DIPARTIMENTO DI SCIENZA E TECNOLOGIA DEL FARMACO
UNIVERSITÀ DEGLI STUDI DI TORINO

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FOOD PROCESSING INNOVATION AND GREEN EXTRACTION TECHNOLOGIES:

RECENT ADVANCES AND APPLICATIONS IN HUMAN HEALTH

BOOK OF ABSTRACT

CATANZARO

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INTERREGIONAL RESEARCH CENTER

FOR FOOD AND SAFETY

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Introduction

Enabling technologies in food processing and green extraction from academia to innovative and large-scale applications: an introduction

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The key aims of the workshop organized in Catanzaro at the Magna Graecia University are to provide a European framework for integrated, multidisciplinary research on innovative technologies in food processing and green extraction and to foster discussion, exchange and research collaboration between academic researchers and industrial partners. Some contributions will deal with the most recent advances on typical nutraceuticals occurring in the Mediterranean diet. The outstanding speakers coming from different areas of expertise will guarantee a wide overview and a high scientific level, the first condition for a successful venue.

The workshop introduction will outlines of the main topics with a short survey of the most recent technological achievements for process intensification, mainly innovative protocols for plant extraction and food treatments.

An introduction on non-conventional energy sources applied in highly efficient and sustainable processes together with a series of last-generation reactors will be presented highlighting the scalability at industrial level.

Beside the scientific achievements and the potential business opportunities, the location of the workshop in the heart of the Mediterranean Sea should also spend a thought to the recent tragic events linked to the migrants drama and see which kind of contribution could offer the scientific community to inspire international actions.

Plenary Lectures

PL1

Production of Plant Extracts – from Evolution to Revolution Resource Efficient Manufacturing in Regulated Industries

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Plants come back into focus also of the chemical industry in a time of increasing impact for sustainability and environmental compatibility. Plant constituents substitute basic chemicals of petrochemical origin more and more. The first step for preparing the desired natural constituents is always an extraction. Today the central aim for research and development of extraction procedures are careful, efficient and successful processes. Only this will guarantee the necessary high quality and attractive economic efficiency in the use of plant extracts. In the last years, process design as well as optimization of existing processes is supported by modeling the unit operations. Therefore, model parameters must be determined in lab scale [1].

On the other hand, new principles in the research are generated, which allow a rapid screening of possible conditions for extraction in the view of basic proceedings, solvents, temperatures and pressures. So also the complex character of plant extracts is considered, which is determined by the multicomponent mixture existing of the group of the interesting constituents and also the side-fractions. The extraction process has to guarantee that side-fractions are not critical in the following use of the plant extract. For that all problematic fractions should not be extracted which will be achieved by selective extractions. [2, 3] Concepts and cost structures for further product purification will be discussed with regard to innovative resource efficient manufacturing technologies. [4]

In addition, the activities of the German Dechema/ProcessNet working group on “plant-based extraction – products and processes” are presented and thereby opened for any participation. [5]

[1] S. Both, I. Koudous, U. Jenelten, J. Strube. Model-based equipment-design for plant-based extraction processes- considering botanic and thermodynamic aspects. C. R. Chimie (2013). http://dx.doi.org/10.1016/j.crci.2013.11.004

[2] I. Koudous, S. Both, G. Gudi, H. Schulz, J. Strube. Process design based on physicochemical properties for the example of obtaining valuable products from plant based extracts. C. R. Chimie (2013). http://dx.doi.org/10.1016/j.crci.2013.11.003

[3] Tegtmeier, M. (2012). Plant Extraction: Key Technology for Sustained Use of Bio-Ressources; Chemie Ingenieur Technik 84 (6)

[4] Chem. Ing. Tech. 2014, 86, No. 5, 1–9; Efficient Engineering and Production Concepts for Products in Regulated Environments – Dream or Nightmare?, Jochen Strube, Reinhard Ditz, Holger Fröhlich, Dirk Köster, Thomas Grützner, Jörg Koch, and Rüdiger Schütte, DOI: 10.1002/cite.201300081

[5] http://www.processnet.org/en/Plant+based+Extracts+ +Products+and+Processes-p-222.html

PL2

Natural ProductsfromHerbs by UltrasonicallyAssisted Extraction. A Green Extraction Technique

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"In one wayitcouldbesaidfairly, thatSonicsisdaughter of musical Harmony,becauseitis in thatwayit came intobeing" [1].

Ultrasonically Assisted Extraction (UAE) of natural products has proved to be one of the most popular topics over the latest 10 years, over 1,700 articles being published in this intervaland over 330 in 2013.
The lecture will present a historical point of view on natural products extraction with theaccent on latest developments in the field:

- Herbal extracts introduction
  - Definition
  - A short history of herbal extracts
  - Extraction procedures

Ultrasonically Assisted Extraction will be presented in more details including:

- Ultrasonically Assisted Extraction
  - UAE, general unit operation scheme
  - Processing of herbs

Some good practice rules will be emphasized:

- Swelling index
- Extractive value
- Residual moisture
- Particles size [2]
- Extraction parameters:
  - Type of extraction:
    - Continuous
    - Batch
  - Ultrasonic – real – power [3]
  - Extraction time
  - Extraction temperature

with the hope that in the near future this green technique – UAE – will became a routine and accepted technology as part of the phytopharmaceutical industry.

[1] Constantinescu, G., Theory of Sonics: A Treatise on Transmission of Power by Vibrations, The Admiralty, 1918, London.
[2] M. Vinatoru, unpublished results, part of DumitruBadica Ph.D. thesis, 2001, Polytechnic University, Bucharest, Romania
[3] TakeyoshiUchida, TsuneoKikuchi, Ultrasonic power measurement by calorimetricmethod by using water as heatingmaterial - Comparisonwith radiation balance method -, Proceedings of Symposium on UltrasonicElectronics, Vol. 33 (2012) pp. 55-56 1P2-10 13-15 November, 2012.

PL3

Green Extraction of Natural products. From innovations to industrial applications

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This presentation will introduce a new and innovative area in the frontiers of chemistry, biology and processing: green extraction with special emphasis on medicinal and aromatic plants. Green extraction is a part of the sustainable development concept; its history, concept, principles and fundamentals will be described. We will pay special attention to the strategies and the tools available to make biorefinery greener. The representation will present the innovative research in this area these past five years in term of innovative techniques (microwave, ultrasound, pulse electric field...) and alternative solvents (ionic liquids, sub and supercritical fluid, agrosolvents, water...) applied to this new area green extraction of natural products with special examples applied to biorefinery concept.

A general definition of green chemistry is the invention, design and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances. In relation of green extraction of natural products, this definition can be modified as follows: "*Green Extraction is based on the discovery and design of extraction processes which will reduce energy consumption, allows use of alternative solvents and renewable natural products, and ensure a safe and high quality extract/product*". The listing of the "six principles of Green Extraction of Natural Products" should be viewed for industry and scientists as a direction to establish an innovative and green label, charter and standard, and as a reflection to innovate not only in process but in all aspects of solid-liquid extraction. The principles have been identified and described not as rules but more as innovative examples to follow discovered by scientist and successfully applied by industry.

F. Chemat
Eco-Extraction du Végétal : procédés innovants et solvants alternatifs.
DUNOD, Paris, 2011.
F. Chemat, M. Abert-Vian, G. Cravotto
Green Extraction of Natural Products: Concept and Principles
International Journal of Molecular Sciences, 2012, 13, 8615-8627.

PL4

Analytical Tools For The Analysis And Quality Control Of Herbal Material

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In the past decades, major efforts have been made to study the effects and efficacy of single herbs and of combinations of herbs used in traditional medicine systems. Herbs or botanicals used in folk medicine usually contain dozens of bioactive compounds and in many cases it is not clear which of these compounds underlie an herb's medical use. Besides identification of the active principles from the multitude of constituents analytical assessment of plant material and the products thereof is of major importance. Gas chromatography/mass spectrometry (MS), liquid chromatography (HPLC/UHPLC)/MS or capillary electrophoresis/MS have been proven to be efficient separation techniques. More sophisticated methodologies are LC-SPE-nuclear magnetic resonances (NMR), NMR based metabolic profiling as well as quantitative NMR. In recent years also supercritical fluid chromatography was becoming more and more popular. The suitability of some of these techniques for the quality control of natural products will be demonstrated by means of selected examples.

Lectures

L1

Green extractions to improve yields and final quality of two botanicals: the case of rosemary and lemon balm.

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The recovery of characterized herbal extracts from raw material is commonly obtained by applying traditional standardized processes, some of which are described in the European Pharmacopoeia. The phenolic compounds are a group of phytochemicals well recognized for their health properties in humans. Nevertheless, there is little information available on the features of phenolic extracts obtained by ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). These techniques can certainly be considered green processes as they help to accelerate the extraction procedure and reduce energy consumption.

In this study different extractive procedures applied to aromatic plants and food by-products will be discussed. The efficiency and selectivity of UAE and MAE in recovering the phenolic fractions from *Rosmarinus officinalis* leaves and *Melissa officinalis* L. aerial parts using the last generation of dedicated equipment will be discussed.

Two groups of compounds are mainly responsible for the numerous biological activities of *Rosmarinus officinalis* L.: the volatile fraction and phenolic compounds, mainly constituted by rosmarinic acid and some diterpenoids derived from carnosic acid. Several extractive sequences were tested to optimize the recovery and/or test the ability to fractionate the rosemary phenols. The phenolic content of dried rosemary leaves under MAE and UAE was more than 3 times higher than the classic solid-liquid extraction, that requires ten-fold more time to perform [1]. When comparing final yields, it was pointed out that high-intensity ultrasound is the most effective and versatile method and avoids the oxidation processes that produce carnosol and rosmanol from the precursor carnosic acid. Among the 20 different samples, we selected an ultrasound extract characterized by a high concentration of terpenoids (mainly carnosic acid) and by the absence of rosmarinic acid. Moreover, the extractive ability of a common organic solvent such as hexane was strongly modified by the use of UAE as observed for the rosemary leaves.

Melissa officinalis L, well known for its sedative, spasmolytic, antitumoral and antioxidant properties, contains protocatechuic acid, caffeic acid and rosmarinic acid as the most representative phenols in the aerial parts [2]. As expected, rosmarinic acid was always the main component in all the obtained fractions of lemon balm while ethanol was the best solvent for both UAE and MAE procedures.

1-Mulinacci N., Innocenti M, Bellumori M, Giaccherini C, Martini V, Michelozzi M. *Talanta* **2011**, *85*, 167-176

2. Miron T.L., Herrero M., Ibanez E. *Journal of Chromatography A*, **2013**, 1288, 19

L2

Food processing and human health: the novel redoxomic approach.

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The continuous challenge of food processing is to warrant a balanced solution between benefits and possible drawbacks for human health. On the general background of chemical modifications than can affect both nutritional quality and safety of vegetable or animal-derivative foods in such process oxidative changes may play a relevant role. Main targets of oxidation are unsatured/polyunsatured fatty acids that are widely distributed in seeds and their derivatives (e. g. oils), fishes and animal meats. When the natural antioxidant barrier of raw material is destroyed by reactive species like reactive oxygen species (ROS) unsatured lipids undergo to isomeric conversion and therefore to peroxidation that leads from lipid peroxides to small oxidised end-products including hydrocarbons and aldehydes. This uncontrolled daily burden of oxidants by improperly processed foods may have a strong negative impact on human health, including the acceleration of aging and the triggering/worsening of oxidative stress-related diseases (e. g. atherosclerosis, diabetes, neurodegenerative disorders, cancer and so on).

Therefore one of the aims of food processing is to avoid or slow down unwanted oxidative reaction chains by reducing the impact of oxygen (e. g. with packaging adaptations) and/or improving antioxidant barrier against oxidation (e. g. with preservatives) from the raw materials to the edible end products.

Redoxomics is a novel branch of "applied biochemistry" and "molecular diagnostics" having the following aims:

- to analyse the structure, the physiological role and the distribution of reactive species and antioxidant systems in a living organism;
- to identify the reciprocal interactions of oxidant and antioxidant systems – in the general flow of information – in a biological system (cell, tissue, organ, apparatus, system, whole organism) in a defined step of its development, in basic conditions as well as after potentially stressful stimuli;
- to evaluate the implications of these findings by the view-point of epidemiology, patophysiology, clinics, pharmacology and so on.

The ambitious goal of redoxomics (as well as for other "-omics" in other fields) is "to map" dynamically – by means of all the available and sophisticated analytical techniques – the whole oxidative-antioxidant repertoire, i. e. the "redoxoma" of a living unit in different conditions. Redoxomics approach includes chemical analyses aimed to identify and to quantify oxidation biomarkers in edible vegetable and animal foods (e. g. dosing of total lipid peroxides in extra-virgin olive oil, determination of total vegetable polyphenols) as well as in blood samples (e. g. d-ROMs test, BAP test). Among the innovative tools of redoxomics is the lipidomics of plasma membrane that allows to measure the level of trans fatty acids in erythrocytes.

[1] Iorio EL, Marin MG. Redoxomics. An integrated and practical approach to genomics, metabolomics and lipidomics to manage oxidative stress. 2008. Gen-T. 2: 67.

L3

Pharmaceutical Nanotechnology in Nutraceuticals: from Food to Health Sciences

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From the end of 90s the pharmaceutical nanotechnology prompted colloidal drug delivery systems to move from the scientific speculation to the clinical use, thus providing suitable nanomedicines for the efficacious treatment of a number of diseases. The advantage of last generation nanomedicines is based on the possibility to modulated the device as a function of the therapeutic requirements. Recently, the pharmaceutical nanotechnology drove the attention also to natural active compounds coming from both medical plants and foods thus allowing the possibility to achieve nanomedicines and nutraceuticals based on natural products. Therefore, the use of innovative drug delivery systems can provide an advancement in functional foods and natural compounds by improving their biopharmaceutical features [1].

The topical application of natural substances to prevent or treat skin disorders represents an interesting and innovative field of application. The potentiality of ultradeformable vesicles, i.e. ethosomes®[2] and niosomes[3,4], as a possible topical delivery system for *Glycyrrhiza glabra L.* extract was evaluated. These highly-deformable vesicles allowed the greatest *in vitro* skin permeation of ammonium glycyrrhizate by showing a 10-fold increase with respect to the free drug solution, thus improving the anti-inflammatory activity of the delivered natural compound as demonstrated using an in vivo model of skin erythema on human volunteers. The use of vesicular nanocarriers allowed also the potential application of *Citrus Bergamia* extracts for dermo-cosmetic use to treat non-pathological disorders, i.e. photo-ageing and photo-induced erythema. This is also the case of oleuropein and its active metabolite hydroxyl-thyrosol, that formulated as a nanodevice dosage form can be applied for both topical and systemic administration. In fact, not only topical application but also other administration routes are possible for nanomedicines based on food-derived natural compounds as well as their application for the treatment of severe pathologies, i.e. cancer disease. It was the case of *Citrus Bergamia* extract loaded within a liposomal carrier [5], which allowed a significant increase of the *in vitro* anticancer activity.

The real advantage of the application of pharmaceutical nanotechnology in the field of food-derived natural compounds is the fact that it can provide a concrete chance for the application of these compounds for therapeutic use, thus ensuring the right dose at the right time in the right place.

[1] Paolino D, Cosco D, Cilurzo F, Fresta M. Curr Bioactive Comp 2007; 3: 262-277.
[2] Paolino D., Lucania G, Mardente D, Alhaique F, Fresta M. J. Control. Rel. 2005; 106: 99-110.
[3] Marianecci C., Rinaldi F., Mastriota M., Pieretti S., Trapasso E., Paolino D., Carafa M. J. Control. Rel. 2012; 164: 17-25.
[4] Marianecci C, Rinaldi F, Di Marzio l, Mastriota M, Pieretti S, Celia C, Paolino D, Iannone M, Fresta M, Carafa M. Int J Nanomedicine 2014; 9: 635-651.
[5] Celia C., Trapasso E., Locatelli M., Navarra M., Ventura C.A., Wolfram J., Carafa M., Morittu V.M., Britti D., Di Marzio L., Paolino D. Colloids Surf. B Biointerfaces 2013; 112: 548-553.

L4

Subcritical Water Extraction as a Green Extraction Technique in Food Samples

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The Green Chemistry Centre of Excellence (GCCE) aims to promote the development and implementation of green and sustainable chemistry and related technologies with new products and processes. Subcritical water extraction (SWE) is a promising "green" technique based on the use of water as the sole extraction solvent, at temperatures between 100 and 374 °C and at a pressure high enough to maintain the liquid state. Temperature is one of the critical factors that affect the extraction efficiency. The use of high temperatures improves the extraction efficiency as it helps the disruption of analyte-sample matrix interactions caused by van der Waals forces, hydrogen bonding and dipole-dipole interaction. Increasing temperature means water becomes less polar. The polarity of subcritical water is measured by the value of the dielectric constant. When water is heated above 100 °C its dielectric constant decreases and water becomes similar to organic solvents. The reduction of the polarity of water generally leads to an increase in the solubility of organic molecules in it. When the temperature of water was raised from 25 °C to subcritical conditions at 250 °C, the solubility of chlorothalonil increased by a factor of 130 000. Some compounds from plant samples can degrade around 175-200 °C during extraction so care should be taken to find the optimum temperature for each type of sample. SWE as a method, is non-toxic, non-flammable, cheap, readily available, safe, environmentally friendly and uses a green solvent. The extraction of phenolic compounds, carotenoids, flavonoids, flavor, fragrances and essential oils have been carried out using SWE and both qualitative and quantitative results obtained. We have recently efficiently extracted pectin from citrus peel, pea vine and mango peel samples; flavonoids from citrus peel and potato peel; protein from potato peel and essential oils from lavender and oregano using SWE.

L5

Bergamot polyphenol fraction prevents non-alcoholic fatty liver disease via stimulation of lipophagy in cafeteria diet-induced rat model of metabolic syndrome.

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Non-alcoholic Fatty Liver Disease (NAFLD) is the most common liver disease in industrialized countries. Defective autophagy of lipid droplets in hepatocytes, also known as lipophagy, has been recently indicated as a possible pathophysiological mechanism of NAFLD. Experimental and epidemiological evidence suggest that dietary polyphenols, such as flavonoids may prevent NAFLD. To address this hypothesis and analyze the underlying mechanisms we supplemented Bergamot Polyphenol Fraction (BPF) to cafeteria (CAF) diet-fed rats, a good model for pediatric metabolic syndrome and NAFLD. BPF treatment (50 mg/kg/day supplemented with drinking water) potently counteracted pathogenic increase of blood triglycerides and had moderate effects on blood glucose and obesity in this animal model. Importantly, BPF strongly reduced hepatic steatosis as documented by ultrasound examination and histological analysis of liver sections. The morphometric analysis of oil-red stained sections confirmed a mean 48.5% reduction in size and in numbers of lipid droplets accumulated in hepatocytes. BPF-treated livers showed increased levels of LC3 and Beclin-1 and reduction of p62, suggesting autophagy stimulation. Higher levels of LC3II in the lipid droplet subcellular fractions from BPF-treated animals indicated lipophagy stimulation by polyphenols present in BPF. This was confirmed by *in vitro* studies. BPF and BPF-derived flavonoid aglycons dose-dependently enhanced autophagic flux and AMP kinase activation in HepG2 cells. This correlated with the ability of BPF to counteract the accumulation of palmitic acid-induced lipid droplets. This study suggests that the liver and its lipid metabolism are the main targets of bergamot flavonoids, indicating BPF as an effective strategy to prevent NAFLD.

L6

Waterplant Constitutional, novel pharmaceutical form, topical and systemic in functional medicine.

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Plant Cell Water: a new and exclusive natural ingredient for health products. The plants are naturally rich in water 80 - 90%. In this drying process is dissipated into the atmosphere in vapor form.

The Valverbe Company, through a process of dehydration in cold edge of the fresh organic herbs, is able to extract the fraction of constitutional water so highly conservative.
This water contained within the plant tissues is pure distilled water, which are the imprint of many useful substances of the mother plant. Essential oils, trace elements, and active ingredients are present in a reduced but detectable. Analyzes conducted in university research laboratories have shown the presence of these substances.

In contrast to hydrolates, obtained by distillation in steam current, these waters have not undergone the process of vaporization at high temperature and therefore the active costituens, although in trace amounts, do not undergo any deterioration.

These waters constitute a simple but innovative ingredient that lends itself to be used for both preparations for oral use that topical: solvent extraction of the same plants from which the water is obtained in such a way as to obtain the truly "phytocomplex" of the plant extracted, see hydroalcoholic extracts, macerated glycerol, prepared syrups or soft drinks, or as an aqueous phase of lotions, cleansers, mouthwashes.

Are many possible applications not counting the value of "energy" inherent in a "constitutional water"

The study program is based on two lines of research, such as *biochemistry* and quantitative analysis of fractions of active ingredients and nutrients important for metabolic activity, although within the framework of Functional Medicine; that the *frequency*, similar to the known pharmacological and medical subjects: Oligoterapia, Schussler salts, Flower Therapy, Homeopathy, for whom efficacy and desirable to encode is to be assessed according to the methods of imprinting the frequency of each specific water plant of the constitutional available selected plants.

The research team, at the time he made a first study on the water plant constitutional Bergamot, relative to metals of Oligotherapy; significant results are the quantitative presence of Manganese and Copper. The work in progress of the study program expects to be able to assess the clinical efficacy according to the directions on the second head of Menetriere diathesis, both orally and for topical use; as the same Oligotherapy expected.

L7

Alternative semi-synthetic routes towards new food additives from olive leaves.

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Numerous evidences suggest a direct relationship between the high consumption of virgin olive oil (VOO) phenols in the Mediterranean diet and a lowered incidence of cardiovascular diseases, cancer and diabetes. The main phenolic compounds in VOO are oleuropein **1**, demethyloleuropein **2**, hydroxytyrosol **3** and aglycones 3,4-DHPEA-EA **4** and 3,4-DHPEA-EDA **6** (Figure 1).[1]

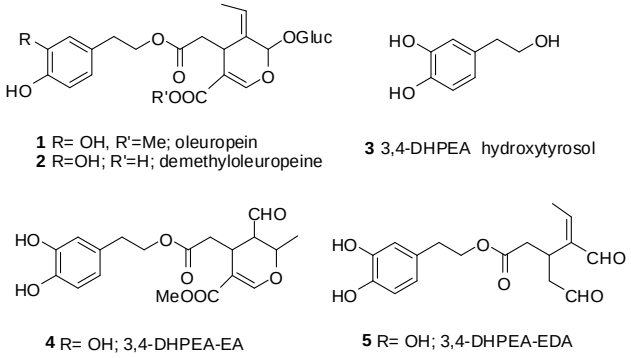

Figure 1

This work summarize our efforts to optimize semi-synthetic catalytic protocols for chemical manipulation of oleuropein towards a new set of natural analogues with improved lipophilicity, whose biological activity as anti-inflammatory, antioxidant or anti-neoplastic agents has been extensively explored.[2] Each synthetic step, starting with the extraction from the vegetal matrix, was performed in a sustainable and scalable way, using eco-friendly reagents and solvents.[33] In particular, peracetylated oleuropeina and peracetylated olive leaves extract were added to olive oil using a US-assisted procedure. Preliminary results of their use as protective food additive for olive oil are presented.

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[2] (a) S. Bulotta, R. Corradino, M. Celano J. Maiuolo, M. D'Agostino, M. Oliverio, A. Procopio, S. Filetti, D. Russo. *J. Mol. Endocrinol.*, **2013**, *51*, 181-189. (b) S. Bulotta, R. Corradino, M. Celano, M. D'Agostino, J. Maiuolo, M. Oliverio, A. Procopio, M. Iannone, D. Rotiroti, D. Russo. *Food Chem.*, **2011**, *127*, 1609-1614; (c) C. Muscoli, F. Lauro, C. Dagostino S. Ilari, L. A. Giancotti, M. Gliozzi, N. Costa, C. Carresi, V. Musolino, F. Casale, D. Ventrice, M. Oliverio, E. Palma, S. Nisticò, A. Procopio, V. Mollace.– *JBRHA*, **2014**, *28*, 107-118.

[3] (a) A. Procopio, G. Sindona, M. Gaspari, N. Costa, M. Nardi. **2008** *International Patent n°* PCT/IT2008/000303. (b) A. Procopio, S. Alcaro, M. Nardi, M. Oliverio, F. Ortuso, P. Sacchetta, D. Pieragostino, G. Sindona. *J. Agric. Food Chem.*, **2009**, 57, 11161–11167. (c) A. Procopio, C. Celia, M. Nardi, M. Oliverio, D. Paolino, G. Sindona. *J. Nat. Prod.*, **2011**, *74*, 2377-2381. (d) A. Procopio, S. Bonacci, G. De Luca, M. Nardi, M. Oliverio, G. Sindona. *Food Chem.*, **2014**, *162*, 89-93.

L8

Effects of Dietary Polyphenol-Rich Extra-Virgin Olive Oil on Oxidative Stress and DNA Damage in Women with Cognitive Decline

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Objectives: A high-fat diet increased reactive oxygen species in the cerebral cortex.[4] Currently, much attention has been focused on the potential of using dietary components as neuroprotective agents. The present study investigated the role of dietary polyphenol-rich extra-virgin olive oil and Mediterranean Diet against oxidative stress and DNA damage in elderly women with mild cognitive decline. **Method**: We studied antioxidant parameters and DNA damage in 59 women enrolled among those participating the "Study of the Mediterranean Diet and Cognitive functions" approved by Italian Health Secretary, having mild cognitive decline. We measured the above parameters at basal, after 3 weeks at 20 g/day and after further 3 weeks at 40 g/day of a polyphenol-rich (301mg/Kg) extra-virgin olive oil and Mediterranean Diet (energy from fat max 30%). Activities of erythrocyte copper zinc-superoxide dismutase (CuZn-SOD), glutathione peroxidase, total antioxidant capacity and glutation peroxidase in the blood were measured. Using the CBMN test, DNA damage on lymphocytes was determined by frequency of micronuclei (MN) per 1000 binucleated cells, nucleoplasmic bridges and nuclear buds. **Results**: An increase SOD activity was observed with 20g compared to basal (see table) however the frequencies (‰) of MN with 40g were statistically significant increased compared to basal condition (see table).

	BASAL	WITH 20g	WITH 40g	p
SOD(UI/ml)	191.5± 53	224.6±41	207.50 ± 53	0.05 (B vs 20g)
MN(‰)*	4.0 ±0.8	5.3 ± 0.06	5.7 ± 0.02	0.02 (B vs 40)
* 5 patients				

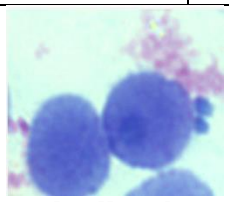

Figure: binucleated cell with micronuclei (MN)

Conclusion: Polyphenol-rich EVO use (20 g/day) improves antioxidant status but high amount (40 g/day) reduce chromosomal stability in mild cognitive decline. The high-fat regimen may overwhelm the beneficial effects of phenolic component[1]

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L9

Nutraceuticals in Pain

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Millions of people worldwide suffer from pain of various etiology as a result of damage to or dysfunction of the nervous system under various disease conditions. Development of effective therapeutic strategies requires a better understanding of molecular and cellular mechanisms underlying the pathogenesis of pain.
In healthy subjects, acute-pain processing consists of a chain of events that begins with stimuli that activate specialized receptive endings on peripheral sensory nerve fibres. However, pain processing is not simply a relay of signals from the body to the brain, it is a redundant, multi-pathway and dynamic system in which profound pain suppression or enhancement can occur at any level of synaptic communication. When dysfunctional pain signalling occurs, pathological pain ensues. Lesions of the nervous system can produce a form of pathological pain, neuropathic pain, which is characterized by unexplainable widespread pain, a sensory deficit, a burning sensation, pain caused by light touch (allodynia), or acute pain in the absence of a noxious stimulus. Furthermore, chronic neuropathic pain can persist for months, without the underlying cause being treatable or identifiable. Severe pain syndromes reduce quality of life in patients with inflammatory and neoplastic diseases, partly because the reduced analgesic effectiveness accompanying chronic opiate therapy (i.e. tolerance) leads to escalating doses and distressing side effects.
Accordingly, there is major interest in new approaches to maintain opiate efficacy during repetitive dosing without engendering tolerance or causing unacceptable side effects. Recent mounting evidence implicates nitroxidative stress caused by the presence of superoxide, nitric oxide and subsequently peroxynitrite during pain development. Here, we provide a nutraceutical basis for developing inhibitors of oxidative stress as potent adjuncts to pain killers in the management of chronic pain, addressing an issue of major clinical and socio-economic importance while laying the basis for interventions with strong therapeutic potential.

Poster Communications

P1

Evaluation of antioxidant and antimicrobial activity of *Sinapis nigra* L. extracts

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http://www.unito.it/unitoWAR/appmanager/dipartimenti3/D025?_nfpb=true

Nowadays there is a growing demand by consumers for the use of natural antimicrobials to enhance safety, shelf life and quality of foods because they are considered less or not harmful to human health.[5]

Glucosinolates present in plants belonging to *Brassicaceae* family are natural antimicrobials because isothiocyanates precursors. *Sinapis nigra* L. seeds and powder contain high levels of glucosinolate sinigrin which is hydrolized to allyl isothiocyanate in the presence of moisture and myrosinase plant enzyme.[6,7]

Mustard seeds were extracted with different solvents (EtOH/H_2O 8:2, H_2O and H_2O/β-CD 1.5%) to evaluate their sinigrin content by HPLC analysis, and their antioxidant activity with DPPH test. The cryogenic grinding of frozen seeds afforded a powder (≈ 300 μm) that was sonicated few minutes in EtOH/H_2O (high-intensity ultrasound at 20 kHz) and left in e maceration. This protocol gave the highest sinigrin yield in the final extract (18 mg/g seeds).

Because of some microorganisms have been found to possess myrosinase-like action,[8,9] the antimicrobial activity of the extract with the higher sinigrin content was evaluated on some common food pathogens (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538 P, *Streptococcus pyogenes* ATCC 12344, *Candida albicans* ATCC 10231 and *Pseudomonas aeruginosa* ATCC 10145) with the disc diffusion test.
The inhibitory effect against *S. pyogenes*, *C. albicans* and *E. coli* prompted us to evaluate the activity of nebulized extract on packaged salad in term of shelf life. The promising results achieved opened the door to further investigation at semi-industrial scale.

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Pulse Electric Field And Utrasound Extraction Of Plant Materials

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Fruit is a natural rich source of antioxidants such as vitamin C, polyphenols, flavonoids and anthocyanins. These compounds have positive effects on human health. It would be therefore interesting to recover these bioactive compounds from vegetable by products to enrich other food products.
The purpose of the research was to compare the extraction efficiency, on grapes skin and plums, of two novel technologies: pulsed electric fields (25kV,10Hz, 6μs pulse) and ultrasound (400W, 24kHz, 120μm amplitude, 30 min, 25°C and 50°C) with the conventional aqueous extraction technique. Extraction yields of polyphenols, flavonoids, anthocyanins and vitamin C, antioxidant capacity, pH, acidity, conductivity, soluble solids, browning index and polyphenol oxidase activity (PPO) were evaluated on the obtained extracts.
The ultrasound treatment at 50°C resulted as the most efficient for the extraction of polyphenols, while the PEF treatment is more efficient respecting anthocyanins, flavonoids and antioxidant capacity equal to (896.3±5.3μM Troloxeq). In the plum skins the best technology was pulsed electric fields, which resulted in the highest extraction of polyphenols (540.22±1.31mg/L). The extracts obtained with this treatment also had the highest antioxidant capacity.The pulsed electric fields allow to preserve the vitamin C, do not promote browning of the extract but not reduce the activity of the PPO.
The data set shows how the application of advanced technologies, such as ultrasound and pulsed electric fields, with water as only solvent, can effectively be used for the recovering of high value biological molecules from vegetables by products in the full respect of the environment.

P3

Totally green and scalable MW- assisted protocol for oleuropein peracetilation

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Acetylation reaction is among the most common transformation in organic synthesis. It is known that acetylation of polar groups in drugs and natural compounds represents a widespread method for increasing the permeability across cell membranes and, consequently, the therapeutic efficacy. In particular, peracetylation of organic compounds bearing catechol groups is recognized as a transient chemical modification which allows to increase the stability and bioavailability of the natural active ingredient[1,2]. The latter may act as a pro-droug as a result of a de-acetylation by endogenous esterases. Our research group has developed an environmental friendly method for the acetylationof oleuropein (Fig.1), related to extraction from natural sources and subsequently transformated into its aglycone e HT, all oleuropein hydrolysis products, both peractylated[3,4].

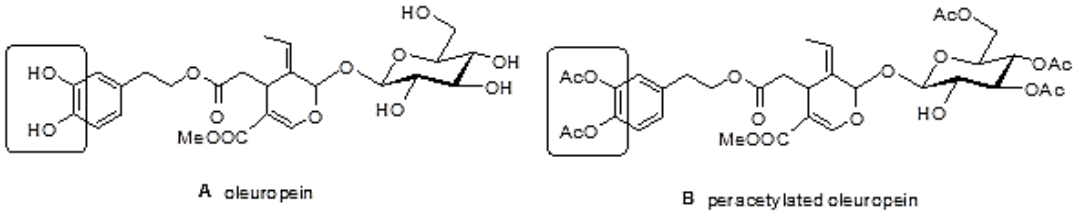

Fig.1.: Chemical structures of oleuropein (A) and peracetylated oleuropein (B). The free catechol moiety responsible for the anti-oxidand activity of compound A, is protected in compound B with the acetyl group.

Optimization peracetylation method starts from the consideration that peracetylated oleuropein, due to its dual purpose as a lipophilic molecule by the action of pro-drug, may find use in industry as a "food additive". The method uses an environment-friendly process: for instance we eliminate the classic distillation for the anhydrification of food grade acetic anhydride, we conducted the reaction under microwaves action without any catalyst and we focused on the recovery of by-products of the peracetylation reaction. Finally, the reaction was performed on pure oleuropein as well as on a commercial extract of olive leaves titled in oleuropein. the characterization of the products was made by LC/MS. The chromatogram mass shows how the process of peracetylation leads to a mainly complete peracetilated oleuropein, besides the presence of homologous species with one or two less acetyl groups. The key point is that the process allows to obtain a simple derivation of molecules of natural sources having biological and pharmacological effects with the aim of improving their bioavailability through enhanced lipophilicity and solubility in fat matter.

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(3) Procopio, G. Sindona, M. Gaspari, N. Costa, M. Nardi. Brevetto italiano numero deposito MI2007A000904; (b) A. Procopio, G. Sindona, M. Gaspari, N. Costa, M. Nardi. Brevetto italiano numero deposito MI2007A000903;
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P4

PAHs detection in red wine using central composite design to optimise the extraction procedure and GC-FID analyses

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The quality control for foods and beverages requires several attentions to reduce risks for human health and detect potential contaminations in manufacture products. The polycyclic aromatic hydrocarbons (PAHs) are environmental compounds, which often contaminates foods and beverages during the industrial manufactures. These compounds can be used as selective markers to control the quality of food products and identify their origin and production. The aim of this work was to develop an analytical method using the chemical fingerprint to screen and quantify the PAHs in selected red wine coming from different countries. The red wines were selected based on the grape fruit origin, the geographical area and the year of vintage. The extraction procedure was performed using central composite design (CCD) in order to improve both the amount of PAHs obtained from different samples and the recovery values. The limit of quantification was 10 mg/mL (n = 7, PAHs, first group) and 25 mg/mL (n = 7, PAHs, second group). The analytical method shows linearity until 400 mg/mL for different standards and linear regression coefficient over 0.9521. The intra and inter-day precision (R.S.D.%) and trueness (bias%) values fit the acceptance guidelines and international standard criteria for full range of analysis. This method demonstrated selectivity and sensitivity to quantify PAHs in commercial red wines.

P5

Aroma Recovery By Pervaporation

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Aroma compounds are a blend of organic and volatile compounds present at very low concentration, usually ppm or ppb levels in many natural sources. Chemically they can be distinguished as esters, alcohols, aldehydes, terpens, amines, mercaptans, lactones etc [1]. They are the main responsible of the fragrance, the taste and the odour of many natural products from which they are usually isolated. Aroma compounds are widely used, for their properties, in the cosmetic and food industry. Above all during some industrial processes for the production of beverages and juices, during the concentration step, the thermal treatment can cause the loss of many of these valuable components. Furthermore, chemical modifications due to thermal degradation, Maillard reactions or oxidation can occur. In order to maintain the original flavour of the product, is important to recover the aroma compounds and add them back to the concentrated juice [2].

Common techniques for aroma recovery are distillation, partial condensation and adsorption. However, some disadvantages are associated to these processes such as degradation of the aroma compounds, high energy consumption and aroma contamination [3]. Pervaporation (PV) can be considered as a valid alternative to other common techniques [4,5]. It allows, in fact, the recovery of volatile compounds at low temperatures, preserving the molecular integrity of the aroma components with a lower energy consumption and higher selectivity.

In this study, a model aroma solution from the citron fruit (*Citrus medica* L. cv Diamante) was studied during PV process by using different types of membranes and the effect of some variables (aroma concentration, temperature) on membrane performances was also evaluated.

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P6

Novel PVDF hollow-fiber membranes for the recovery of valuable compounds from pomegranate juice

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Polyvinylidenfluoride (PVDF) hollow fibers were prepared by the dry/wet spinning technique, as described elsewhere[1]. The effect of the composition and injection rate of the bore fluid on the morphology and properties of the prepared membranes was investigated.

The produced fibres were used to clarify the raw pomegranate juice in order to preserve some high-added value compounds, such as anthocyanins and other phenolic compounds, and to produce a clarified fraction suitable for further purification/concentration processes. The idea was to tailor the morphological characteristics (i.e. structure, pore size, porosity, etc.) of the prepared membranes for evaluating the possibility of producing fractions of interest for cosmeceutical and/or nutraceutical applications (fortification of food and beverages).

The pomegranate juice (*Punica granatum* L.) has been chosen due to its significant anticarcinogenic, antimicrobial, anti-atherosclerotic and anti-inflammatory activities[2]. All these activities can be attributed to its remarkably high antioxidant capacity which is correlated to the presence of different phenolic compounds including gallagyl-type tannins, ellagic acid derivatives and anthocyanins[3].

The produced hollow-fibers were characterized for evaluate their performance by water permeability (PWP), fouling index and rejection (R) towards compounds of interest.

Green extraction of aromas from herbs and spices using alternative solvents: Theoretical and experimental solubility study.

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The use of alternatives solvents has been possible for the extraction of aromas components from herbs and spices. This present study was designed to evaluate the performance of nine alternative solvents (α-pinene, MeTHF, Ethyl acetate, Methyl acetate, Ethyl lactate, Butanol, Isopropanol, Ethanol, supercritical fluid CO_2) for extract of aromas compared to n-hexane. Terpenoids are main constituents of plant. This study of varies terpenoids has been performed via simulation using Hansen solubility methodology for comprehension of dissolving mechanism. Experimentally, the extracts of aromas components in buds Blackcurrant (Ribes nigrum L) were analyzed to compare the solvents performance in terms aromas of compositions. The results indicated that the MeTHF could be the most promising solvent for n-hexane substitution with good yield and selectivity of aromas.

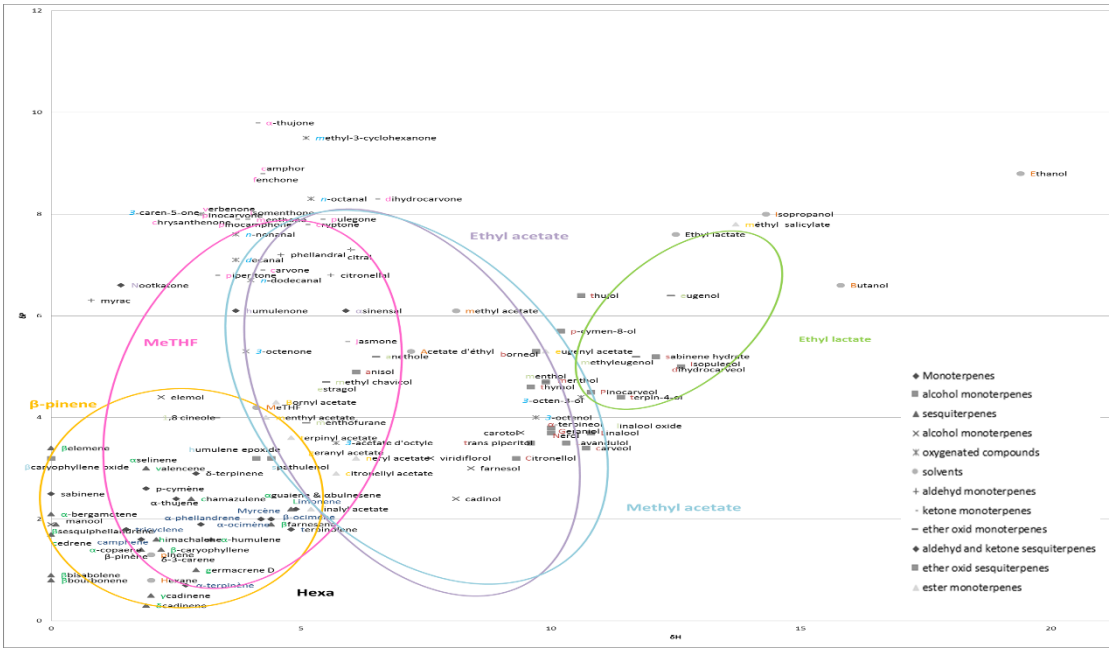

Figure 1: Alternative solvents solubilizing aromas according setting HANSEN

P8

Systematical process design for plant-based substances as a function of characterizing complex mixtures

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In recent years, the selective extraction of active pharmaceutical ingredients from plant materials has increased due to the high demand of consumers. For example, the world trading volume of phytopharmaca was approximately 100 Billion Dollar in 2011 [1]. In other areas, such as cosmetic and food industry, growth rates of up to 6% for the coming years are forecasted. To meet the requirements of the production of herbal substances from an economic and environmental point of view, optimized procedures are necessary. The aim is to develop separation sequences that reflect both the solid - liquid extraction and the subsequent purification as a function of the characterization of complex mixtures.
For mixtures with up to five key-components a combination of experimental tests to determine the physico-chemical properties and a model-based design of the apparatus has been developed for the optimal design of processes [2]. Depending on the model depth several physico-chemical properties and modelling parameters are required. These can be calculated using thermodynamic models such UNIFAC or COSMO-RS [3], extracted from databases such as Dortmunder Datenbank, DIPPR or NIST or experimentally determined. In this work, it will be discussed how this methodology can be transferred to complex mixtures like phytoextracts [4]. The example systems yew needles, fennel fruits and sage leaves are analyzed, which are typical representatives of pharmaceutical, cosmetic and food industry. Furthermore the laboratory equipment to determine modeling parameters and physico-chemical properties will be described in detail.

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P9

Oleuropein exerts antioxidant activity and reduces the expression of COX-2 and proinflammatory cytokines in Caco-2 cells and colonic mucosa from ulcerative colitis patients

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INTRODUCTION: Oleuropein (OLE) is the major secoiridoid of olive tree leaves and its antioxidant and anti-inflammatory activities have been demonstrated in vitro and in vivo animal models.
AIMS & METHODS: The aim of this study was to investigate the activity of OLE in the colonic mucosa from patients with ulcerative colitis (UC). First, the toxicity, proliferative and antioxidant effects of OLE were assessed in Caco-2 cells using the MTT assay and ELISA (xMark™ Microplate Absorbance Spectrophotometer, Bio-Rad). Cells were treated with different concentrations of OLE for 3 h and then with H_2O_2. Untreated cells and cells treated with only H_2O_2 for 1 h stand for negative and positive controls, respectively. Second, biopsies obtained during colonoscopy from 14 patients with active UC (8 M, 39-80 years, median 59; Mayo score 4-9, median 6) were immediately placed in an organ culture chamber and challenged with or without lipopolysaccaride from Escherichia coli (EC-LPS) at 1 μg/mL in the presence or absence of 3mM OLE for 24 h. Levels of cyclooxygenase (COX)-2 and IL-6, IL-8, MCP-1, VEGF, TNFα, IL-1α, IL-1β cytokines were assessed in total protein extracts from treated colonic biopsies and culture supernatant by Western blotting and Biochip Array on Randox Evidence Investigator (Randox Laboratories), respectively.
RESULTS: Treatment with OLE did not show toxicity on Caco-2 cells while significantly improving cell viability when compared with untreated cells (i.e., 121±16 % at 50 mM) or cells treated with only H_2O_2 (i.e., 67±3 % vs 53±6 % at 100 mM). In colonic mucosal biopsies from UC patients, levels of COX-2 were significantly lower in samples treated with OLE when compared with untreated (0.67±0.16 a.u. *vs* 0.84±0.16 a.u., $p = 0.03$) as well as in samples treated with OLE+EC-LPS when compared with those treated with EC-LPS alone (0.80±0.15 a.u. *vs* 1.06±0.19 a.u., $p = 0.003$). Accordingly, the level of each of the cytokines IL-6, IL-8, MCP-1, VEGF, TNFα, IL-1α, and IL-1β was significantly lower ($p = 0.02$ to 0.003) in culture supernatants of colonic samples treated with OLE or OLE+EC-LPS compared with untreated samples or samples treated with EC-LPS alone, respectively.

CONCLUSION: Non-toxic, proliferative and antioxidant activities of OLE have been demonstrated in epithelial colonic cells. Together with the anti-inflammatory effects exhibited in human *ex-vivo* experiments, this suggests the possible use of OLE for treatment of UC patients.

P10

A novel idea in food extract field: Solvent Free Extraction of Polyphenols and Essential oil from orange peels using microwave and ultrasound technologies

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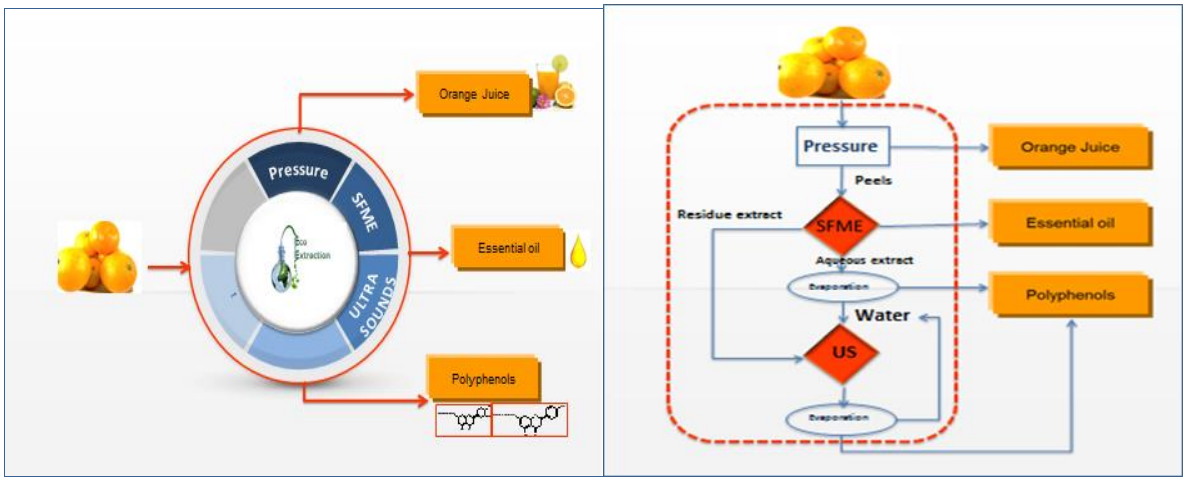
Epidemiologic studies have suggested the beneficial effects of citrus fruits against many degenerative diseases like cardiovascular diseases and some cancers. These positive influences on human health has significant increased the citrus consumption in the last few years and it is continuously increasing with an estimated world production of citrus fruits up to millions tons. The domestic and industrial use of these large quantities of citrus fruits especially for the production of juice results in the accumulation of high amounts of by-products such as peel which account of about half of the fruit weight. Orange peel can be used for the production of essential oil and phenolic compounds especially flavanone glycosides.



With the development of the "Green Chemistry" concept during the last few years, environment-friendly techniques are becoming more and more attractive.

In this work, extraction of essential oil and polyphenols from orange peel has been optimized using microwaves followed by ultrasounds, giving a sustainable process with high yield of extractions where any exogen solvent was needed but only endogen water was recycled was used as solvent.

P11

Simultaneous characterization of saponins from extract of Gynostemma pentaphyllum by liquid chromatography- high resolution mass spectrometry (LC/HRMS)

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Gynostemma pentaphyllum (Thunb.) Makino is a herbal drug of extreme versatility. The plant belongs to the Cucurbitaceae family and is a perennial creeping herb of the genus Gynostemma. Various biological activities have been attribute to this plant including hyperlipidemia, general weakness, palpitation and shortness of breath, spontaneous perspiration[1]. The active species responsible of this activities are the "gynosaponins" which exist mainly as dammarane type-triterpene glycosides (fig.1).

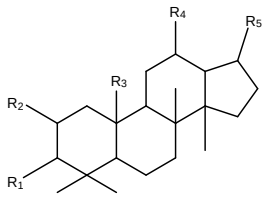

Fig.1 Dammarane skeleton of Gynostemma pentaphyllum with possible substituent position.

Commercial extracts of this plant are available. Its derived for different countries and for this reason the saponins profile and relative identification of the species is an important tool for discriminate them. In fact, over 100 saponins are known and derived for extraction of various part of the plant, but the profile can be very different. Many analytical methods have been using during the past for the identification and characterization of this species, including LCMS, NMR and GCMS[2]. Moreover in the literature there are no paper regarding the use of high resolution mass spectrometry for the study of the gynostemma extract, but sometime it used only for afford the NMR data by showed of quasi-molecular ion peak in analysis of pure compounds. Instead, this technique is able to provide information on the structure of the species both high accuracy and different online HR-MS/MS experiments too: this permit simultaneous identification and unknown recognition. In this work, commercial extract standardized at 98% in saponins, comes from China, have been analyzed by Orbitrap mass spectrometer (Qexactive) coupled with uhplc system. The experiment was carried out in negative ESI ionization and full scan/data dependent MS/MS spectrometric condition. It reveal the presence of known poly-glicosylated saponins along with new species whose structures have been hypotised on the basis of fragment recognition detected in high resolution

[1] Chemistry and pharmacology of Gynostemma pentaphyllum, Phytochemistry Reviews (2005) 4: 197–219.
[2] Dammarane-Type Glycosides from Gynostemma pentaphyllum, J. Nat. Prod. 2004, 67, 942-952.

P12

Impact of water based extraction process (hydro-distillation, steam-distillation, sub-critical water extraction) on essential oils composition and anti-oxidants recovery from extraction residues

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Essential oils are extracts majorically obtained by hydro-distillation (HD) or steam-distillation (SD) of aromatic plants. Apart from essential oils, residual cakes which may account for 99% by mass could be promising resources of natural antioxidants which are increasingly search after. In previous studies, we examined effects of hydro-distillation and steam-distillation on both extraction of essential oils and recovery of antioxidants from residual water and extraction cake of Tussilago farfara [1].
In recent years, another water-based technique called subcritical water extraction (SWE) has been used for extraction of essential oils from aromatic plants. Subcritical water , corresponding to a fluid under pressure at a temperature comprised between boiling point (100°C) and critical temperature (374°C) , can extract different molecules due to specific extracting conditions.
In this study, besides liquid water and vapor water in the case of HD and SD respectively, subcritical water was also employed to examine its effects on essential oil extraction and antioxidants recovery. Choosing Calendula arvensis as model plant and ASE apparatus as generator for subcritical water, HD, SD and SW were applied to its aerial parts to obtain essential oils which were then characterized by GC-MS and GC-FID.
The antioxidant capacity (AOC) of different extracts from residual water or extraction cakes was evaluated by DPPH radical scavenging assay and total phenolic compounds (TPC) of these extracts were calculated. Results reported that if HD, SD and SWE exerced limited impacts on composition of essential oils but they had significantly different impacts on recovery of antioxidants. Indeed, SD appeared to be a better and simplier method for recovery of antioxidants from extraction cakes while for HD and SWE, antioxidant molecules seemed to be distributed in the residual water as well as in extraction cakes [2].

1. Zhao T M, Menut C. , Venskutonis R, Talou T. (2012), In: Proc. 43rd International Symposium on Essential Oils, (Figueiredo AC, Barroso JG, Pedro LG, eds.), Lisbon, FFCL, pp 166-167
2. Zhao T.M. (2014), Université de Toulouse- INP Doctorat thesis, "Chemical and biological characterizations of forgoten aromatic and medecinal plants extracts origined of Midi-Pyrénées (France) and Chongqing (China) regions", 180p

P13

Green Concrete and Absolute using Ultrasound technology for perfumery application

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Concrete and absolute are used extensively in fragrance chemistry using hexane and ethanol. However these extraction methods may leave trace amounts of solvents which are considered undesirable for perfumery. In this work, ultrasound extraction of thyme (*Thymus vulgaris*) was conducted using sunflower oil as a natural solvent to produce Green concrete and Green absolute. Ultrasound extraction was optimized and compared to conventional and n-hexane extraction. The absolutes were analyzed by GG-MS and HPLC and concrete tested for their antioxidant activities (total phenols and DPPH). The sunflower oil used as solvent was also analyzed after extraction of thyme. Different antioxidant tests such as frying oil (added with thyme extracts) and rancimat were also conducted on sunflower oil.
The aim of this study was to determine the possible use of sunflower oil, a natural environmentally solvent, as alternative to petroleum solvents for eco-concrete and eco-absolute extraction.

P14

Green Ultrasound- and Microwave - Assisted extraction of carotenoids using vegetable oils as alternative solvents

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Green Extraction, based upon the principles of Green Chemistry, Green Engineering and the Bio-refinery concept, has arisen as a new trend in natural resource separation techniques. [1] The carotenoids, which are abundant in fresh carrots, are of great interest to food industries. Although various innovative techniques (e.g. supercritical fluids, etc.) have been developed and applied to the extraction of vegetables or fruits, no extraction technique can be considered a truly green, inexpensive and easily carried out method for extraction of such components. Vegetable oils, which can be extracted by natural terpene solvents [2], have proved their potential of being substitutes to organic solvents in this study for the extraction of bioactive compounds from natural plant materials. A greener extraction process which is in line with Green Extraction has then been achieved in a bio-refinery way (Figure 1). The extraction of lipophilic carotenoids was intensified by ultrasound and microwave using vegetable oils as substitutes to organic solvents, which has been optimized by response surface methodology and mutually compared and with conventional solvent extraction as well in terms of procedures, kinetics, yields and environmental impacts [3]. Besides, the laboratory-scale extraction has also been successfully performed at a pilot scale.

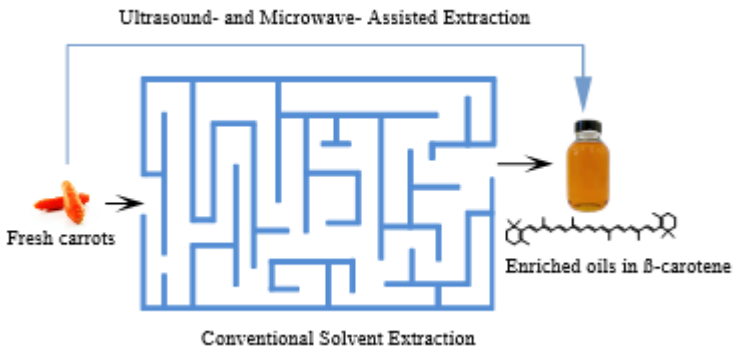

Figure 1. Conventional and innovative extraction for carotenoids

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[2] Li Y.; Fine F.; Fabiano-Tixier A.S. et al. *C. R. Chim.*, 17. **2014**.

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P15

Co-encapsulation of Resveratrol and 5-Fluorauracil in Ultradeformable Vesicles for the Topical Treatment of Non-Melanoma Skin Cancer Diseases

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A number of papers have discussed the use of innovative lipid vesicles to topically deliver chemotherapeutics and bioactive compounds. Ultradeformable liposomes represent useful formulations able to increase the skin permeation of drug compounds and their potential transport into the blood stream[1,2]. In this study, resveratrol- and 5-fluorouracil-loaded ultradeformable liposomes were investigated for the potential treatment of non-melanoma skin cancer.

The *in vitro* anticancer activity of ultradeformable liposomes was tested on human skin cancer cells through viability-, cell cycle- and apoptosis-analysis. Furthermore, we tested the percutaneous permeation of ultradeformable liposomes using human *stratum corneum* and viable epidermis.

The co-encapsulation of resveratrol and 5-fluorouracil (multi-drug carrier) in ultradeformable liposomes improved their anticancer activity on skin cancer cells as compared to both the free drug form and the single entrapped agents. These multi-drug ultradeformable liposomes arrest cell proliferation in G_1/S, thus modifying the action of 5-fluorouracil and increasing the activity of resveratrol. This effect might depend on the ultradeformable liposomes, which may accumulate in deeper skin layers, thus generating a cutaneous depot from which resveratrol and 5-fluorouracil are gradually released.

Resveratrol and 5-fluorouracil co-loaded ultradeformable liposomes could be a new nanomedicine for the treatment of squamous cell carcinoma, i.e. actinic keratosis, Bowen's disease, and keratoacanthoma.

[1]Cosco *et al.* Colloidal carriers for the enhanced delivery through the skin.. Expert Opin Drug Deliv. 2008;5(7):737-55.
[2]Paolino *et al.* Improved in vitro and in vivo collagen biosynthesis by asiaticoside-loaded ultradeformable vesicles. J Control Release. 2012;162(1):143-51.

P16

Green microwave extracts from waste matrices for cosmeceutical and nutraceutical applications

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Nowadays, in a context of sustainability, studies on the exploitation and recycle of solid organic waste are gaining increasing interest. The agro-alimentary sector produces a large amount of organic residues that often are both highly polluting and quite expensive to be treated. Their conversion from worn out plant matrices with high environmental impact to recycled sources with significant added values represents a great challenge in an eco-sustainable industrial context, due to the general need of a "Responsible Care" of the environment, together with a high quality of products and processes. Microwaves assisted extractions (MAE) can be considered relative new "green" procedures possessing several advantages over more conventional extraction techniques such as reduction of extraction times, solvent volumes and energy consumption, coupled to a better extraction efficiency as well as high extract quality.

On the basis of all these statements, the aim of this research was the development of a sustainable approach to the obtainment of new green extracts from exhaust organic matrices in order to recover new bioactive compounds with potential nutraceutical and cosmeceutical properties. Microwaves were exploited for the extraction of worn out plant matrices, such as grape marc, apple and raspberry residues and rosemary leaves allowing to recover the inner water phases of the plant with different water-soluble principles. Microwave hydro-diffusion and gravity (MHG), without the addition of solvent, was exploited for the extraction of some worn out plant matrices such as grape marc (from wine making process) apple and raspberry residues (from fruit juice industry), rosemary leaves (residues from foodstuff production). Microwave extractions were carried out exploiting the recovery of the inner water phases of the plant with different water-soluble principles. The microwave applicator used for this study consists of a multipurpose prototype (multimode cavity) equipped with a specially designed Pyrex reactor, two optical fibers for temperature measurement and a control unit which allows managing and varying different parameters of the process such as emitted power, time and temperature. The furnace presents two holes on the walls of the cavity which allow exploiting the prototype applicator for several applications, also for continuous flow reactions/extractions. The peculiar green extracts obtained ("Essential waters"- EW) were characterized and tested for their potential Radical Scavenging Activity (RSA), by means of DPPH (free radical diphenylpicrylhydrazyl) test.

Taking into account several principles of Green Chemistry (natural and renewable sources, use of industrial waste, alternative energetic source, mild processes, safety assessment), this project represents a useful tool for industries involved in a global Responsible Care Program.

P17

Effects on proliferation and migration of flavonoid fraction of *Citrus reticulata* juice in anaplastic thyroid carcinoma cells

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In this study we evaluated the effects of flavonoids extracted from Citrus reticulata (mandarin) juice on proliferation and migration of three human anaplastic thyroid carcinoma (ATC) cell lines.

By uHPLC flavonoid components of Mandarin juice extract (MJe) were analyzed. Proliferation of CAL-62, C-643 and 8505C cells, measured by MTT and cell count, was significantly reduced by MJe in a concentration- and time-dependent way, with maximal effect elicited at 0.5 mg/ml concentration after 48 h. Again we observed, by cytofluorimetric analysis, a block in the G2/M phase of the cell cycle, accompanied by low cell mortality owed to autophagic death. The extract caused also a reduction of cell migration, associated to decreased activity of the metalloproteinase MMP-2.

Our results demonstrate the effectiveness of flavonoid fraction of mandarin juice in inhibiting growth of ATC cells associated with a reduction of migration, suggesting for such a functional food, a potential use as adjuvant in the treatment of thyroid cancer.

Granulometric Analysis of Different Flour Types By Mastersizer 2000

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Food powders represent a large variety of materials that differ in their chemical composition and physical characteristics. These powders can be used as primary, intermediate or final products in food industry. Flour and dairy powders are important ingredients that are used for many types of foods and their physico-chemical properties during the manufacturing process and the storage must be carefully controlled [1]. Their main characteristics are the particle size (granulometry) and particle shape (morphology). Technological properties of powders (bulk density, flowability, surface area, etc.) as well as the potential areas of their application depend on these characteristics. The aim of the present work was to study the particle size and the granulometric distribution of four different types of flour (durum wheat, whole wheat flour, wheat flour type 0 and type 00) by using a laser diffraction particle size analyzer. In particular, the volume and the particle size distribution were measured by a Malvern Mastersizer 2000® equipped with a Scirocco with a suitable Standard Operating Procedure (SOP), for dried analysis, or a Hydro S System with manual measurement, for wetted analysis. This approach allowed to perform the analysis in different conditions. The particle size distribution was expressed as the mass median diameter (*d(0.5)*); in particular, obtained data showed that durum wheat and whole wheat flour present similar values of *d(0.5)* but different homogeneity; this is probably due to their different manufacturing processes. On the other side, the granulometric profiles of type 0 and type 00 wheat flours evidenced similar features.

[1]Al Mahdi *et al.* Morphological and mechanical properties of dried skimmed milk and wheat flour mixtures during storage. Power Technology. 2006; 163:737-55.

Bergamot Oil Extract Activity On Free Radicals Overt Production Determined By Hplc In A Rodent Model Of Opiate Tolerance

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The mechanisms of such opiate-induced hyperalgesia and antinociceptive tolerance are unclear but a role for superoxide-derived peroxynitrite ($ONOO^-$) and subsequent nitroxidative stress at the level of the spinal cord has been recently reported: morphine tolerance is associated with the overt production free radicals in a rodent model of opiate tolerance [1]. Indeed, the development of morphine-induced hyperalgesia and antinociceptive tolerance was associated with increased activation of NADPH oxidase and superoxide release [2].
In this study, oxidative stress is determined in mice using chronic administration of morphine to induce tolerance. Pretreatment with flavonoid fraction (1-10 mg/kg, i.p.) of Bergamot essential oil abolished morphine tolerance in a dose dependent manner together with free radicals formation.
The development of tolerance is associated with increased oxidation of hydroethidine (HE) and malonildialdeide (MDA) formation in the spinal cord as evaluated by chromatographic analysis. Then, we developed a new HPLC method to determine, in homogenate CD-1 mice medulla, the reaction product of superoxide radical species and hydroethidine (HE) as a sensitive substrate of oxidation that is converted in ethidium (E^+). LC/MS/ESI has been used to study the oxidative reaction. Moreover, MDA, as a measure of liperoxidation, is determined after derivatisation with dinitrophenylhydrazine (DNPH).
We demonstrate that the phenolic components derived from Bergamot exerts antioxidant activities *in vivo*. These studies have unraveled a novel pathway in the nociceptive signaling cascade associated with opioid tolerance and support the potential clinical application of natural antioxidant in reinstating opioid efficacy.

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