INNOVATION FOR HEALTH

SOCIETÀ CHIMICA ITALIANA
Divisione di Spettrometria di Massa

@ **Aboca** – Sansepolcro

September 21st - 22nd, 2015

Natural Molecules and Molecular Complexes: Characterization and Biomedical Effects

@ **Aboca** – Sansepolcro

September 21st - 22nd, 2015

Organised by

SOCIETÀ CHIMICA ITALIANA
Divisione di Spettrometria di Massa

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SCIENTIFIC PROGRAM

Monday September 21st, 2015

12:00 Shuttle bus from the Arezzo railway station

11:30-14:30 Registration

13:00-14:30 Lunch @ ABOCA

14:30-15:00 **WELCOME & OPENING REMARKS**

Valentino Mercati - Aboca President
Luisa Mattoli - 1st MS NatMedDay Chair
Gianluca Giorgi - President DSM

15:00-15:40 PL1 **PHYTOTERAPY: ART AND/OR SCIENCE? MULTIDISCIPLINARY APPROACHES FOR ITS STUDY**

P. Traldi, V. Mercati
IENI CNR, Padova, Italy

Session 1 - Supramolecular studies
Chairman: P. Traldi

15:40-16:20 PL2 **SUPRAMOLECULAR MASS SPECTROMETRY: ASSOCIATION OF MASS SPECTROMETRY METHODS TO COMPUTATIONAL CHEMISTRY TO ACCESS, AT A MOLECULAR LEVEL, SYSTEMS RELEVANT TO HOST-GUEST CHEMISTRY**

P. Gerbaux, G. Carroy, V. Lemaur, J. De Winter, J. Cornil
Université de Mons, Belgium

Session 2 – Biological studies and pharmacokinetics
Chairman: S. Vittori

16:20-16:40 OC1 **IDENTIFICATION AND QUANTIFICATION OF GLUCOSINOLATES IN DIFFERENT TISSUES OF RAPHANUS RAPHANISTRUM BY MASS SPECTROMETRY**

M. Maldini, M. Foddai, F. Natella, G. Petretto, M. Chessa, G. Pintore
Department of Chemistry and Pharmacy, University of Sassari, Italy

16:40-17:30 Poster Session - coffee break

17:30 ***End of session***
Shuttle bus to Aboca Museum in Sansepolcro: guided tour
20:00 Social Dinner

Tuesday September 22nd, 2015

Session 3 - Metabolomics
Chairman: C. Franz

9:00-9:40 PL3 **CHEMICAL CHARACTERISATION OF PLANTS USING NMR-BASED METABOLOMICS: ITS PROS AND CONS COMPARED WITH MS-BASED METHOD**

Y. H. Choi
Leiden University, The Netherlands

9:40-10:20 PL4 **APPLICATION OF LC-MS BASED PLANT METABOLOMICS AND BIOMETRIC ANALYSIS FOR THE IDENTIFICATION OF ACTIVE CONSTITUENTS IN MEDICINAL PLANTS**

S. Ortmann, M. Monschein, E. Maria P.-Wenzig, C. Huber, E. Heiss, C. Malainer, A. G. Atanasov, J. Hartler, Y.-M. Zhao, J.-H. Miao, G. G. Thallinger, V. Dirsch, R. Bauer
Karl-Franzens-Universitaet, Graz, Austria

10:20-10:40 OC2 **METABOLOMIC ANALYSIS OF NATURAL COMPLEX PRODUCTS: UNTARGETED VS TARGETED APPROACHES BY MEANS OF MASS SPECTROMETRY**

S. Tamimi, L. Mattoli, M. Burico, G. Fodaroni, A. Gaetano, S. Bedont, S. Propersi, P. Traldi, E. Ragazzi, M. Stocchero
Aboca Società Agricola Spa, Sansepolcro, Italy

10:40-11:15 Poster Session - coffee break

Session 4 - MS and MS/MS studies
Chairman: G. Bianco

11:15-11:55 PL5 **LC-ESI-MS AND LC-MS-MS FOR THE ANALYSIS OF HERBAL DRUG PREPARATIONS**

A. R. Bilia
Department of Chemistry "Ugo Schiff", University of Florence, Sesto Fiorentino, Italy

11:55-12:15 OC3 **STUDY OF NATURAL COMPOUNDS OF ROSMARINUS OFFICINALIS AS ADDITIVES IN FOOD ACTIVE PACKAGING**

V. Sirocchi, G. Sagratini, M. Ricciutelli, M. M. Coman, C. Cecchini, G. Caprioli, S. Vittori
University of Camerino, School of Pharmacy. Camerino Italy

12:10-12:25	OC4	**CHINESE ASTRAGALUS ROOT EXTRACTS: A CASE STUDY** *M. Menicatti, G. Bartolucci* NEUROFARBA, University of Florence, Sesto F.no, Italy
12:25-12:40	OC5	**SOYASAPONINS WITH HYPOCHOLESTEROLEMIC ACTIVITY IN TRADITIONAL FAGIOLI DI SARCONI BEANS INVESTIGATED BY LC-ESI-IRMPD-FTICR-MS** *G. Bianco, A Buchicchio, T. R.I. Cataldi, C. F. Carbone, R. Pascale* University of Basilicata, Potenza, Italy
12:40-13:00		Concluding Remarks ***End of session***
13:00-14:30	Lunch @ ABOCA	
14:30	***Shuttle bus for a guided tour of the production facility and laboratories at the headquarters of Aboca in Pistrino***	
16:30	Shuttle bus to the Arezzo railway station	

POSTER COMMUNICATIONS

P1 **ANALYSIS OF THE SPONTANEOUS VOLATILE EMISSION OF DIFFERENT AERIAL PARTS OF CAPER (CAPPARIS SPINOSA L.)**

R. Ascrizzi, P. L. Cioni, G. Flamini
Dipartimento di Farmacia, Università di Pisa (Italy)

P2 **CHARACTERIZATION OF BIOACTIVE COMPOUNDS OBTAINED FROM ABRUZZO AUTOCHTHONOUS PLANTS WITH ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES**

M. Pellegrini, A. Ricci, C. Lo Sterzo, G. Mazzarrino, S. D'Amato, A. Serio, A. Paparella
Faculty of Biosciences, Food and Environmental Technologies, University of Teramo (Italy)

P3 **POLYPHENOLS PROFILE OF GENETICALLY MODIFIED TOMATO Del/Ros1-N USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (UPLC-Q-TOF)**

F. Giusti, S. Achterfeldt, M. Philo, I. Colquhoun, M. Genangeli, S. Vittori, G. Sagratini, G. Le Gall, P. A Kroon
University of Camerino, School of Pharmacy. Via S. Agostino, n° 1, Camerino (Italy)

P4 **PROBING INTRINSIC PROPERTIES OF BIOMOLECULES IRMPD SPECTROSCOPY OF BARE PROTONATED PANTOTHENIC ACID**

D. Corinti, B. Chiavarino, S. Fornarini, M. E. Crestoni
Università di Roma "La Sapienza", Roma (Italy)

P5 **STUDY OF THE QUALITY AND BIOACTIVITY OF WILD AND CULTIVATED GREEK ORIGANUM VULGARE L. SUBSP. HIRTUM**

V. Nanni, A. Gismondi, A. Canini
Department of Biology, University of Rome "Tor Vergata", Rome (Italy)

P6 **STUDY ON THE POLAR FRACTION OF AN EUROPEAN MUSHROOM: *SUILLUS BELLINII* (INZENGA) KUNTZE**

C. Frezza, A. Venditti, F. Sciubba, L. Lombardi, I. Serafini, A. Ciccola, M. Guiso, M. Serafini, A. Bianco
Dipartimento di Chimica, Università di Roma "La Sapienza", Roma (Italy)

ORAL COMMUNICATIONS

PL1

PHYTOTERAPY: ART AND/OR SCIENCE? MULTIDISCIPLINARY APPROACHES FOR ITS STUDY

P. Traldi, V. Mercati

IENI CNR, Padova, Italy

Medicine based on medicinal plants developed over generations within various societies before the era of modern medicine. In China, *traditional medicine holds great potential to improve people's health and wellness.* Particularly, two special issues of Science (**346** and **347,** published between the end o 2014 and the beginning of 2015) are dedicated to this medicine[1].

There is an important point worthy of discussion: *What is often observed is that natural extracts exhibit a biological activity higher than that due to their active components*. Williamson, in the review "Synergy and other interaction in phytomedicine"[2], underlines that synergic phenomena can be invoked to justify this aspect: a plant extract is more than the sum of its part. For these aims multidisciplinary approaches must be developed and employed.

Many examples are available in literature on this aspect and this can be explained by the holistic view of complex natural extract derided from medicinal plants. Consequently the development of analytical methodologies giving an holistic view of natural extracts are surely of interest and the mass spectrometric methods nowadays available (based on soft ionization methods privileging the formation of molecular species) are particularly effective for this aim. Some examples of this approach will be given. But can the highest activity of natural extracts be explained only in terms of synergic effects or some other aspects must be considered?

These concepts are shared with the Traditional Medicine world . TM is considered of high interest to improve the quality of life, leading to a therapeutic approach following a holistic way, alternatively and/or in parallel to the reductionist approach of modern medicine.

Life is based on molecular interactions. In an interesting recent guest editorial of Accounts of Chemical Research[3], the origins of chemical evolution, the essential basement for life development, has been discussed. It is emphasized that "*chemical evolution include the capture, mutation, and propagation of molecular information and can be manifested as coordinated chemical networks that adapt to environmental change…….. A dynamic exchange of network component structures and assemblies, via both covalent and noncovalent associations, is fundamental for the network's ability to learn, to capture and integrate information about an environment that ensures the network's future response to similar conditions, as an inherent part of chemical evolution.*"

Then every biological process is a multitude of proteins, nucleic acids, carbohydrates, hormones,lipids, and cofactors, binding to and modifying each other, forming complex frameworks and assemblies, and catalyzing reactions.

A natural extract is a complex mixture of hundreds of neutral organic molecules, organic ions, oligoelements and inorganic salts. It is reasonable to assume that they interact each other, following the rules of the interactions between molecular units.

By the above considerations it follows that the enhanced activity of a natural extract, higher than that expressed by the single active component, could be ascribed to the presence of non-covalent complexes of the active component(s) with other species present in the natural substrate.

The presence of these non-covalent complexes has been described in literature and consequently the higher bioactivity of natural extracts can be justified by the presence of complexes between the active compound and other molecules (or oligoelements) present in the natural environment exhibiting conformations more effective for the interaction with the receptor. ^{1}H and ^{13}C nuclear magnetic resonance data support this hypothesis. The differences observed between the spectra of the synthetic pure compounds and those present in the natural extracts indicate that in the latter case some interactions with other molecular species or with oligoelements are present. This is under investigation to obtain further information on this behavior, which necessarily reflects on the view of the pharmacological effects of natural extracts.

Now let us come back to the title: considering that art can be defined as the expression and the application of human creative skill and imagination and that science is the intellectual and practical activity encompassing the systematic study of the structure and the behaviour of the physical and natural world through observation and experiment, it follows that phytomedicine is based on both these aspects which will be essential for developing approaches effective and valid for its study.

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PL2

SUPRAMOLECULAR MASS SPECTROMETRY: ASSOCIATION OF MS METHODS TO COMPUTATIONAL CHEMISTRY TO ACCESS, AT A MOLECULAR LEVEL, SYSTEMS RELEVANT TO HOST-GUEST CHEMISTRY

Pascal Gerbaux,[a] *Glenn Carroy*,[a] *Vincent Lemaur*,[b] *Julien De Winter*,[a] *Jérôme Cornil*[b]

[a] Organic Synthesis and Mass Spectrometry Laboratory, University of Mons UMONS, Belgium
[b] Laboratory of Chemistry of Novel Materials, University of Mons UMONS, Belgium

Host-guest chemistry represents one of the major topics of Supramolecular Chemistry and concerns the design, the synthesis and the characterization of selective/specific receptors able to strongly entangle target molecules within dedicated cavities. Perfect associations between the host and the guest partners rely on the complementarities between their topologies and functionalities. Numerous spectroscopic methods are developed to investigate those non covalent associations with particular interests on the measurement of binding constants and the determination of the structure of the association. NMR and UV-vis spectroscopies are often used to investigate such systems. Nowadays, mass spectrometry has been demonstrated to be a valuable and elegant way of studying non covalent associations extracted from the condensed phase to gas phase by means of a soft ionization method, such as Electropray.

In the context of our investigation, the experiments are conducted by using conventional mass spectrometry methods such as ESI-MS and ESI-MSMS (CID). In addition, more sophisticated approaches such as energy-resolved CID, ligand exchange experiments and of course ion mobility mass spectrometry were implemented in our work to obtain an in-depth description at the molecular level of the energetics and structure of the gas phase non covalent associations. Beside the experimental part of the project, it is really important to obtain corresponding theoretical data such as optimized structures, energetics, isomerization and decomposition thresholds and collisional cross sections. This aspect of the project relies on the use of state-of-the-art computational methods such as molecular mechanics and dynamics approaches and DFT calculations.

For the present lecture, we would like to give an overall overview of the results that were obtained in our laboratory and that are related to host-guest chemistry with different receptors such as a chiral crown ether [ChemEuJ 2008], cucurbiturils, a bitopic cucurbituril that present homotropic allosterim capabilities [chempluschem 2013] and chiral receptors. Those examples were selected to demonstrate that the association of high level theoretical chemistry with state-of-the-art MS methods represents a powerful tool for investigating host-guest complexes from structural and energetic points of view. Such studies are important to further design specific receptors or sensors for targeted molecules.

Numerous data are reported dealing with non covalent associations between large biomolecules and their ligands. Fewer studies are related to the host guest chemistry domain of research probably because of the availability of the systems to organic chemists nowadays but also since non specific associations are more likely to appear with small molecules than with bigger.

OC1

IDENTIFICATION AND QUANTIFICATION OF GLUCOSINOLATES IN DIFFERENT TISSUES OF *RAPHANUS RAPHANISTRUM* BY MASS SPECTROMETRY

Mariateresa Maldini[1], *Marzia Foddai*[1], *Fausta Natella*[2], *Giacomo Petretto*[1], *Mario Chessa*[1], *Giorgio Pintore*[1]

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Raphanus raphanistrum (Brassicaceae) and its subspecies are widely employed in human nutrition, not only as food but also for medicinal uses [1-2].
Plants belonging to the Brassicaceae family are a very rich source of bioactive compounds such as glucosinolates. Although glucosinolates themselves possess limited biological activity [3], enzymatic degradation by the endogenous enzyme myrosinase results in the formation of a number of biologically active compounds including ionic thiocyanate, isothiocyanates, nitriles, oxazolidinethione, epithionitriles and organic thiocyanates.
Over the past two decades great interest has been focused on glucosinolates and their breakdown products as a promising dietary means of cancer chemoprevention and treatment, since it has been demonstrated that they are able to activate protective mechanisms within the body, reducing risk of cancer as they stimulate the induction of phase 2 detoxification enzymes, and inhibition of phase 1 activation enzymes [4]. It has been also realised that all GL derivatives are not equal in their biological potential. Consequently, the identification and quantitative determination of individual glucosinolates in plant tissues has become of great importance [4-5].
Thus, liquid chromatography-mass spectrometry (LC-MS and LC-MS/MS) qualitative and quantitative analyses were performed for the identification and/or determination of glucosinolates in the different parts of *R. raphanistrum*, in particular, leaves, flowers, fruits and roots.
Furthermore, quantitative results were analysed with a chemometric approach (principal component analysis-PCA). By using PCA, it was possible to distinguish extracts from different tissues and identify differences and similarities among samples and identify which variables characterize one sample over the others.

Acknowledgements
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PL3

CHEMICAL CHARACTERIZATION OF PLANTS USING NMR-BASED METABOLOMICS: ITS PROS AND CONS COMPARED WITH MS-BASED METHOD

Young Hae Choi

Natural Products Laboratory, Institute of Biology, Leiden University, Leiden, The Netherlands

Investigation of metabolites has a long history compared with those of genes and proteins but ironically metabolomics is the youngest 'OMICS' technology and only recently it became an area of major research interest. This might be because metabolomics has to handle large numbers of very different compounds in a highly dynamic system. In fact there is no single analytical method to meet the ultimate goal of metabolomics: the profiling all metabolites in an organism. In any metabolomic research, the important step is the selection of an appropriate analytical method aiming at unbiased profiling of metabolites present in the target organism.

Among the possible candidate technologies, nuclear magnetic resonance spectroscopy (NMR) together with MS-based technology is currently considered one of the promising techniques for metabolomic analysis. NMR has some unique advantages over other analytical methods in terms of quantitation and qualification (structure elucidation) of metabolites. It can provide a detailed analysis on the biomolecular composition (diverse group of metabolites) very quickly with relatively less sample preparation steps. The intensity of all proton signals is absolutely proportional to the molar concentration of the metabolite. In addition, NMR spectra are uniquely based on physical properties of a molecule which results in unsurpassed signal robustness which makes it easier to perform further multivariate data analysis.

However, despite these undoubted advantages of NMR, low sensitivity (mmol in NMR compared with nmol in MS spectrometry) is the most important obstacle in comprehensive profiling of metabolites. Moreover, complexity of the spectra is another problem. In recent days, to overcome these problems of NMR-based metabolomics methods, many technical approaches and multidimensional methods have been applied.

In this presentation, compared with MS-based metabolomics, the potential and limitations of NMR in the field of plant metabolomics will be shown using several examples of medicinal plants application.

PL4

APPLICATION OF LC-MS BASED PLANT METABOLOMICS AND BIOMETRIC ANALYSIS FOR THE IDENTIFICATION OF ACTIVE CONSTITUENTS IN MEDICINAL PLANTS

Sabine Ortmann[1], Marlene Monschein[1], Eva Maria Pferschy-Wenzig[1], Claudia Huber[1], Elke Heiss[2], Clemens Malainer[2], Atanas Georgiev Atanasov[2], Jürgen Hartler[3,4], Yi-Min Zhao[5], Jian-Hua Miao[5], Gerhard G. Thallinger[3,4], Verena Dirsch[2], Rudolf Bauer[1]

[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria; [2]Department of Pharmacognosy, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria; [3]Bioinformatics Group, Institute for Knowledge Discovery, Graz-University of Technology, Petersgasse 14/V, 8010 Graz, Austria; [4]Omics Center Graz, Stiftingtalstrasse 24, 8036 Graz, Austria; [5]Guangxi Botanical Garden of Medicinal Plants, 189 Changgang Road, Nanning, China

Searching for the active compounds in medicinal plants has always been like searching for the needle in the hay stack. Activity guided isolation has been a very effective but tedious approach to identify the active constituents. In recent years, plant metabolomics based strategies have been increasingly used. LC-MS based metabolomics and multivariate data analyses allow correlations between the chemical profiles of plants and the observed pharmacological activities, and to predict which compounds may contribute to the activity. Moreover, LC-MS can be used for dereplication.

The aim of a study funded within the NFN *Drugs from nature targeting inflammation* was to identify the anti-inflammatory active constituents of Chinese medicinal plant extracts by matching the UHPLC-HR-MS metabolite profiles with their *in-vitro* activity. Based on the results of a screening, the genus *Lonicera* was chosen for this study. Ethanolic extracts from 36 Lonicera samples were included. The extracts were tested for their inhibitory effects on NO production in RAW 264.7 macrophages, IL-8 expression in HUVECs, and NF-κB activation in HEK 293 cells, as well as their effect on the activation of PPAR-β/δ in HEK 293 cells. In parallel they were analyzed by UHPLC-ESI-HRMS in the negative mode at the NAWI Graz Central Lab "Environmental, Plant & Microbial Metabolomics" using a Q ExactiveTM Hybrid Quadrupole Orbitrap-MS (Thermo Fisher).

The LC-MS data were processed in an untargeted approach by MZmine 2 [1]. Peaks were identified and quantified by Lipid Data Analyzer [2]. The abundance of the peaks was linked to the pharmacological activity of the extracts using SIMCA 13 [3]. Correlations were modeled using orthogonal partial least squares-discriminant analysis (OPLS-DA) and the results were visualized by means of the S-plot. This led to the identification of compounds which were predicted to be most relevant for the *in vitro* anti-inflammatory activity in the particular bioassays. Finally the results need to be verified by testing the pure compounds. Isolation of minor compounds turned out to be challenging. Nevertheless, this approach is a straight forward strategy for identification of relevant active constituents including synergistic effects.

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OC2

METABOLOMIC ANALYSIS OF NATURAL COMPLEX PRODUCTS: UNTARGETED *vs* TARGETED APPROACHES BY MEANS OF MASS SPECTROMETRY

Sara Tamimi,[a] *Luisa Mattoli,*[a] *Michela Burico,*[a] *Giada Fodaroni,*[a] *Anna Gaetano,*[a] *Stella Bedont,*[a] *Simona Propersi,*[a] *Pietro Traldi,*[b] *Eugenio Ragazzi,*[c] *Matteo Stocchero*[d]

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Crude plant extracts and natural compounds deriving from them have been used since ancient times in the folk medicine for treating a broad range of diseases.[1] Although the last decade has witnessed a marked growth in the market of plant-based natural products,[2] their high complexity in terms of composition still makes a challenging task the guarantee of quality, efficacy and safety requirements. Accordingly, advanced technologies and robust analytical protocols are needed both for evaluating the composition in metabolites and for reaching the required quality standards.[2,3]

In such scenario, two complementary mass spectrometry-based approaches are currently adopted in our research laboratory for the metabolomic investigations of natural complex products.[4] The first approach, based on an untargeted fingerprinting, is aimed to obtain a broad picture of the whole metabolome by detecting as many groups of metabolites as possible, without necessarily identifying nor quantifying. This untargeted metabolomic approach represents a valuable analytical tool to generate a sort of "identity card" useful for assessing the quality endpoint of final formulated natural products. On the other hand, targeted metabolomic

approaches allow the quali-quantitative determination of specific sets of metabolites as well as the identification of known compounds by using *in-house* metabolites databases.

Based on these considerations and following our interest in the development of innovative analytical solutions,[4,5] in this communication we discuss the development of an untargeted approach for monitoring the quality of formulated natural products by direct infusion electrospray ionization mass spectrometry (FIA-*ESI-MS*) and the application of a LC-MS-based targeted protocol for the quali-quantitative characterization of our best-selling products.

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PL5

LC-ESI-MS AND LC-MS-MS FOR THE ANALYSIS OF HERBAL DRUG PREPARATIONS

Anna Rita Bilia

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Standardization of material derived from plants represents a major challenge, especially for reproduction of biological effects.
Quality of an herbal drug preparation is strictly related to the quality of the botanical source (herbal drug) defined by the botanical name of the plant according to the binomial system (genus, species, variety and author) and the part used (e.g. leaf, root or fruit). In addition other factors should be considered such as the method of preparation (extraction process, solvents used; solubility and stability of the plant constituents), the drug extract ratio (DER), time and temperature operations, which could be crucial not only for safety but also for the efficacy of the product (Bilia et al., 2010).
The chemical standardization of plant products, like extracts and fractions, demands the application of methods and techniques that aim at detecting and characterizing substances that guarantee the quality of the plant material used, by the aspect of the metabolic composition, the phytochemical profile. The methods used in the standardization of the starting herbal material and its preparations must be validated considering the following parameters: specificity/selectivity, linearity, interval, precision, limit of detention, limit of quantification, accuracy and robustness, which must be determined and verified.
However, some minor or unexpected constituents cannot be detected with the analytical tools, even if they contribute to the pharmacodynamic effects leading to additive, synergistic, antagonistic effects or can interact with the pharmacokinetic profile of the extract (absorption, distribution, metabolism and excretion) modifying the bioavailability of active constituents.
In this lecture the HPLC-DAD and HPLC-MS analyses of preparations based on Violae flos (Karioti et al., 2011) and Tilia Flos (Karioti et al., 2014) using a classical phytochemical approach based on isolation and structure elucidation of constituents by NMR and MS combined techniques, is reported.
The developed analytical methods were validated for limits of detection and quantification, intra- and inter-day precision and accuracy.

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OC3

STUDY OF NATURAL COMPOUNDS OF *ROSMARINUS OFFICINALIS* AS ADDITIVES IN FOOD ACTIVE PACKAGING

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The use of natural extracts categorized as flavorings by the European Union (EU) and generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA), represents an interesting approach in the active packaging technology. Considerable research, especially from a biological point of view, have been carried out on the assessment of the antioxidant and antimicrobial activity of many herbs [1]. For example, the Rosemary (*Rosmarinus officinalis* L.) essential oil (REO) contains bioactive compounds having antimicrobial activities [2]. On the other side, the natural extracts (E392) from Rosemary plant contains molecules (carnosol and carnosic acid) that have antioxidant activity. The latter is regulated from European Commission (2010/67/UE) as food additive for processed meat. The aim of this research work was:

- to characterize chemical profile of REO (GC/MS) and E392 (HPLC/MS ion trap and SPME-GC/MS);
- to analyze their antimicrobial and antioxidant properties;
- to produce an innovative active packaging enriched with these natural additives;
- and finally, to study the storage of packaged food.

The shelf life of meat and derivatives has been widely studied by monitoring the follows freshness markers: biogenic amines (BAs), hexanal, microbial growth, pH and organoleptic properties. The major attention was focused on BAs, because they are considered markers of freshness and hygiene during storage of meat product, not only, an high level of BAs can form carcinogenic nitrosamines [3-4]. Ten BAs were analyzed and quantified by HPLC-DAD and a new fast method was developed with HPLC-MS/MS (Figure 1) [5]. REO results showed a good preservation of food, because the level of BAs, hexanal and bacteria were lower on food wrapped in the active packaging (Figure 2). Not only, the REO has a good inhibitory action against bacteria responsible to the formation of BAs [6]. The strong aroma of REO can change organoleptic qualities of meat, and this could affect consumers acceptance. To improve the shelf life of food, without changing the sensory properties, the active packaging was formulated with E392. This extract showed antioxidant properties, and sensorial analyses results confirm the quality of the active packaging, which does not changes the taste and flavor of packaged food. Preliminary analyses about BAs and bacteria showed interesting results on meat wrapped in active packaging enriched with E392. Finally, monitoring carnosol and carnosic acid concentrations, the conservation of E392 was investigated.

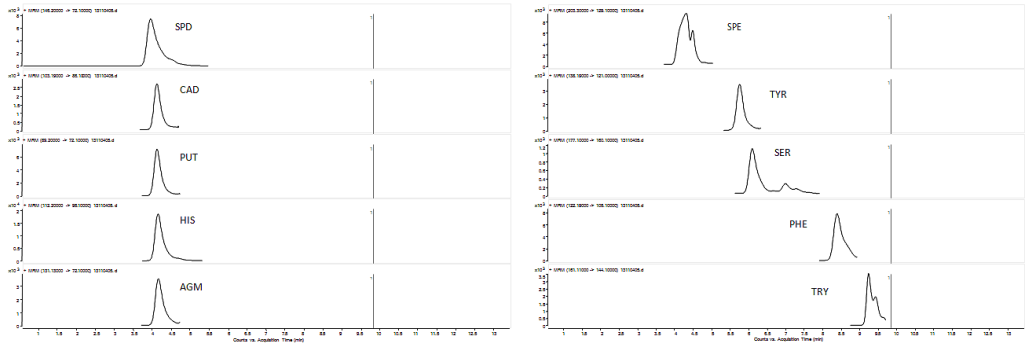

Fig. 1. HPLC-MS/MS chromatogram (triple quadrupole) acquired in MRM mode of a spiked meat sample at concentration of 0.25 mg kg-1. Single BAs are reported in the picture.

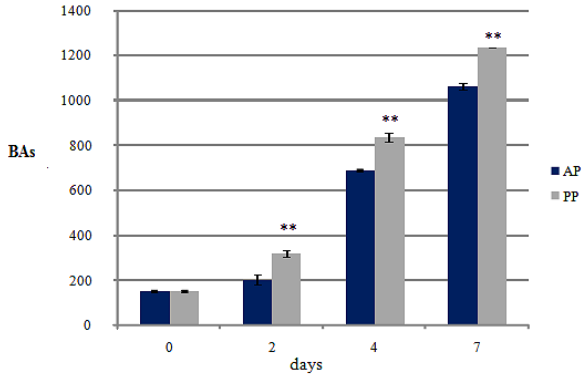

Fig. 2. Shelf life study of chicken meat wrapped in active packaging enriched with REO (AP) in comparison with chicken meat wrapped in a classic packaging (PP), taking into account total BAs content expressed in mg kg-1. *: data were significant for $t<0.05$ **: data were significant for $t<0.01$.

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OC4

CHINESE ASTRAGALUS ROOT EXTRACTS: A CASE STUDY

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Astragalus (Astragalus membranaceus) is an oriental plant, belonging to the Fabaceae family, whose roots are able to strengthen our immune system. In traditional Chinese medicine it is used from the dawn of time. Medicinal preparations based on astragalus are reported in a Chinese text of Materia Medica written around 120 BC.
This plant is widespread in asian countries, particularly in China, Korea, Mongolia and Siberia. The root is the medicinal part of the plant, and is usually harvested from 4-year-old plants. The dried roots, generally without branching, it are characterized with a length of 30-90 cm and a diameter of 1-3.5 cm. The taste is slightly sweet, slightly similar to the bean.
According to the latest research, the medicinal benefits of the Astragalus are due to the presence of more than 40 components essential for the human organism, among which: saponins, bioflavonoids, polysaccharides, and coumarins. Many pharmacological studies report that regular intake of extracts or preparations containing of astragalus performs a real therapeutic action.
The quality of astragalus preparations needs a special investigations due to the wide diffusion in commercially food supplements.
The reported case study uses the mass spectrometry for characterization of some commercial extracts of astragalus. For this purpose, we have analyzed the natural extract by an LC-MS system to identify the main components. The complex experiments have been carried out by mass spectrometry (MS/MS) in order to study their structure through the analysis of fragment ions.
The obtained results demonstrate how it is important the characterization of the components of a natural extract.

OC5

SOYASAPONINS WITH HYPOCHOLESTEROLEMIC ACTIVITY IN TRADITIONAL FAGIOLI DI SARCONI BEANS INVESTIGATED BY LC-ESI-IRMPD-FTICR-MS

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Soyasaponins are secondary metabolites, which play a role in the protection of plants against microorganisms, moulds and insects [1]. More generally, saponins are responsible of diverse physiological effects such as lysing of red blood cells by increasing plasma membrane permeability, thus they are highly toxic when injected into the blood stream. However, saponins are relatively harmless when taken orally, and some of our valuable foodstuffs, e.g. beans, lentils, soybeans, spinach, and oats, contain significant amounts of these phytochemicals [2]. Saponins have been shown to have a number of biological activities in animal systems including chemoprotective, hypocholesterolemic, haemolytic, immunostimulatory, antiviral, and anticarcinogenic activities. These activities are only just beginning to be characterized in cell culture and animal feeding studies. Recent research has shown that these compounds induce apoptosis and macroautophagy in cultured cancer cells [3]. The common bean exhibits a rich family of soyasaponins at high, medium and low abundances [4]. The present study deals with the identification of major soyasaponins from traditional Fagioli di Sarconi beans of different ecotype (*Phaseolus vulgaris L.*) by reversed-phase liquid chromatography/mass spectrometry (RPLC-MS) using high-resolution Fourier-transform ion cyclotron resonance (FTICR) MS upon electrospray ionization (ESI) in positive ion mode. Fagioli di Sarconi beans are protected by the European Union (Reg. CEE n° 1263/96) with the mark PGI (Protected Geographical Indication) and are cultivated in Basilicata (southern Italy). IRMPD tandem mass spectrometry in the ICR cell was employed as an effective tool for establishing the identity of all examined compounds. On the basis of their fragmentation behavior eight soyasaponins were successfully identified including: Soyasaponin Ba $[C_{48}H_{78}O_{19}]^+$, Soyasaponin Bb $[C_{48}H_{78}O_{18}]^+$, Soyasaponin Bb' $[C_{42}H_{68}O_{14}]^+$, Soyasaponin Bd $[C_{48}H_{76}O_{19}]^+$, Soyasaponin Be $[C_{48}H_{76}O_{18}]^+$, Soyasaponin αg $[C_{54}H_{84}O_{22}]^+$, Soyasaponin βg $[C_{54}H_{84}O_{21}]^+$ and Soyasaponin γg $[C_{48}H_{74}O_{17}]^+$. This study demonstrated an additional successful example of utilizing HRMS-based strategy to investigate unambiguously bioactive compounds in traditional foodstuffs with very high selectivity [5].

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POSTER COMMUNICATIONS

P1

ANALYSIS OF THE SPONTANEOUS VOLATILE EMISSION OF DIFFERENT AERIAL PARTS OF CAPER (CAPPARIS SPINOSA L.)

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Capparis spinosa L. is a perennial eremophyte shrub, widely distributed in Mediterranean Europe: in Italy it is commonly found as a ruderal species growing on and covering walls of the cities. Its highly developed root and xylem systems are the reason of its ability to survive in both cold and heat stressed environments, as they confer the plant the capability of using the groundwater resources (Yin et al., 2014) and the ability of growing on very dry spots, like walls and rocky coastal areas (Kulisic-Bilusic et al., 2010).
We analysed the spontaneous volatile emission of different aerial parts of caper (*Capparis spinosa* L.) by means of HS-SPME-GC/MS. The samples included leaves, buds, sepals, seeds, fruits, pistils, stamens, petals and flowers; they were randomly collected from specimens growing as wild, ruderal plants on the medieval walls of Pisa (Italy).
We identified 178 different compounds of which, in different proportion based on the type of sample, the main ones were (*E*)-β-ocimene, methyl benzoate, linalool, β-caryophyllene, α-guaiene, germacrene D, bicyclogermacrene, germacrene B, (*E*)-nerolidol, isopropyl tetradecanoate and hexahydrofarnesyl acetone.
We carried the multivariate statistical analysis of the results, with both the HCA (Hierarchical Cluster Analysis) and PCA (Principal Component Analysis) method. The analyses seem to point out that the parameter leading the emission patterns is the function of the studied sample: the flowers samples showed differences in the emission profile between their fertile and sterile portion, and between the other parts of the plant. The green parts emission profiles group them together in a cluster of their own and they are different from those of seeds and fruits.
Furthermore, we hydrodistilled caper fully bloomed flowers and analysed its composition. Literature reports analyses of the essential oil of caper seeds (Ara et al., 2014; Yin et al., 2014), fruits (Afsharypuor et al., 1998; Yin et al., 2014), roots (Afsharypuor et al., 1998), leaves (Afsharypuor et al., 1998) and floral buds (Kulisic-Bilusic et al., 2010). The bloomed flowers essential oil volatile profile significantly differs from those of the essential oils obtained from other parts of Capparis spinosa reported in the literature: it is the only volatile oil rich in oxygenated sesquiterpenes (mainly (*E*)-nerolidol and (*E*)-nerolidol acetate) and oxygenated monoterpenes (mainly linalool).

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P2

CHARACTERIZATION OF BIOACTIVE COMPOUNDS OBTAINED FROM ABRUZZO AUTOCHTHONOUS PLANTS WITH ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES.

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Food spoilage can be defined as the process or change leading to a product becoming undesirable or unaccepted for human consumption; the manifestations of food spoilage are many and varied, and may be caused by microbial, chemical or physical mechanisms. These mechanisms can lead to illness and poisonings. There are many effective preservation strategies against them, that involves synthetic preservatives. However, the increasing negative consumer perception of synthetic preservatives and the worldwide growing problem of allergies, require more effective preservation strategies. One of the alternative preservation strategies, involves the bioactive compounds contained in the essential oils (EOs). The interest in EOs and their application in food preservation, has been amplified in recent years due to consumer awareness of natural food products. EOs are a valuable fraction of low-boiling aromatic compounds, contained in a lot of different plants. According to European Pharmacopeia, the EOs can be obtained by steam distillation or by hydrodistillation. The composition of any plant EO is influenced by the presence of several factors such as: location, climate, plant species, methodology and experimental conditions. Since 1881, when De la Croix first evaluated the antibacterial properties using essential oil vapors, EOs or their components, have been shown to possess: antibacterial, antiparasitic, insecticidal, antiviral, antifungal, and antioxidant properties. The extraction of bioactive compounds from plants, can be obtained also from other different extraction techniques. One of the most innovative strategies is the RSLDE – Rapid Solid-Liquid Dynamic Extraction. The RSLDE is performed by "Naviglio Estrattore®" extractor. This extractor works on a new extractive principle: Naviglio's Principle, based on the generation of a gradient pressure between the inner and the outlet of solid matrix. This technique allows the extraction of bioactive compounds in short time, regardless of the solvent used and at room temperature, important for thermolabile compounds.

The natural origin of the extracts, is more safety to people and environment than chemical preservatives; moreover, they can be considered at low risk for development of microbial resistance. Thus, this work was directed to the production of extracts with interesting antimicrobial and antioxidant capacity that can be used on foods. Furthermore, were employed plants obtained from Abruzzo farmers and compared to commercial plants, in order to evaluate the differences between them. Solvents, extraction techniques and operations involved in the process, were chosen according to the food legislative criteria. The extracts that showed interesting antimicrobial activity, were evaluated for the antioxidant capacity and characterized. The antimicrobial activity was carried out using Broth dilution and Disk diffusion tests. The antioxidant capacity was evaluated through FRAP, DPPH, ABTS assays. The characterization of the chemical composition were carried out with chromatographic techniques, GC and

GC/MS for volatile fractions, and HPLC and HPLC/MS for non-volatile fractions. The results of this study could provide basis for a future evaluation of the effectiveness of these extracts applications on foods (evaluation of applications forms, applied concentrations, way of action, storage temperatures). These further studies may lead to the production of biopreservatives.

P3

POLYPHENOLS PROFILE OF GENETICALLY MODIFIED TOMATO Del/Ros1-N USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (UPLC-Q-TOF)

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Tomato is an excellent candidate for transgenic enhancement of flavonoid content; it is an important food crop worldwide and its levels of flavonoids are considered suboptimal. The genetically modified tomato Del/Ros1-N, developed by Dr. Cathie Martin's research group at the John Innes Centre in Norwich, has been demonstrated to express high levels of polyphenols. The effect of the transformation is that tomatoes are purple because they are synthesising enhanced levels of anthocyanins in the ripening fruit [1]. These tomatoes have been shown to have potential health benefits, for example extending lifespan in a mouse model of tumourigenesis and the shelf life of the tomatoes is also extended with delayed grey mould occurrence [2].

The present study aims to undertake a comprehensive metabolites characterization of the transformed tomatoes, of their wild type comparators and of tomatoes from many commercial varieties.

The extracts were analysed by reversed phased UPLC coupled to a Quadrupole-Time of Flight (QToF) spectrometer. Samples were analysed in positive and negative electrospray ionisation (ESI) modes to encompass detection of as many chemical species as possible.

Chromatographic and mass spectrometric data were data mined to identify characteristic differences in the extracted compounds between the transformed tomatoes and the wild type counterpart.

The output of differences, generated by XCMS software [3] and given as a list of specific masses and associated retention times, were identified using the accurate mass observations along with any supporting information such as chromatographic characteristics and any observed associated mass fragments. The identity of twenty metabolites including benzoic acid, caffeic acid, chlorogenic acid, sinapic acid, ferulic acid, m-coumaric acid, p-coumaric acid, o-coumaric acid, shikimic acid, naringenin, vanillic acid, naringenin chalcone, quinic acid, nicotinic acid, protocatechuic acid, pantothenic acid, rutin, quercetin, hydrocinnamic acid, cinamic acid, has been confirmed by the use of standards.

All these compounds were more expressed in the genetically modified tomatoes rather than in the wild-type comparators.

This comprehensive metabolite profiling study shows that the main changes in transformed purple tomatoes are related to the phenylpropanoid pathway.

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P4

PROBING INTRINSIC PROPERTIES OF BIOMOLECULES: IRMPD SPECTROSCOPY OF BARE PROTONATED PANTOTHENIC ACID

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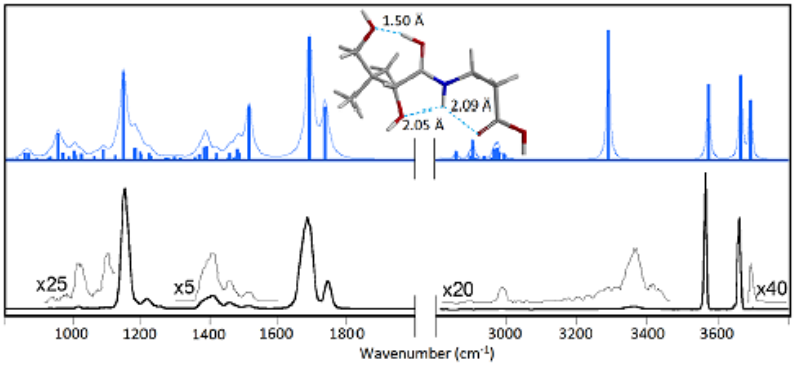
Mass spectrometry (MS) has widely proven its importance for the study and analysis of compounds of nutritional and pharmaceutical interest. The combination with lasers has allowed to develop an "action" spectroscopy performed by recording the mass-resolved photofragmentation activated by resonant absorption of multiple infrared photons (IRMPD).[1] Such highly sensitive vibrational spectroscopy achieves a direct experimental assay of the structural features of biomolecular ions in the isolated state.

In this contribution, the same methodology is applied on the bare protonated pantothenic acid, [panto+H]+, also called vitamin B5, an essential, ubiquitous nutrient implied in many relevant metabolic pathways. Given the relevance that acid/base properties may exert on molecular function, the vibrational features of [panto+H]+, generated by electrospray ionization, have been investigated in both the fingerprint region (800-2000 cm-1), at the FEL beamline of the CLIO facility, and the NH, OH stretching range (2800-3800 cm-1), using a tunable OPO/OPA benchtop laser assembled in our laboratories.[2] Calculations of optimized geometries, thermodynamic properties and linear IR spectra have been carried out at the B3LYP/6-311++G(d,p) level. Comparison of the experimental IRMPD absorptions with the calculated vibrational modes of a number of plausible isomers and conformers suggests that the lowest energy [panto+H]+ structure protonated at the amide carbonyl moiety significantly contribute to the averaged ion population at 298 K.



This combined procedure, already applied for the characterization of several biologically active molecules,[3] does confirm here its potentiality to elucidate the conformational landscape of flexible molecular ions including nutrients and natural compounds.

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P5

STUDY OF THE QUALITY AND BIOACTIVITY OF WILD AND CULTIVATED GREEK *ORIGANUM VULGARE* L. SUBSP. *HIRTUM*

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In the last decade, the food nutritional quality has become a hot topic in the scientific world and in the agrifood industry. In fact, the principal prevention strategy of chronic diseases, such as age-related pathologies, tumors, diabetes, obesity and inflammation, is nowadays represented by the consumption of foods with high nutritional value, especially vegetables and fruits. For this reason, farmers and industries are continuously looking for new food production techniques suitable for preserving or increasing the nutritional and nutraceutical properties of foods. In light of these considerations, our research had the aim to compare the quality and bioactivity of wild and organic-farmed O*riganum vulgare* L. subsp. *hirtum*, supplied by Greek farmers. From the whole plants, grown in both conditions, we obtained hydroalcoholic extracts. By preliminary HPLC-DAD and spectrophotometrical analyses, we observed that cultivated oregano extract was richer in total phenolic acid and flavonoid content, with respect to the extract of the wild one. Moreover, ABTS and FRAP free radical scavenging assays showed that cultivated plant extract had a slightly higher antioxidant capacity in comparison with the wild oregano one. Since our oregano samples showed very interesting data about their antiradical power and melanomas are the principal human skin pathology associated to death, caused by DNA damage and oxidative stress [1], we decided to investigate the bioactivity of both these extracts on B16F10 murine melanoma cell line, Respectively, MTT assay and cytofluorimetric analysis demonstrated how both oregano extracts, but in particular that obtained from cultivated plants, were antiproliferative, cytotoxic and able to arrest the cell cycle in G2-M phase. Finally, DCFH-DA test indicated that the plant extracts exerted a pro-oxidant activity on cells, in a time dependent manner. According to all these initial evidences, we can suppose that Greek oregano extracts have an antineoplastic activity and that the organic-farm techniques, applied by these producers, can preserve and also increase the plant nutritional quality.

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P6

STUDY ON THE POLAR FRACTION OF AN EUROPEAN MUSHROOM: *SUILLUS BELLINII* (INZENGA) KUNTZE

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Suillus bellinii (Inzenga) Kuntze (originally Watling) is a mycete belonging to the Boletaceae family. It can be found prevalently in the coastal pine forests of southern Europe. It generally lives in mycorrhizal form with two-needled pines like *Pinus pinea* L. Its fruiting bodies are characterized by an irregular cap, large pores, a short and squat stem and lastly a whitish dampish flesh with a very sweet taste [1,2]. Right for these features it is considered one of the most appreciated species of *Suillus* group. This species, as well as several other edible fungi, is often employed as component of low caloric diets [3] even if, in this case, the removal of its slimy cap cuticle is highly recommended since it is toxic and may result into a laxative action. Moreover it's widely utilized for its high tumor inhibiting and cytotoxic properties [4].

In this work we report the first study on the composition of the methanolic fraction obtained from the fruiting bodies of this mushroom collected in the Lazio region, Central Italy (geographic coordinates: N: 41°61'58"; E: 13°54'79") at an altitude of about 170 m a.s.l., in January 2014.

Three major classes of compounds were isolated: terpenoids, amino acids and polyols.

The isolation of these constituents was performed through a series of chromatographic separations on silica gel columns using several eluting systems while the identification of the chemical structures was, instead, accomplished by means of spectroscopic and spectrometric techniques in particular mono and bidimensional NMR Spectroscopy and Mass Spectrometry using an instrument equipped with an ESI source operating both in positive and negative ion mode.

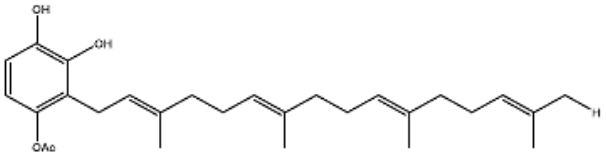
Among terpenoids were identified suillin (**1**) [5] and ergosterol (**2**). The former is constituted by a diterpenic chain linked to an aromatic ring in the position 3 and it was found for the first time in this species although not in the genus [5]. Indeed, together with isosuillin, it is deemed to be responsible for the pharmacological activity of this mushroom [4] i.e. antitumoral and cytotoxic. The latter is a provitamin form of vitamin D_2 which adds an important nutritional value to this fungus.

For what concerns the amino acidic part, instead, six free amino acids were identified namely glutamic acid (**3**), isoleucine (**4**), leucine (**5**), threonine (**6**), tyrosine (**7**) and valine (**8**). In particular, compounds **4**, **5**, **6**, **8** are essential amino acids and must be supplied.

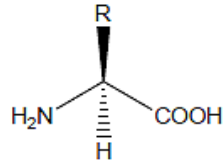
Lastly, as for the polyols, mannitol (**9**) which is the reduced form of the sugar called mannose was evidenced in big amounts. This alditol represents a common sweetener and is contained in many foods as a cooler. In medicine it is often given to patients suffering with diabetes.

These whole results fully explain, also from a phytochemical point of view, the recent uses of *S. bellinii* both in medicine and in diets and especially in this last field the presence of ergosterol,

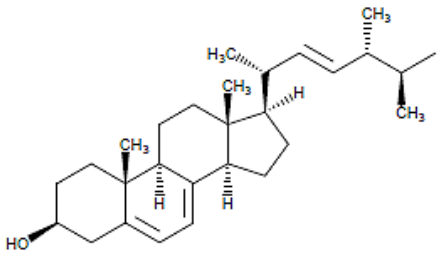
the free amino acids and mannitol is important due to their sudden availability for the consumer against the reduced digestibility associated to all mushrooms in general.



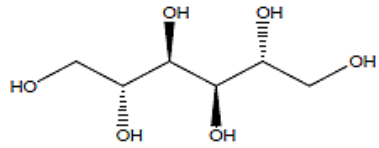
suillin (**1**)



R = CH_2CH_2COOH : glutamic acid (**3**)
R= $CH(CH_3)CH_2CH_3$: isoleucine (**4**)
R= $CH_2CH(CH_3)_2$: leucine (**5**)
R= $CH(OH)CH_3$: threonine (**6**)
R= CH_2-phenol: tyrosine (**7**)
R= $CH(CH_3)_2$: valine (**8**)



ergosterol(**2**)



mannitol (**9**)

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AUTHOR INDEX