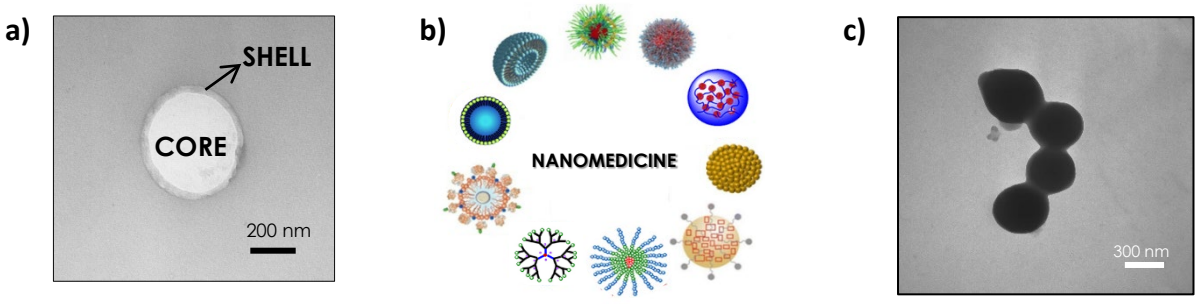

FIGURE. A) TEM IMAGE OF OXYGEN-LOADED NANOBUBBLES; B) REPRESENTATION OF DIFFERENT NANODELIVERY SYSTEMS; C) TEM IMAGE OF Albumin-based Doxorubicin-loaded nanoparticles.

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Computational strategies for structure-based lead optimization and analysis of SAR transfer

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THE TOPIC OF THIS PHD RESEARCH IS THE APPLICATION OF COMPUTATIONAL CHEMISTRY IN DRUG DISCOVERY AND DESIGN. THE PROJECT WAS CONDUCTED AT THE *Department* of *Drug Science and Technology* of UniTo and at the *Department of Life Science Informatics* at b-it, University of Bonn. The aim of the thesis is the design of new small molecules effective in cancer therapies as well as the developing of innovative computational methodologies aimed at hit-to-lead and lead optimization. The target of these studies was *human* dihydroorotate dehydrogenase (*h*DHODH), a flavin-dependent mitochondrial enzyme involved in *de novo* pyrimidine biosynthesis. *h*DHODH overexpression has been associated with acute myelogenous leukemia, a disease for which the standard of intensive care has not changed over decades.[ref] In this study, computer-aided lead optimization were carried out on a potent *h*DHODH inhibitor.[1] Structure-based approaches were used to generate working hypotheses to modify further the lead compound and improve its molecular properties. A series of analogues were designed, synthetized and biologically evaluated.[2] Moreover, this doctorate focused also on the identification of new computational strategies for lead-optimization. The exploration of structure-activity relationships (SARs) is central relevance in drug design. In such situations, one would ideally like to build upon priori knowledge, utilize available SAR information, and evaluate the possibility of an "SAR transfer", i.e., the exploration of an alternative chemotype that displays similar SAR characteristics and potency progression. So far, few studies have computationally (and indifferent ways) analyzed SAR transfer events.[3-5] However, currently lacking are structure-based approaches for the assessment and prediction of SAR transfer. In our analysis, we investigated SAR environments with the aid of experimental structures and compound binding data (Fig.1), introducing a computational method for the structure-based identification of SAR transfer events and their systematic assessment.

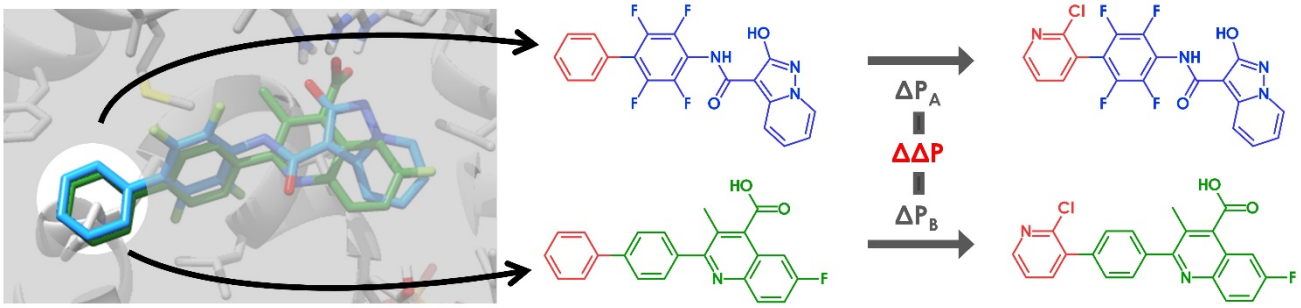

Figure 1: The representation illustrates principles of computational structure-based SAR transfer exploration. On the left, complex X-ray structures of *h*DHODH with two inhibitors are superimposed (PDB ID: 6FMD and 1D3G). The circle highlights a shared phenyl ring. On the right, the two inhibitors are displayed together with corresponding active analogues, red substructure represents the shared fragment. From the ΔP (potency difference) value of each X-ray inhibitor – analogue pair, ΔΔP is determined.

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Identification of new products with biostimulant action and evaluation of their effects on plant growth and development by using genomic and metabolomic approaches

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Biostimulants are substances and/or microorganisms that, when applied to a plant at very low doses, improve natural physiological mechanisms such as nutrient uptake and use efficiency, response to abiotic stress and crop traits (1). These products represent a good tool to increase crop yield, in a moment in which to feed the growing world population with the decrease of arable land became a necessity (2). The main goal of this PhD thesis was to identify new matrices with a biostimulant action and investigate their effects on plants by using multidisciplinary approaches. Moreover, the chemical characterization of these matrices was of paramount importance to identify active compounds involved in the biostimulant activity in order to understand their mode of action. In this perspective, the PhD project was divided in two subprojects, both concerning the development of new products able to act on plant at different levels.

In the first one, the effects of **KIEM®**, a pre-sowing biostimulant, based on lignin derivatives, amino acids of plant origin and molybdenum were evaluated on soybean (*Glycine max* L.) and cucumber (*Cucumis sativus* L.) seed germination. Biometric parameter measurements, followed by transcriptomic and biochemical analyses performed on seeds germinated in optimal and heat stress conditions, suggest the role of KIEM® as a priming product, able to protect the seed from heat damage and improve plant growth. Based on these studies, KIEM® is now available on the market.

In the second project, the effects of **GHI_18_120**, a new prototype based on hydrolysable and condensed tannins, were evaluated on tomato (*Solanum lycopersicum* L.) plants. Biometric parameter measurements, transcriptomic and metabolomic analyses carried out on treated and control plants, showed that this biostimulant, tested under standard and salt stress conditions, was effective in improving the root system development and plant growth and in enhancing salt stress tolerance .

In conclusion, in this work we tried to get insights on the potential of two biostimulants, under different conditions and crops. These products are normally not species-specific and the idea is to make them able to reduce the use of more polluting and dangerous fertilizers.

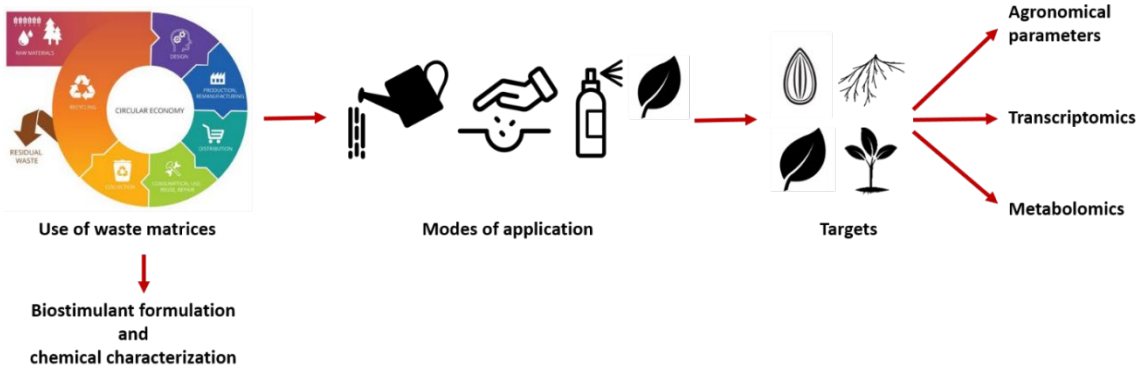

Fig.1 Graphical representation of the main steps involved in the study of biostimulant effects. Biostimulants are often obtained from industrial waste and then chemically characterized to identify active compounds. Methods of application and target depend on the nature of the biostimulant. Finally, to get insights on the mechanism of action, a multidisciplinary approach including biometric parameter measurements and transcriptomic and metabolomic analyses is usually applied.

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Design and testing of novel *in vivo* imaging probes for SPCCT and MRI

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Spectral photon-counting CT (SPCCT) is an emerging X-ray imaging technology that extends the scope of available diagnostic imaging tools. The photon-counting detector has the capability to detect K-edges and to distinguish simultaneously between different attenuation profiles, allowing multi-contrast agent imaging (1). Essentially, K-edge imaging offers exciting potential to transform CT into a true molecular imaging technology, but numerous challenges must be overcome for ultimate success in the clinic. Namely, SPCCT is relatively insensitive (10-1 to 10-3 mol/L). Therefore, the design of ideal contrast media that can provide maximum concentrations of suitable metals without toxicity is demanded (2). Lanthanides, most notably gadolinium, are predicted to afford an optimal signal level with SPCCT imaging. A number of Gd(III) chelates, routinely employed in clinical MRI practice, can find new diagnostic applications in SPCCT imaging without requiring extensive safety assessment. Molecular imaging of biological targets requires the specific accumulation of contrast media at the target site. However, the density of the molecular target might be inherently low, limiting the amount of contrast agent that can be accumulated and eventually the sensitivity of the imaging technique. An interesting approach to increase the concentration of contrast media at the target site is to link several Gd(III) chelates to the same targeting vector, in order to accumulate multiple copies of the contrast agent within a single target binding event. The aim of this project is to develop a novel bifunctional agent, carrying one functional group for the bioconjugation to targeting vectors and four Gd(III)-DOTA-like functions as contrast agent. This compound can be used as versatile building block to insert a pre-formed Gd(III)-multimer to biological targeting vectors (3). In particular, the tetramer has been used to label a tropoelastin-binding peptide. Tropoelastin may represent a promising imaging biomarker for non-invasive detection of atherosclerosis progression and lesion instability resulting in earlier diagnosis (4). Actually, dysfunctional matrix turnover, occurring in atherosclerosis progression, leads to the accumulation of monomeric tropoelastin rather than cross-linked elastin (5). Such Gd(III)-labelled peptide can be considered as a dual-modality molecular imaging probe, as it can be used both for K-edge imaging and MRI. In conclusion, this thesis is devoted to investigate the feasibility of monitoring lesion progression and rupture-prone plaques, in a pre-clinical level, by means of MRI (Fig.1) and SPCCT imaging.

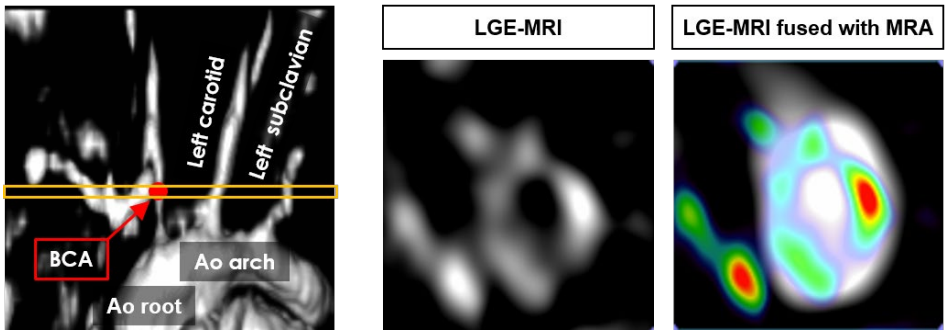

Figure 1: *In vivo* MRI (vessel wall enhancement) using the tropoelastin-binding contrast agent in an atherosclerotic apolipoprotein E-deficient mouse. MRI of the brachiocephalic artery (BCA) showed enhancement of the vessel wall after administration Gd_4-TESMA because of the presence of tropoelastin in the atherosclerotic lesion.

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Structural and functional characterization of Mycobacterium tuberculosis Suf machinery

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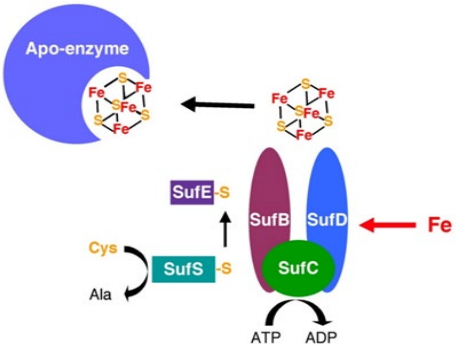
The worldwide recrudescence of tuberculosis and widespread antibiotic resistance have strengthened the need for the rapid development of new antituberculous drugs targeting essential functions of its etiologic agent, Mycobacterium tuberculosis (Mtb). The recently identified mycobacterial SUF machinery constitutes a new potential target as being implicated in the pathogen's survival. The Suf pathway is one of the three main pathways for the biosynthesis of Fe-S clusters in bacteria. While utilized under iron limitation and oxidative stress in E. coli, it is the unique way to assembly and repair the [Fe-S] clusters in M. tuberculosis. The mycobacterial Suf locus encodes SufB, SufC, SufD, SufU, and SufS ortholog proteins, which present high homology with the E. coli proteins. In contrast, no ORF coding for orthologs of SufA and SufE from E. coli was found in the mycobacterial locus. Generally, in bacteria SufE (in gram negative) or SufU (in gram positive) accelerates the cysteine desulphurase activity of SufS.

So the first aim of this thesis was to identify a new ortholog sequence that shares high homology (60%) with E. Coli SufE. That was not part of the suf operon but it was expressed in the same growth dependent pattern of the other suf proteins. The next step was to go deeply and understand why a microorganism such Mtb possesses both SufU and SufE while every microorganism has SufE or SufU. Therefore, we decomposed the Suf system in its basic components: SufS, SufU and SufE. Each component was cloned in an expression vector and expressed as phusion-protein in E. coli heterologous system. Protein purification was performed through different chromatrographic techniques (affinity, ion exchange and size exclusion chromatrography). Once we obtained the purified proteins we were able to characterize them structurally and test their stability by circular dicroism. Afterwards, we studied SufE/SufU function by enzymatic assays in presence and absence of SufS to test if they were able or not to enhance its desulfurase activity.



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Carla Ayala-Castro, Avneesh Saini, and F. Wayne Outten*, Fe-S Cluster Assembly Pathways in Bacteria. MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Mar. 2008, p. 110–125.

Process development for the adsorption/desorption of pharmaceuticals and other organic pollutants from industrial wastewater

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Water contamination caused by hazardous organic compounds, represents a serious environmental problem that requires urgent actions. As first, the development of robust, efficient, economically viable and environmentally friendly water treatments should be considered. Due to the simple design, low cost, and easy operation towards noxious pollutants, adsorption can be considered a method of choice [1]. Moreover, activated carbon (AC) has been globally recognized as the oldest, widely used and most popular adsorbent in water and wastewater treatments [2]. The investigation carried out in this doctoral thesis aimed to evaluate the potential application of specific AC as an adsorbent in highly polluted wastewater with polyphenols (PP) and other corkwood extracts, phenolic compounds (PC) and pharmaceuticals, as well as residual veterinary antibiotics in low concentrations.

Specific treatments involving purification/removal, recovery of substances and desorption studies from wastewater were designed. This project comprised three main fields: i) Treatment of cork industrial wastewater (CW) with high chemical oxygen demands (COD) and PP content. A novel flocculation/adsorption method for CW purification and microwave efficient regenerated of spent AC (Figure 1 (a)) have been described [3]. ii) Treatment of aqueous solutions with high concentration X-ray contrast agent Iopamidol (IOP). An efficient adsorption/desorption process of IOP, and recycling of the adsorbent AC via methanol or ethanol elution in a semi-continuous flow mode (Figure 1 (b)) have been reported [4]. iii) Treatment of PC at high concentrations. Different solvents for desorption of 4-nitrophenol were carried out in batch (Figure 1 (c)) and semi-continuous flow modes, which suggested the desorption feasibility and mechanism. In summary, AC used in this study exhibited excellent results for the high concentration organic removal and the efficient recovery from aqueous solution or wastewater.

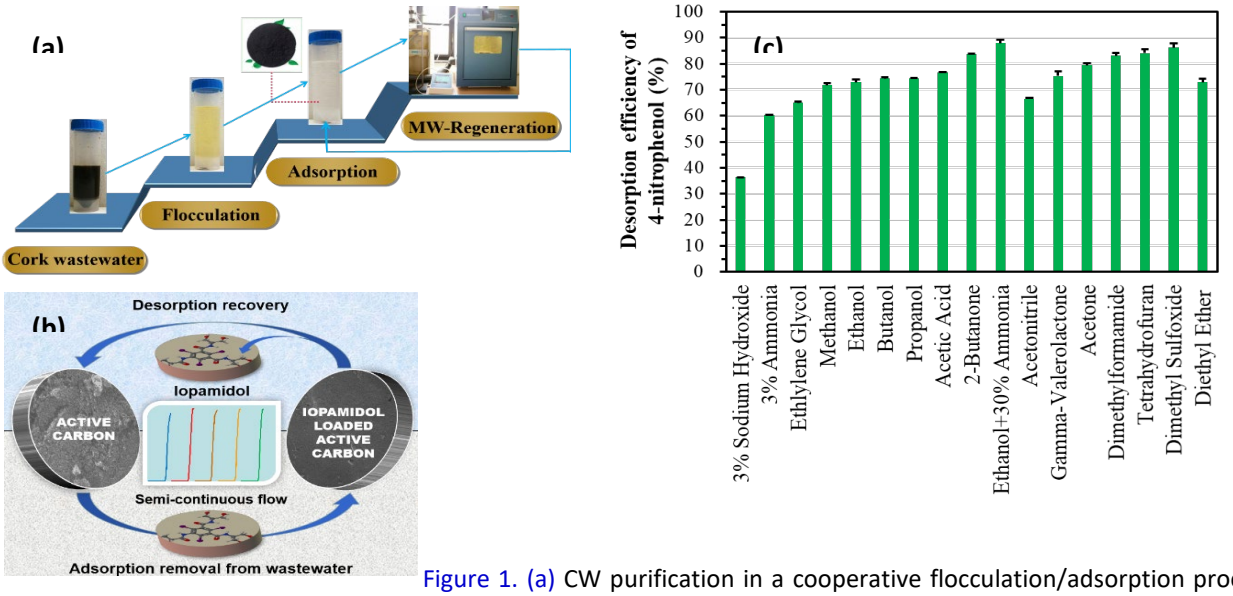

Figure 1. (a) CW purification in a cooperative flocculation/adsorption process with microwave-regenerated AC; (b) Adsorptive recovery of IOP from aqueous solution and reuse of AC; (c) Desorption of 4-nitrophenol from spent AC with solvents.

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Design of non-conventional chemical processes for biomass valorization

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Despite chemical industry still runs mainly with fossil feedstock, the demand for renewable sources has steadily increased over the last two decades. In this frame, biomass and bio-wastes have been recognized as renewable source of fixed carbon for the production of valuable platform chemicals for industrial applications. In the meantime, new enabling technologies for processes intensification have been developed and successfully applied in this field. Microwaves (MW), ultrasound (US), hydrodynamic cavitation (HC) may generate high-energy microenvironments. The aim of this PhD thesis was the design of new processes based on non-conventional technologies for biomass extraction and selective conversion. The investigation started on batch reactors provided suitable data for systems scale up, towards industrial applications. Furthermore, the target is a zero-wastes protocol to treat different biomasses following a circular economy approach. The combination of new green solvents, sustainable heterogeneous catalysts and non-conventional chemical reactors opened new paths for biomass conversion. Well-balanced cascade process enabled a full valorization of biomass constituents and by-products into high-value-added chemicals. According to this strategy, four pivotal steps have been deeply investigated: (I) Metabolites Extraction, (II) Pre-treatment, (III) Structural Conversion and (IV) Platform Modification.

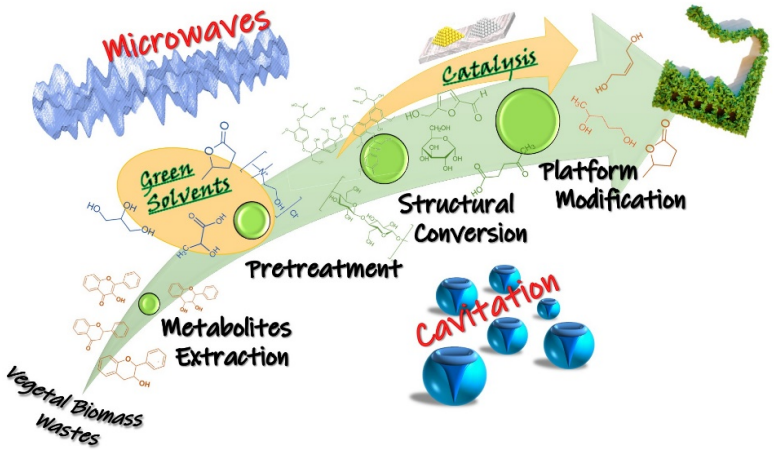

Fig. 1: Biorefinery cascade approach for vegetal residues.

Extractions (I) can provide several valuable secondary metabolites, such as polyphenolic compounds. Different protocols have been optimized, by kinetic studies, among vegetal residual matrixes: cocoa shells, curcuma, tea, grape stalks and pomace. Structural biopolymers, as alginates from algae, pectins from citrus wastes, and lignin from lignocellulosic materials could be isolated within the same protocols. Green solvents play a key role for new and sustainable pre-treatment (II), showing surprising synergies with non-conventional techniques to achieve fractions enriched in lignin-derived polyphenols and solid residues suitable for the conversion into fermentable sugars, after enzymatic hydrolysis. Pre-treatment can also be implemented before the extraction phase, in order to cripple recalcitrant biomasses, enhancing the final metabolites recovery. Enabling technologies exhibit synergistic effects also with heterogeneous catalysts, mainly due to hot-spots generation on the active sites. This feature can be applied for structural biopolymers conversion (III) into valuable building blocks: hydrolysis and isomerization have been exploited to produce monosaccharides, hydroxymethylfurfural (HMF) or levulinic acid from cellulose and hemicellulose. For these purposes, solid acid catalysts as sulfated zirconia and zeolites have been studied. A further modification of biobased derivatives could be carried out *via* reduction reactions under heterogeneous catalysis with Au, Pd or Ru nanoparticles supported on TiO_2, ZrO_2, Al_2O_3 or AC. The main products obtained were γ-valerolactone (GVL) and 1,4-pentanediol. The best performing protocols of the cascade process have been chosen for the scaling up, increasing batch volumes towards pilot scales (75L), or moving to flow systems, with pre-industrial flow-rates (from 1L/h to 20L/min). Final tests were carried out in a plant working with 50 kg/die of lignocellulosic residues to obtain fermentables, boosting the delignification percentage of the matrix. On the other hand, the biomass delignification was also achieved through an integrated process: biowastes were treated in GVL to recover pure lignin and to convert cellulose into levulinic acid, which was then hydrogenated to GVL. Following a circular approach, the solvent was regenerated and recycled for next delignification steps.

Plant magnetoreception:
ionomics and lipidomics of plant responses to reduced magnetic fields

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The Earth magnetic field (or geomagnetic field, GMF) is a natural component of our planet and variations of the GMF are perceived by plants by a still uncharacterized magnetoreceptor. In particular, (near null magnetic field, NNMF) caused an early downregulation of clock genes, photoperiod, gibberellin, and vernalization pathways resulting in a delay in the transition to flowering (1). The overall goal of this project is to provide new findings about magnetoreception mechanism of plants. Therefore, the impact of MF intensity variation on i) plant growth and development; ii) plant metabolism (lipid metabolism) and iii) on nutrients homeostasis were carried out to reach our aim. Arabidopsis plants were grown under normal (GMF) and near null magnetic field (NNMF), which was generated by using a triaxial Helmholtz coil system (Fig.1). Morphological, biomolecular, metabolome and ionome analyses were performed on both root and shoot tissues. NNMF-grown plant showed i) a decrease in plant growth, leaf area index and in the number of rosette leaves and ii) a delay of flowering time in reproductive stage. Such impairment of plant growth parameters could be reflected also at the metabolic level. Particularly, plants exposed to NNMF conditions showed a general increase of plant surface alkanes and the increase of unsaturated fatty acids and associated gene expression. Lipids are one of the major components of biological membranes and act as a signaling molecules for abiotic stress, indicating that MF intensity variation is perceived by plants as a stress condition. Moreover, metal homeostasis of plant exposed to NNMF was affected: in particular, a strong induction of genes involved in the Fe uptake was observed. Under Fe deficiency, the Fe uptake genes were activated at lower extent, while Cu and Zn strongly accumulates in NNMF compared to GMF plants. These results indicate that lowering MF intensity impairs metals homeostasis, particularly at the root level. Furthermore, we provide evidence that by transferring plant from GMF to NNMF root Fe reductase activity (which is responsible for the Fe mobilization in the rhizosphere) is affected in vivo. These results indicate that NNMF affects Fe uptake process directly at the root level. Since, it is known that Fe is involved in the maintenance of the Circadian period (2) the effect of NNMF on Circadian clock might be linked to the observed impact of NNMF on Fe homeostasis in plant (3). The obtained results highlight the influence of MF on plant growth. Understanding the nature and function of the plant magnetoreceptor will be important for future space programs involving plant growth in environments with a reduced MF.

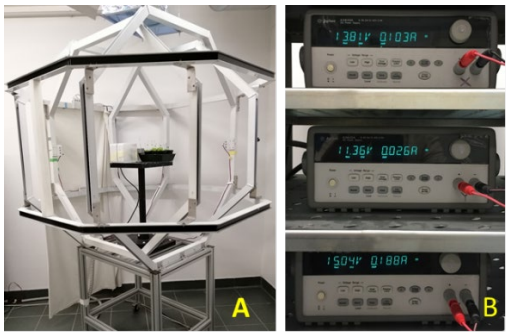

Fig.1. Geomagnetic field compensation system. (**A**) Triaxial coils (comprised of a Helmholtzpair of octagonal coils for each of three perpendicular axes) for cancelling the geomagnetic field. (**B**) 3 DC power supply.

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Improving the Diagnostic Efficacy in Pathological Models with Novel High Relaxivity Gadolinium Chelates

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Gadolinium based contrast agents (GBCAs) have been widely used in clinic to enhance the quality of images acquired during Magnetic Resonance Imaging (MRI) acquisitions. Currently, GBCAs are used in 40% of MRI scans and 60% of neuro MRI scans, corresponding to approximately 40 million worldwide administrations per year (1). GBCAs associated fatalities are really rare (40 serious events and 0.9 deaths per million). Nevertheless, during the last five years their safety has been under discussion after the reported experimental evidence of MR signal hyper-intensity in certain brain regions in unenhanced images (2) due to the presence of gadolinium retention following repeated administrations of GBCAs (3). In this scenario, where the GBCAs are largely used in clinic, but at the same time new concerns are emerging, the research of new Gd-chelates with high relaxivity (to improve the detection of small lesions and/or reduce the administered dose) and improved kinetic inertness and chemical stability (to decrease the amount of gadolinium retained) is undergoing to a new boost. This PhD thesis is inserted in this challenging context, and specifically is aimed to test two novel high relaxivity GBCAs on healthy animals and on different pathological models as a first step for translation to clinical trials. All the pathological models were selected to guarantee a suitable preclinical development and the induction procedure was optimized to obtain a feasible and reproducible protocol, associated to a reduced pain and stress of the animal, in accordance to the 3Rs guidelines. In the first part of the thesis, a linear, dimeric, albumin binder GBCA was fully characterized in vitro in terms of relaxometric properties and then its bio-distribution was evaluated on healthy animals and on a pathological model of ischemia. $(Gd\text{-}DTPA)_2Chol$, presented a series of features, such as good affinity for albumin, high number of binding sites, properties of carrying two Gd ions per molecule, and limited hepatobiliary elimination. These properties contributed to an unexpected long blood elimination half-life, resulting into an optimal confinement in the vascular space and thus into an extension of the available time window for MR angiography. In the second part, a dimeric macrocyclic GBCA, selected as possible candidate for clinical translation, was tested for its pharmacokinetics and its efficacy on a series of pathological models: glioma, meningioma, cerebral ischemia and breast cancer (Fig.1). The compound, namely Lead, presented good relaxometric properties and stability in vitro, and preclinical studies demonstrated that had the same efficacy of commercial compounds, i.e. Gadovist® and Dotarem®, when injected at the half dose 0.05 mmol Gd/kg and an efficacy approx. two times higher when injected at the same dose, i.e. 0.1 mmol Gd/kg.

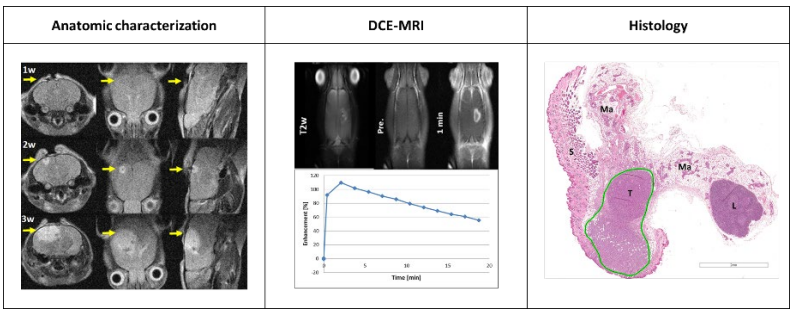

Figura 1 a) MRI anatomic characterization of Convexity Meningioma; B) DCE MRI of a cerebral ischemia ; C) Transversal histological section of a mammary breast tumor.

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Combining ultrasound and microwaves in chemical processes

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The actual environmental concern and the desire of facing the current planetary emergency has opened a door for an extensive number of green research procedures. Conventional protocols are replaced with new efficient processes where safer chemicals, solvent free conditions, natural abundant solvents and alternative energy sources are used to achieve sustainability and scalability. In particular, the fine chemicals and pharmaceuticals industries are in search of simple, environmentally friendly and inexpensive synthetic routes as alternative of using risky conditions and hazardous compounds in which also high E factor are pursued. One of the most direct way to pursue Green Chemistry principles is the use of truly efficient catalytic reactions. The efforts focused on the development of selective catalysts that are able to suppress the undesired noble metals such as Pd and Pt, has awakened an enormous interest in the topic of 3d metals (Fe, Co, Ni and Cu) as "perfect" candidates. In particular, during the last years, copper (Cu) heterogeneous catalysis has gained more and more attention due to important advantages: compared to other transition-metal, copper catalysts are inexpensive, readily available and can be easily handled.

Being part of important building blocks in organic synthesis, the selective reduction of aromatic nitrocompounds represents a fundamental procedure in organic synthesis and many synthetic routes have been described *via* the catalytic hydrogenation. Herein, we report a mechanochemical reduction of aromatic nitro compounds in a stainless steel jar with formate salts without catalyst addition.[1] In addiction, a new efficient and hydride free Cu-catalysed procedure for the selective reduction of nitroarenes[2] to anilines and azocompounds has also been developed, using glycerol as an excellent "sacrificial" hydrogen source. US has been shown to play an important role in the process *via* its ability to enhance copper nanoparticles (CuNPs) dispersion, favour mechanical depassivation and increase catalytic active surface area, while MW irradiation shortened the reaction time from some hours to a few minutes. Moreover, since recovery and reuse of catalysts is an important factor for sustainable process management, heterogeneous copper supported catalysts has been studied. Copper nanoparticles supported over celite proved to be an ideal material for the transfer hydrogenation reaction of nitrobenzene in continuous flow, as well as a new solid supported copper catalyst based on an efficient grafting of β-CD onto the inorganic silica surface in Cu(II)-catalysed alkyne azide reactions in the absence of a reducing agent.[3]

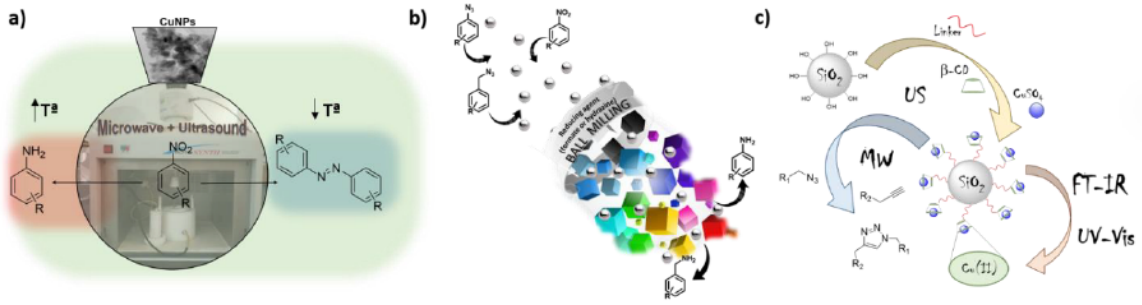

Figure 1: a) Selective copper-catalyzed reduction of aromatic nitro compounds and alkynes under microwave and ultrasound irradiation; b) Highly efficient nitrobenzene and alkyl/aryl azide reduction in stainless steel jars without catalyst addition; c) Sonochemically promoted preparation of silica-anchored cyclodextrin derivatives for efficient copper catalysis.

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Effect of thermal processing on food protein digestibility and allergenicity

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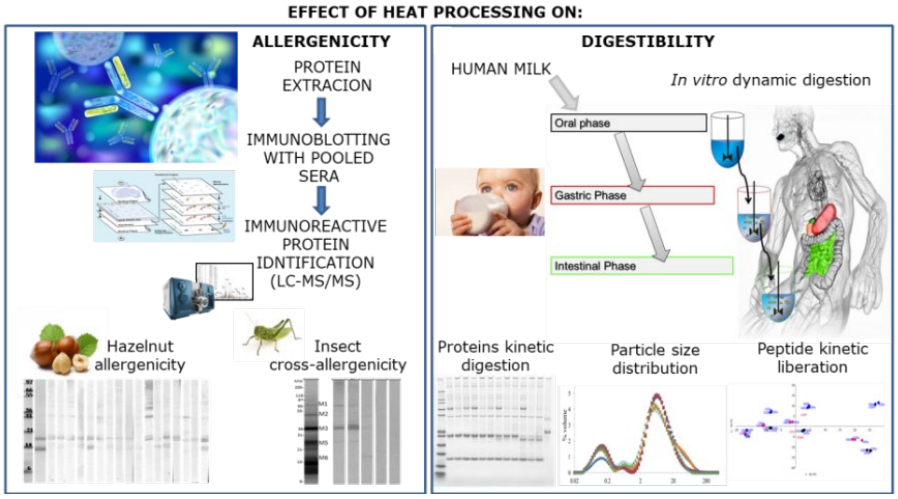
Thermal treatment is the conventional and most commonly used processing technique for many foods in order to reduce their pathogen load, increase shelf life and improve quality and palatability. Different processing methods alter the structure of food proteins in different ways. Structural changes of food proteins induce unfolding of a protein molecule, loss of secondary and tertiary structure, formation of intra and/or inter-molecular covalent and non-covalent interactions between proteins, carbohydrates and lipids. For this reason, food processing may affect to some extent the protein fraction, also by modifying protein solubility, digestibility and allergenicity[1].

The aim of the Phd project was to investigate, by a proteomic approach, the changes in food allergenicity and digestibility induced upon processing of differently raw and processed food matrices: hazelnut, edible insects and human milk. Proteins from hazelnut and insects were extracted and the allergenic profile of raw and processed food was revealed by immunoblotting, using the sera of sensitized patients. Immune-reacting proteins were then identified by Mass Spectrometry. By studying a cohort of pediatric allergic patients in collaboration with Regina Margherita Children's Hospital (Turin), a novel allergen was discovered in hazelnut oil bodies. The sequence was **SUBMITTED, ACCEPTED AND ASSIGNED WITH THE OFFICIAL NAME BY THE I.U.I.S. ALLERGEN NOMENCLATURE SUB-COMMITTEE** (Cor a 15). As far as edible insects are concerned, major allergens of house dust mites (HDM) and shrimp correspond to ubiquitous proteins that are widely distributed among the arthropod[2]. In this respect, the effect of boiling and frying on the possible insects cross-allergenic reaction in a cohort of HDM and shrimps allergic patients was investigated in collaboration with the Mauriziano Hospital (Turin). Processing resulted to slightly affect the cross-allergenicity potential of edible insects and it showed to be protein-, species- and treatment-specific. Human milk in vitro gastro-intestinal digestion was performed in order to assess whether a difference in the human milk digestive kinetics exists, depending on the type of applied pasteurization techniques (Holder vs HTST). An in vitro dynamic model[3] was used and the digestion kinetics of proteins and peptides was studied in collaboration with the institute of Science and Technology of Milk and Eggs- INRA (Rennes, France). **A** better retention of native lactoferrin and milk fat globule associated proteins was found in the HTST treated milk compared to the Holder pasteurized milk. During digestion, a closer amino acid release profile was found between HTST pasteurized and raw human milk, when compared to Holder pasteurized milk, suggesting a better suitability of HTST pasteurization in preserving the original protein bio-availability of raw human milk.

The results of the Phd research allowed to discover a new hazelnut allergen and to obtain novel evidences on the effect of processing and gastro-intestinal digestion on food protein modifications and allergenicity in different foods matrices. In addition, the new evidence will be useful to highlight the biochemical basis of the immunoreaction of sensitized patients to the food allergens.



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Advanced analytical approaches for "omic" investigations of high quality food matrices of vegetable origin

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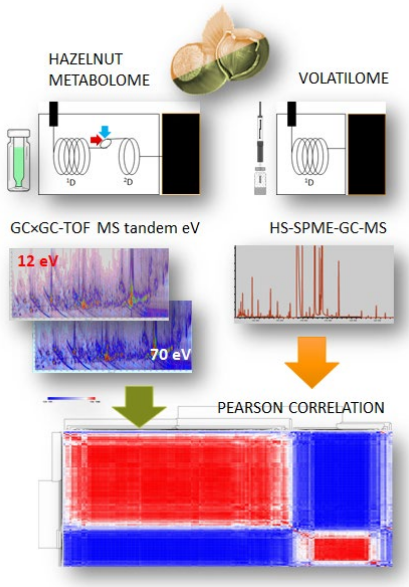
In recent years, consumer preferences have been directed at healthier and more flavorsome food with, above all, higher nutritional value and hedonic quality. Modern "omics" disciplines (foodomics, metabolomics, sensomics, flavoromics, etc.) aim at investigating all food constituents (primary and secondary metabolites, compounds generated by thermal treatments and/or enzymatic activity) in a comprehensive way while correlating biological properties with specific chemical signatures. Sensory properties, for example, can be correlated to quali-quantitative distribution of known (or unknown) taste and odor active chemicals.



The goal of this project is the development of analytical strategies, based on multidimensional chromatography and on mass spectrometry, capable of comprehensively characterizing the food metabolome with particular emphasis on those components directly correlated to sensory properties and hedonic quality[1]. The entire analytical process is considered as an integrated work-flow that includes: (a) dedicated sample preparation; (b) analyte separation and detection in a multidimensional information space; (c) dedicated data processing, and (d) chemometrics for results interpretation and rationalization.

Hazelnuts (Corylus avellana L.) are taken as reference food matrix because of their chemical complexity in terms of primary and secondary metabolites, their relevance in Piedmont economy and for the peculiar sensory profile of particular interest for confectionery industry.

These fruits are characterized by a complex array of primary metabolites strictly correlated to key-volatile components[2] produced during storage and after thermal processing, i.e., roasting. It is indeed well known that some primary metabolites are precursors of (key)-aroma compounds and potent odorants (lysine and proline/1-pyrroline; lysine and arginine/2-acetylpyrroline; leucine/3-methylbutanal; alanine/pyrazines) developed by Strecker degradation and Maillard reaction.

The information potential of comprehensive two-dimensional gas chromatography combined with time of flight mass spectrometry (GC×GC-TOFMS), featuring hard and soft ionization in tandem, is integrated in a work-flow based on pattern recognition algorithms (template matching) developed for the 3D-array of data to achieve a comprehensive untargeted/targeted (UT) chemical fingerprinting[3]. Chemical characterization of key-metabolites is then completed by enantiomeric recognition of key-odorants by dedicated ES-GC-MS and by an accurate profiling of esterified and free fatty acids. The temporal evolution of hazelnuts chemical signature is studied over two harvest years and 12 months of shelf-life. The sampling design includes also reference cultivars of economic relevance and different production areas.

Results pose solid foundation for a rational evaluation of hazelnuts quality "potential" based on advanced analytical work-flows capable of exploring Nature's complexity with high effectiveness.

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Ultrasound assisted C=C bond activation

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The doctoral study arose from the COSMIC (European Training Network for Continuous Sonication) project. The main goals of ultrasound application covered 1) the oxidative cleavage of unsaturated fatty acids to obtain bifunctional monomers and 2) the catalyst reactivation of hydrogenation of bifunctional monomers to obtain the perfect building blocks for polyamides synthesis.

Mono Unsaturated Fatty Acids (MUFAs) from biosourced oils, as castor oil, have attracted a growing interest as a specialty monomer precursor, motivated not only by its renewability, but also by the outstanding polymer technical properties. Being a world leader of biobased polymer production, Arkema (France) designed the polyamide 11 (Rilsan®) and the polyamide 10.10 (Rilsan®T); extraordinary examples of 100% biobased polymers from castor oil seeds. Owing to its unique physicochemical properties such as high flexibility, high resistance to wear due to a low coefficient of friction, these polymers find application in several industries such as textile, electronic, automotive and energy.

Oxidative cleavage of fatty acids and derivatives proved to be an attractive way to obtain bifunctional monomers, which can be used for polycondensation reactions to obtain specialty polymers. Among all the oxidants, hydrogen peroxide proved to be easier to handle than ozone, but the kinetics of the catalyzed reactions are still slow. The time-consuming reaction (24h) requires higher temperatures (85-95°C) to cleave C-C bonds (347-356 kJ/mole, at 298 K) and obtain the desired monomers. In addition, several studies highlighted major limitations in mass transfer leading to undesired by-products and lack of reproducibility at larger scale. Arkema has selected mono-unsaturated fatty nitriles from MUFAs as precursors of a new synthetic route to obtain monomers for PA11 and PA12 using hydrogen peroxide[4]. The solventless multiphasic system requires a phase transfer agent to promote the difficult mass transfer under several published processes. Since 1927 ultrasound technology confirmed its potential in a variety of catalytic processes. It has been also proved that mass transfer across the phase boundary of a multiphasic system is substantially enhanced through acoustic emulsification. The aim of our work was to exploit sonochemical methods to overcome mass transfer and time limitation of conventional oxidative cleavage reaction in UFAs and derivatives. Experimental data obtained in an ultrasonic tank and an ultrasonic horn were compared with results obtained in a conventional batch reactor. Significant results were obtained with an ultrasonic horn, working at frequency of 25 kHz and 100W of input power. With this system we were not only able to reduce 1) the reaction time from 24h to 5h, 2) the concentration of H_2O_2 from 70% to 35%, 3) reduce the working temperature from 90°C to 60°C but also 4) to achieve the desirable monomers in the absence of an emulsifier. Besides the safer alternative of using lower concentration of oxidant and no need of emulsifier, with this new technology, the monomers can be obtained in higher yield at shorter reaction time. Studies in a continuous system to prove the applicability or not for industrial implementation are also highlighted. The application of ultrasounds in Ru-based and Raney-Nickel® catalyst reactivation of hydrogenation of mono unsaturated nitrile esters to amino-esters is also compared with conventional methodologies.

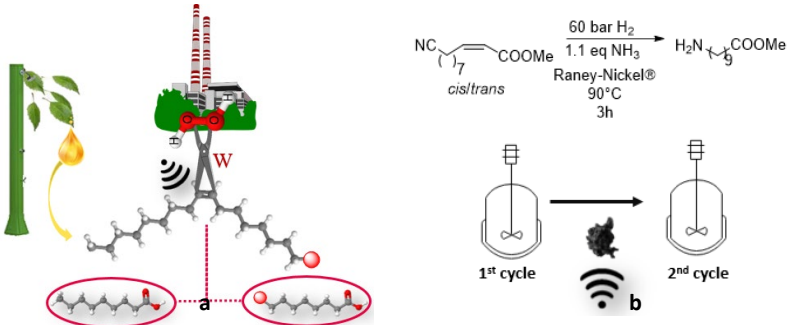

Figure 1. a- Ultrasound-assisted oxidative cleavage of fatty compounds with H_2O_2 to obtain bifunctional monomers, **b-** Application of ultrasound technology in regeneration of Raney-Nickel® catalyst applied in the hydrogenation of unsaturated nitrile-esters.

Molecular and chemical fingerprinting of high oleic sunflower hybrids. "A systems biology approach"

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Helianthus annuus L. (sunflower) is one of the most cultivated oil crops in the world, indeed the world production is estimated at 21 million hectares in 60 countries. It is the second largest hybrid crop, and the fifth largest oilseed crop, with an annual value of over 40 billion $. The main use of sunflower seeds is oil production. The quality of sunflower oil depends on the quality and content of fatty acids. Besides Helianthus annuus, different species or cultivars of the same genus are present, each one characterized by small differences in their genome. These can affect not only the phytochemical oil profile but also the whole seed composition. Because species of the same genus have very similar phenotypic characteristics, molecular and chemical fingerprinting is one of the most important instruments to screen among varieties. On the other hand, sunflower oil production generates a dramatic waste flow, that represents an economic problem for companies for its disposal. A challenge can be represented by the reuse of residues from oil production, into a virtuous circular economy (CE) strategy. Recently, environmental CE is increasing the trend of using food waste byproducts as alternative source of bioactive compounds. Moreover, several studies have shown how from this waste byproducts polyphenols, including chlorogenic acids, can be extracted. These bioactive compounds showed the same biological activities (antioxidant, antiproliferative, antinflammatory, antidiabetic properties). The development of new methods for the characterization of sunflower cultivars and hybrids is crucial for their unequivocal identification. This project, financed by the SO.RE.MO. S.A.M. of the Ferrero Group and scientifically supported by Biosfered Srl, aims to combine a molecular approach with chemical data to define a unequivocal and rapid method fpr sunflower screening.

Figure 1. Economic enhancement, molecular and chemical standardization of sunflower waste by-products; a) Neochlorogenic acid; b) Oleic acid

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ANALYSIS AND FOOD PROCESSING OF SUNFLOWER (Helianthus annuus L.) NATURAL EXTRACTS

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The cultivated sunflower (*Helianthus annuus* L.) is one of 67 species in the genus *Helianthus*. It is a dicotyledonous plant and a member of the Compositae (Asteraceae) family, having a typical composite flower. Originating in subtropical zones, sunflower has been made highly adaptable through selective breeding to various climates, in particular to temperate regions.
Sunflower seeds are a good source of all essential nutrients. Currently available information on sunflower seeds is still insufficient. These observations have led to continuing research aimed at identifying specific bioactive components. The chemical composition of sunflower oil is one of the major determinants. Sunflower refined oils contain 98% fatty acid triacylglycerols, the other 2% being mainly micronutrients. Sunflower, is also a source of phenolic compounds, coenzyme Q9, tocopherols, phytosterols and proteins. Among alternative plaint proteins, sunflower seeds are particularly interesting in view of their widespread availability in areas where soy is not or only sparsely produced. Compared with other sources of vegetable proteins, sunflower seeds have been reported to have a low content of antinutritional factors.
Therefore, the project aims to:

1. Selection of sunflower seed varieties
2. Determination of key chemical features
3. Chemical characterization of sunflower components by HPLC-MS, GC-FID and GC-MS.
4. Analysis of protein lipid content, focusing particularly on amino acid and fatty acids composition
5. Analysis of sunflower hull non-starch sugar and polyphenolic content of two high-oleic cultivars.
6. Characterization of products derived from roasting and processing of sunflower and Piedmont hazelnut

Over the three years, a study was carried out on sunflower seed with the final aim of evaluating its application in industrial products.
The analysis conducted in the first year showed, as from bibliographic data, a greater suitability for the industrial use of high oleic sunflower hybrids which denote a greater resistance to oxidation, a chemical process which, if persistent, can lead to an acceleration of the organoleptic decay of the product.
In order to enhance the sunflower hull, a by-product of seed squeezing, fiber and polyphenol composition analyses were performed.
The objective of this research was to enhance this by-product obtained from the squeezing of the seed, to date destined solely for the livestock industry. The results obtained have revealed a high potential due to the high content of fibers and polyphenols, opening new paths for new possible food uses.
The last phase of the work consisted in comparing the aroma components of the hazelnut with those of the sunflower in order to evaluate the common points and the possible organoleptic differences. The results of this survey indicated a modest overlap between the volatile chemical component of sunflower melanoidins and that of the Piedmont hazelnut.
However, these values are a good starting point to understand if and how the roasting process can affect the development of the aroma of these two noble raw materials.