Spettrometria di Massa

DSM-IL-01 Gas Phase Reactivity of Bioorganic Ions

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The study of properties and reactivity of organic and bioorganic ions in the gas phase is an interesting challenge [1]. Radical cations, even-electron cations or anions can be easily formed by a wide range of ionization techniques and their intrinsic properties and reactivity, free from solvation and reticular forces, can be studied by different methodologies. Further to structural characterization, regio and stereochemistry can be investigated together with chiral recognition, conformation, spectroscopic properties. Decomposition reactions, both unimolecular or induced by photons, electrons or collisions, together with ion/molecule and ion/ion reactions can be carried out and their pathways can be elucidated together with their kinetic and thermodynamic features.

Different approaches and methodologies used in studying the gas phase reactivity of bioorganic ions, taken from the literature and from own research activity [2-3], will be presented.

The gas phase behavior of radical cations and of even-electron positively and negatively charged species produced by perfluoroalkyltriazines has been studied. Their decomposition reactions are strictly dependant upon their structure and type of ion. In particular, protonated species show competitive hydration/elimination reactions involving nucleophilic addition of water [4].

The second study concerns the characterization of a cyclic peptide and the study of its reactions owing to collisions and infrared multiphoton dissociations. The rationalization of the different pathways has been carried out by theoretical calculations.

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DSM-KN-01 Infrared spectroscopy and structure elucidation of mass selected ions

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The survey of gaseous ions by vibrational spectroscopy is a rapidly growing field, taking advantage from the development of widely tunable infrared laser sources. The so-obtained IR multiple photon dissociation (IRMPD) spectroscopy may now easily cover a wide portion of the vibrational spectrum ranging from the mid-IR (fingerprint region) to the X-H (X = C,N,O) stretching region. This methodology, combined with kinetic studies of ion molecule reactions, offers a powerful tool to obtain information about ion structure and dynamics [1]. In this communication an overview is given of few systems studied recently.
The interaction of simple anions with unsaturated molecules bearing electron withdrawing groups may lead either to covalently bound complexes or to non-covalent adducts. IRMPD spectroscopy allows to discriminate between alternative structures for the adducts between RO- (R= H,CH3,C2H5) and halide ions with neutrals such as trinitrobenzene and CF2=CF2.
The post-translational modifications known to severely affect the behavior of an aminoacid residue in a biological matrix may be similarly revealed by characterististic vibrational signatures. An example is shown in Figure 1 where protonated nitrotyrosine displays highly active modes assigned to asymm and symm stretching of the nitro group. Potential interest may thus be envisioned also for diagnostic purposes.

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DSM-KN-02 Diagnostica ed analisi chimica per il monitoraggio del nostro intervento sul manufatto artistico

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Le sofisticate strumentazioni analitiche oggi disponibili trovano sempre più frequente utilizzo nel campo della conservazione e restauro dei Beni Culturali, allo scopo di acquisire informazioni a vari livelli..
Innanzitutto, per lo studio della composizione del manufatto, dei suoi materiali originariamente costitutivi e di quelli eventualmente aggiunti nel passaggio dell'opera attraverso i secoli. In secondo luogo, per lo studio dei processi di degrado che inevitabilmente avvengono nel tempo, a carico dei materiali dell'opera. In terzo luogo, per il monitoraggio dell'intervento di restauro dell'opera stessa, quando necessario. Quest'ultimo aspetto, sebbene di importanza fondamentale al fine di preservare il più fedelmente possibile i valori materiali e di forma dell'opera, è purtroppo quello che fin'ora ha ricevuto la minor attenzione.
In particolare, tra tutte le operazioni passive e attive della conservazione e del restauro, speciale attenzione merita quella genericamente, e imprecisamente, definita "pulitura" di opera policrome e non, proprio per il suo carattere peculiare: intrinsecamente irreversibile, in quanto mirata alla rimozione di materiali, presenta il più alto rischio di interagire con l'opera, di modificarne quindi la composizione, la morfologia e la percezione da parte dell'osservatore.
Se condotta col semplice monitoraggio sensoriale del controllo visivo, come ancor oggi troppo spesso accade, l'assenza di fenomeni macroscopicamente percepibili (solubilizzazione di materiali, perdita di colore…) induce l'operatore a ritenere di aver compiuto un'operazione selettiva, a minima interferenza coi materiali costitutivi dell'opera. Troppo spesso, se valutata con criteri analitici più oggettivi, la stessa operazione mostrerebbe invece un ben diverso grado di interazione: un'irreversibile modificazione del manufatto.
Questo è particolarmente accentuato per opere policrome mobili, come dipinti su tela e tavola e sculture lignee policrome, che sono manufatti compositi: successioni di strati, originari e aggiunti successivamente, in parte compenetratisi. I materiali organici di questi strati, con l'invecchiamento tendono ad acquisire proprietà (dimensioni molecolari, polarità e acidità) sempre più simili a seguito di processi chimici, principalmente ossidativi e idrolitici; gli strati, di conseguenza, divengono difficilmente differenziabili, a scapito della selettività dell'intervento di rimozione.
Nella presentazione si prenderanno in considerazione casi rappresentativi di intervento, discutendo i materiali utilizzati e le tecniche analitiche impiegate per il monitoraggio. Infine, sarà esemplificata una considerazione di importanza fondamentale: sempre più numerose divengono le tecniche analitiche per lo studio dei Beni Culturali, e tutte sono potenzialmente utili. Per esserlo però davvero, occorre che il dato analitico ottenuto sia correttamente interpretato e contestualizzato. Questo spetterebbe a figure professionali dotate di entrambi i tipi di conoscenze e competenze: quelle scientifiche/analitiche e quelle inerenti l'opera d'arte e il restauro. Sfortunatamente, queste figure professionali sono ancora largamente inesistenti…

DSM-OR-01 Determination by ICP-MS of silver concentrations in different dressings used in burns care and evaluation of their release kinetics

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Silver in the ionic form of silver nitrate has been used for the treatment of chronic wounds, ulcers, burns and infections since mediaeval times. Recently the interest in using silver to heal wounds has increased due to the rise of antibiotic resistant bacteria, so that several different silver dressings have been commercialized and are now widely used in burns centres. The dressings are typically composed of a polymeric scaffold impregnated with metallic or ionic silver, the declared silver concentrations are at percentage levels. Different mineralization methods were developed to determine the total silver concentration in the dressings by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The instrument parameters and the experimental conditions were optimized using ammonia solution to obtain the best signal stability and to reduce the memory effect of silver. The samples were analyzed using both external calibration and isotope dilution analysis (IDA). Our results confirm the declared silver concentrations in the dressings. After chemical characterization, we assessed the kinetics of silver release in different matrices of increasing complexity:: ultra pure water, normal saline solution (made in house from silver free analytical grade 0.9% m/v NaCl) and a serum substitute (Hit serum substitute, STEMCELL technologies, Vancouver, Canada). These matrices represent the possible environments in which the silver can act during the routine application of the dressings. The concentration of silver released in ultra pure water represents the simplest matrix for studying silver release. The saline solution chemically simulates the wound environment, in which a considerable fraction of the available ionic silver could precipitate from solution as insoluble silver chloride. The serum substitute contains human serum albumin, insulin and transferrin and reflects the protein composition and concentration observed in human serum. We used this solution to assess if these proteins, and particularly albumin, could enhance the solubility of silver by shifting the equilibrium towards more silver in solution by actively competing with chloride as a complexing agent.

DSM-OR-02 CHARACTERIZATION OF CARBONYL BY-PRODUCTS DURING UNIBLU-A OZONATION BY LC-ESI/Qq/TOF/MS AND IC-ESI/Qq/TOF/MS

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Ozonation is one of the most effective chemical oxidation technologies to remediate textile wastewater containing bio-recalcitrant dyes and other persistent organics [1]. However, even though dyes removal by ozonation is often achieved successfully, their complete oxidation is seldom obtained. Therefore, ozonation by-products should usually be expected in ozonation effluents [2].

QqTOF/ESI/MS both in single and tandem MS, coupled to liquid chromatography (HPLC) and ion chromatography (IC), was employed for the characterization of several intermediate as well as end by-products formed during an extensive ozonation of hydrolyzed Uniblu-A (Uniblu-OH), i.e the compound found in the spent bath resulting from dyeing process employing the reactive dye Uniblu-A. The obtained results demonstrated the effectiveness of accurate mass measurement to identify low molecular weight ozonation by-products arising from by-products formed in the early stage of the ozonation process [3]. For achieving such a goal QqTOF/ESI/MS was interfaced to LC, employing a preliminary derivatization step with 2,4-dinitrophenylhydrazine, or to IC. In addition, the employment of spectral accuracy [4] was investigated to identify by-product structures as well as to get detailed information about their fragmentation patterns.

Most of by-products were characterized by mono or double CO_2 and water losses consistent with assignment to aldheydes-, keto- and poli-hydroxylated carboxylic acids of low molecular weight.

The employed experimental approach also allowed the identification of both nitrogen- and sulphur-containing carbonyl by-products. This result is of environmental relevance for the balance of both organic nitrogen and sulphur, as these atoms do not undergo complete mineralization. Owing to the complexity of the reactions occurring during ozonation, it was possible to assess the presence of correlations between different identified by-products which in turn decompose, through decarboxylation and further oxidation, to smaller homologues and then to complete mineralization.

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DSM-OR-03 Sheep cheeses and cashmere pullovers: MS-certified authenticity through peptide analysis

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The authenticity of quality products is an issue of paramount importance in many fields. In foods, the authenticity is usually intended as the adherence to defined production methods, the use of particular ingredients or a production done in a well defined place. Typical examples include olive oils produced starting from defined cultivars, cheeses produced in mountain regions, cheeses produced with milk of defined species, sausages and cheeses having a defined ripening period, fruits and vegetables of defined varieties. But also in other fields, authenticity is important. As an example, dresses of pure cashmere wool are usually considered of very high quality as compared to similar garments made of standard sheep wool. In all these cases, the detection of potential frauds and the objective assessment of authenticity are essential, also because of the high market price of the "authentic" quality products

In the present communication, it will be shown how the use of the MS analysis of proteolytic peptides can rapidly and reliably provide a tool for determining the authenticity of food and non-food products at the molecular level.

Proteolytic peptides, generated from the casein breakdown which takes place during the production and the ageing of cheeses, can be used as markers for the mammalian species from which the milk is produced. In particular, LC/ESI-MS analysis of homologous, but not identical, proteolytic peptides derived from α_{S1}-casein allows to rapidly and reliably assess the presence of cows' milk in cheeses supposedly made only from sheep milk [1] or water-buffalo milk [2].

Enzymatic digestion of keratin extracted from wool's dresses and peptide analysis by LC/ESI-MS allows to determine not only the presence of wool derived from different species (Yak, Cashmere, Sheep) but also to assess the relative percentage of usage.

In all these cases MS-based analysis allow to obtain objective data on the specified products more reliably than currently applied methodologies.

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DSM-OR-04 Molecular characterization of bio-oil through mass spectrometry: GC-MS and APPI FTICR MS analysis.

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The increasing demand for petroleum by emerging economies, the predicted shortage of fossil fuels, as well as related environmental concerns, are pushing up the search for new sources of liquid fuels. It is expected that a significant part of future renewable fuels will be represented by biomass resources.
In this work GC-MS and FTICR MS direct analysis have been used to characterize a Bio-Oil sample obtained by thermochemical conversion of waste biomass. The bio-oil has a high percentage of carbon and a high content of etheroatoms, in particular nitrogen and oxygen.
The sample has been first analyzed in GC-MS with a single quadrupole mass spectrometer. Although the gas chromatographic separation yields a partial coelution of many different compounds, the main classes of compounds have been identified, considering the electron ionization mass spectra.
Direct analysis through APPI-FTICR MS, recently utilized for the molecular characterization of crude oil,[1] has been used for the characterization of bio-oil. The ion source is connected to a LTQ-FT Ultra (Thermo Scientific) instrument with a FT-ICR cell surrounded by a 7 Tesla magnet. Photoionization[2] is very effective for the ionization of low-medium polarity and aromatic molecules. The spectrum has been acquired in positive mode with an average resolving power of 400000. Very high mass accuracy together with high resolution allows the attribution of thousands of molecular formulas per mass spectra. In this bio-oil mass spectrum we have assigned a molecular formula to more than 6000 peaks through a custom built software (ISOMASS).
The main classes found contains one or two nitrogen atoms and 0-2 oxygen atoms(N2,O1N2,O1N1,O2N2,O2N1) (Fig.1). By GC-MS we have detected some molecules with the same DBE value as those found in the APPI spectrum, but with lower molecular weight. Using these two MS techniques we have made structural hypothesis for some of these classes.

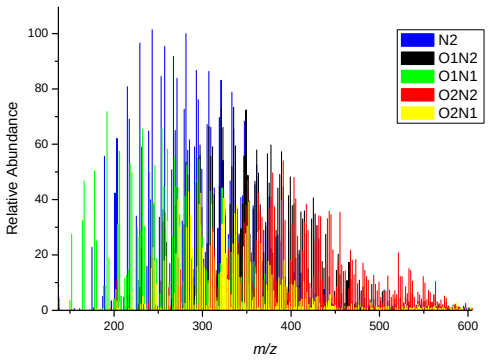

Fig.1 : Bio oil APPI positive mass spectrum.

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DSM-OR-05 Maldi –Tof Mass Spectrometry as a tool for the detection of sheep and goat milk adulterations

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Adulteration consists of the addition or the removal of some components in food in order to obtain a larger profit without significantly changing the product. It includes all the actions aimed at modifying the analytical composition of food. Due to economic convenience unscrupulous producers prefer substituting expensive raw materials with more abundant and cheaper ingredients.
Besides the utilization of casein or powdered milk for the production of cheeses and the selling of skimmed milk and semi-skimmed milk instead of whole milk, common fraud in the milk and dairy field is the illegal addition of cow's milk to goat's and sheep's milk and of goat's milk to sheep's milk.
These practices have commercial, ethical and also serious sanitary consequences because consumers can be exposed to allergens as for example cow's milk proteins, especially αs1- casein (αs1-CN) and β-lactoglobulin. Sheep's and goat's milk differs from cow's milk in terms of allergenic properties.
In order to protect human health and the product quality, European Union (EU) has developed a specific policy relevant to the authenticity of foodstuffs.
Regulation EC 178/ 2002 aims to protect consumers and to defend consumer rights to food safety and accurate information. Indeed, a strict number of modifications such as the addition of certain minerals, vitamins, proteins and changes in the fat content are officially allowed.
Analytical techniques employed in the dairy food quality control are near-infrared (NIR), mid-infrared (MIR), nuclear magnetic resonance (NMR) spectroscopies, liquid chromatography (HPLC) [1], immunoenzymatic assay, polymerase chain reaction (PCR), electrophoresis and sensory analyses. These techniques are time consuming and labor intensive so they can't be used for routine analyses. A possible alternative could be represented by mass spectrometry (MS) techniques; among the soft ionization techniques, Matrix assisted Laser Desorption Ionization (MALDI)-time of flight (ToF) has recently shown to be a useful tool for the detection of milk adulterations through the study of intact proteins [2].
Here, we propose a fast and sensitive method to detect cow's milk adulteration in sheep's and goat's milk and goat's milk adulteration in sheep's milk.
In particular, the tryptic digestion has been performed on milk samples as such and intentionally adulterated at levels of 50%, 20%, 10% and 5% and the resultant digests have been analyzed by means of MALDI TOF MS using the new MALDI matrix 4-Chloro-α-cyanocinnamic acid [3].
Many peptide markers of cow's proteins (β-lactoglobulin, α-S1- and α-S2 caseins) and goat's protein (κ-casein) have been identified in adulterated fresh milk and commercial milk samples.

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DSM-OR-06 *In mesopore* protein digestion a new strategy for mass spectrometry-based proteomics

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As a part of an on going project aimed to the development of convenient and sensitive procedures for proteomics applications [1-3], our group has recently rationally designed and developed a new enzymatic mesoreactor for ultrafast protein digestion [4].
The high surface areas, the ordered periodicity of the pores with controllable dimensions and morphology, render mesoporous silicate (MPS) optimal supports for a great variety of catalysts, from small molecules catalysts (such as metals, metal complexes, metal oxides) to large molecule catalysts such as enzymes.
MPS SBA-15 together with N-(2-aminoethyl)-3-aminopropyl and aminopropyl (indicated as AAPTES and APTES, respectively) functionalized derivatives were prepared with pore dimensions of about 4 nm, slightly larger than the diameter of trypsin (3.8 nm), to obtain a well-fitting physical entrapment. Myoglobin was added to trypsin meso-reactor, with a molar enzyme/substrate ratio of 1:3. Within 1 min of digestion, a rich pattern of proteolytic fragments was obtained for SBA-15-AAPTES and SBA-15-APTES, which allowed unambiguous myoglobin identification. The best performance was achieved for trypsin adsorbed in SBA-15-AAPTES with 100% sequence coverage obtained in just 1 min.
The effect of organic functionalities such as AAPTES and APTES grafted on SBA-15 on in situ-proteolysis are examined. In addition we address the suitability of these bionanocatalysts, for a convenient "proteomic scale" procedure consisting of few sample-handling steps, with increased proteolytic efficiency (1000 times faster and an improved performance compared to the conventional *in solution* procedure), making them promising for high-speed and high-throughput protein identification.

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DSM-OR-07 APULIAN TABLE GRAPES: A COMBINED STUDY BY DIRECT INFUSION HIGH-RESOLUTION MASS SPECTROMETRY AND NUCLEAR MAGNETIC RESONANCE

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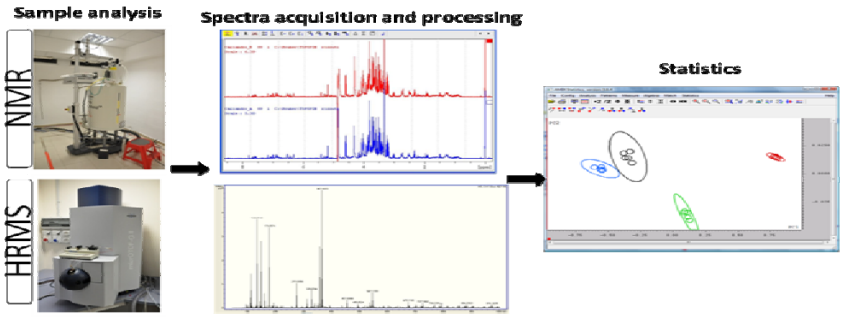
In the table grapes world market, Italy is the fourth largest producer and the second largest exporter. Apulia is in first place of the national context with approximately 70% of annual production. Efficient cultural practices, currently adopted to improve the quality of the table grape, are dependent on the skills of the growers and the use of robust scientific tools for quality control of the table grape is quite rare in the production stages. Even though the direct infusion HRMS and the solution NMR have been proven as fast and reliable techniques for the characterization of food matrices and fruit juices such as grapes and grape juices [1], these spectroscopic methods have not yet been applied to studies on the table grape. This contribution describes the results of a combined study on Apulian table grapes by direct infusion HRMS and NMR. The application of multivariate statistical analysis will also be discussed in order to show the potentialities of HRMS and NMR as discriminating techniques for quality control of the Apulian table grapes.



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DSM-OR-08 High-Throughput Protein Glycomics by Glycoblotting and MALDI mass spectrometry.

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The complex sugar chains of glycoproteins and glycolipids are believed to play important roles in the control of cellular functions and in recognition between the cell and its cellular and fluid environment. Protein glycosylation has long been recognized as a common post-translational modification and has been increasingly recognized as one of the most prominent biochemical alterations associated with malignant transformation and tumorigenesis. Thus, detailed knowledge of protein glycosylation at the proteomics level involving structural information of both the glycan microheterogeneity and the backbone peptide sequence, is of growing importance in clinical research [1].
However, the enrichment and direct determination of individual glycopeptides derived from naturally or engineered glycoproteins in vivo have not been routinely possible to date, because they typically require tedious and time-consuming separation of the glycosylated peptides from extremely complex mixtures before analysis [2, 3]. These limitations, resulting in reduced identifications of proteins and posttranslational modifications, have led to the development of alternative "off-gel" strategies. The "front-end" approaches and its specific implementations, including chemical probes, can selectively tag and facilitate subsequent isolation of a target protein subpopulation such as glycosylated proteins. In particular, any technique that sequesters a glycoprotein bearing a reducing oligosaccharide from a sample and allowing its further manipulation while immobilized on a solid phase support, represents an automated method that greatly facilitates glycoproteomic analysis of a wide range of naturally occurring and engineered glycoproteins.
The proposed method reduces proteome complexity by segregating the *N*-linked glycoproteins by the conjugation of glycoproteins via hydrazide chemistry using commercial fischer-type-functionalized gel and the specific release of formerly *N*-linked glycosylated peptides via peptide-*N*-glycosidase treatment. A single analysis leads to the identification of the glycoprotein, the site(s) of N-linked glycosylation and the characterization of the N-linked carbohydrates by high throughput MS and MS/MS analysis.

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DSM-OR-09 Fragmentation pathways of synthetic peptides investigated by infrared multiphoton dissociation (IRMPD) and Fourier-transform ion cyclotron mass spectrometry (FTICR MS)

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Over the last two decades, there has been a tremendous effort towards the application of mass spectrometry to the field of proteomics/peptidomics, in large part because of the success of tandem mass spectrometry for elucidation of primary sequences and modifications of peptides and proteins [1, 2, 3]. Several ion activation methods have been developed in this context, including collision induced dissociation (CID) which is the most widely used due to its relatively well-understood underpinnings [4]. In most MS/MS experiments, protonated peptides are excited collisionally to induce dissociation (CID) and the fragment ion spectrum is used to elucidate peptide sequences [3]. Here, electrospray ionization and tandem mass spectrometry (ESI-MS/MS) using Fourier-Transform ion cyclotron resonance (FTICR) MS and infrared multiphoton dissociation (IRMPD) was used to perform the characterization of two autoinducing peptide precursors CVGIW and LVMCCVGIW involved in the *quorum sensing* of *L. plantarum* [5]. The IRMPD experiments were performed using a 20 W in-built CO_2 laser (70 ms at 60% energy). Both protonated peptides dissociate to produce *b* and *a* ions as well as abundant fragments arising from further backbone fragmentations (immonium and especially internal ions) or loss of small neutrals. For most fragment ions, the mass resolution defined as $m/\Delta m$ (Δm is the full peak width at half-maximum) achieved >100000 was more than sufficient to resolve the isotopes of the ions and to determine the charge states from the isotope spacing, with routine sub-ppm mass accuracies (-0.5 ppm). Reversed phase liquid chromatography with ESI coupled to a hybrid quadrupole linear ion trap (LTQ) and Fourier-transform ion cyclotron-resonance mass spectrometry (FTICR-MS) was employed for the identification of CVGIW and its dimeric form in cell-free culture of *L. plantarum WCFS1* grown in Wayomonth's Medium Broth (formulation without sulphur organic compounds).

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DSM-OR-10 1,8-bis(Dimethyl-Amino)Naphthalene / 9-Aminoacridine Binary Matrix for Direct Analysis of Intact *Gram-positive* Bacteria by MALDI – TOF – MS

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Lipids and phospholipids (PLs) are important components of all biological cell membranes involved in many significant metabolic and biochemical processes such as energy production and storage, formation and functioning of cellular membranes, and signal transduction [1-3]. Perturbations in the environment and cellular activity can change dramatically the lipid metabolic network affecting the cell's lipidome. For instance, thermophilic and mesophilic bacteria grown at different temperatures have been shown to alter the fatty acid composition of their membrane lipids. This change in lipid composition could influence the cell membrane integrity in Gram-positive micro-organisms during freezing [4]. Thus a simple and reliable strategy for comparative lipidomics could be of great interest for complementing biochemical studies [5]. However, in some cases such as *Gram positive* bacteria, obtaining a lipid extract is not a very straightforward procedure. In fact, these bacteria are characterized by having, as part of their cell wall structure, a thick peptidoglycan (heteropolymers of glycan strands) layer as well as polysaccharides and/or teichoic acids which makes difficult the phospholipid extraction [6]. Then a rapid and sensitive method providing all required compositional information on intact bacteria in a single experiment would be of outstanding interest.
Here, we demonstrate the effectiveness of a strong base, 1,8-bis(dimethyl-amino)naphthalene (DMAN; proton sponge) combined with 9-aminoacridine (9AA) as a novel matrix for the direct lipid analysis of whole cell bacteria by MALDI-TOF-MS. Initially, a standard of phosphatidylglycerol was analyzed using DMAN and 9AA separately or in combination as matrix. In all cases only deprotonated analytes signals were observed in the negative-mode MALDI-TOF/MS spectra with the complete absence of matrix-related signals. Then, DMAN/9AA was successfully applied to the analysis of whole cell *Lactobacillus sanfranciscensis* microorganisms. Different components were sensitively identified from a single spot including free acids, glycolipids, phosphatidylglycerols (PGs), glycolipids and cardiolipins. Compared with the single components, the DMAN/9-AA binary matrix provided better results using a lower laser energy, leading to better resolution and reduced fragmentation and a good intra-spot and spot-to-spot repeatability. This method could be very useful in food safety chain since it allows to rapidly verify if the concentration and preservation technologies employed for lactic acid bacteria used as starter cultures in the food industries can cause cell damage or loss of viability to various degrees.

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DSM-OR-11 Exploring the frontiers of synthetic eumelanin polymers by high resolution Matrix Assisted Laser-Desorption Ionization Mass Spectrometry

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The rapidly developing new trends in material sciences and nanotechnologies are the reason for a growing interest in melanin research.[1] Melanins are important bio-polymers comprising functional components of the human pigmentary system. Eumelanin, in particular, is a heterogeneous polymer derived by the enzymatic oxidation of tyrosine with tyrosinase/O_2 via 5,6-dihydroxyindole (DHI) and its 2-carboxylic acid (DHICA). The photoprotective action, the ion-exchange as well as the prooxidant and antioxidant activities, and also electrical transmission are recognized to be prominent functions of the eumelanin macromolecules.[2] Under the technological point of view, the peculiar physicochemical properties of eumelanins make this molecular system a good candidate for the realization of new bio-inspired functional soft materials, with structure-based physical-chemical properties.[3]

An unprecedented breakthrough into the mechanism of synthetic eumelanin buildup has derived from a detailed investigation of the oxidative polymerization of DHI and its N-methyl derivative (NMDHI) by linear and reflectron MALDI-MS.

Regular collections of oligomers of increasing masses, spanning the entire *m/z* ranges up to 5000 Da (>30-mer) and 8000 Da (> 50-mer) for the two buiding blocks, respectively, were disclosed. It is the first time that the in vitro polymerisation of dihydroxyindoles to form synthetic eumelanins is explored up to its high mass limits, giving at the same time information on the polymerisation mode, whether it follows a stepwise pattern (being this the conclusion in our case) or a staking sequencing of small sized entities.

It is highlighted, also, the influence of the N-methyl substituent on the polymerization process, this opening the way to the production of N-functionalized, synthetic eumelanin-inspired soft materials, for possible future technological applications.

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DSM-PO-01 Characterization of α_{s2}-casein variants in donkey's milk

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Clinical investigations demonstrate that donkey's milk represents a safe and valid alternative to cow's milk for infants affected by cow's milk protein allergy [1]. Although it is reasonable to assume that the reduced allergenic properties of donkey's milk are related to structural differences of its protein components with respect to bovine milk, the mechanism of the tolerance of donkey's milk has not yet been clarified at molecular level. Indeed, the knowledge of the protein composition of equidae milk has been very scant until recent years. In the last years, the investigations carried out in our laboratory on the protein fraction of a individual donkey's milk samples allowed us to improve the knowledge of its protein composition by the identification and characterization of the primary structure of several previously unknown proteins [2, 3, 4, 5].

In the frame of our research line, we report here the determination of the primary structure of four α_{s2}-CNs variants carried out by coupling RP-HPLC and 2DE analyses, enzymatic digestion by trypsin, chimotrypsin and protease V8, and mass spectrometry characterization. The contemporary presence of four variants of α_{s2}–CN in an individual milk sample seems more and more typical of equine milk CNs and appears correlated with the intron/exon structure of their correlated genes, which consists of a large number of short exons that may undergone to differential splicing events and therefore originate numerous isoforms with different amino acid length.

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DSM-PO-02 A mass spectrometry-based proteomic approach for the identification and quantification of potential biomarkers in prostate cancer.

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Prostate cancer (PCa) is the most commonly diagnosed invasive cancer and the second leading cause of cancer-related death among men in Western countries [1]. In the early diagnosis of the disease a crucial role is played by the prostate-specific antigen (PSA), whose value increases in the presence of cancer (> 4 ng/ml). Recent studies show that indeed there is no safe limit value below which it can be assumed that prostate cancer is absent (2). Moreover, the value of PSA has low specificity in differentiating malignant from benign conditions, resulting in overtreatment (3). The identification of new biomarkers that allow early detection of cancer, distinguishing between indolent and aggressive form, and are specific to PCa and PCa recurrence are an urgent, unmet medical need.

The aim of this project is the analysis of differential proteome through the study of tumoral and normal prostate tissue of patients with PCa, to identify potential tissue biomarkers for PCa. Prostate tissue specimens from patient with PCa were collected and analyzed to identify proteomics-based biomarkers useful for prostate cancer diagnosis. We analyzed the tumoral and non tumoral tissue from the same individuals. The hydrosoluble tissue protein extraction and protein chemical fractionation were performed to study the sub-proteome components. Moreover, to remove the high abundant proteins, as albumin, immunoglobulin and transferrin, we developed an alternative methods compatible with a mass-spectrometry analysis. The low molecular weight proteins were proteolitically digested with trypsin, fractionated using a reversed-phase (C18) cartridges and the eluate was analyzed by MALDI-TOF mass spectrometry and MS/MS for protein identification. Differential expression analysis between Normal and Tumoral PCa tissue will be performed in order to identify potential biomarkers in prostate cancer.

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DSM-PO-03 Characterization of the acyl chains of sulfoquinovosyldiacylglycerols (SQDGs) by LC-ESI - tandem mass spectrometry

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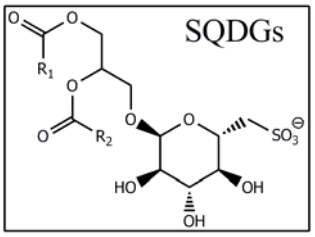
Sulfoquinovosyldiacylglycerols (SQDGs), commonly referred to as the "plant sulfolipids", were first discovered by Benson *et al*. in 1959 [1] and have since been found to exist in all photosynthetic organisms [2]. In contrast to most naturally occurring organosulfur compounds (including sulfur containing lipids), where sulfur occurs in a sulfate ester ($C\text{-}OSO_3^-$), SQDGs contain a very stable and strongly acidic sulfonic acid group, with sulfur linked to the carbon at the 6 position of glucose. Recently, 23 SQDGs have been identified during a global characterization of photosynthetic glycerolipids in a marine alga by LC-ESI-qToF-MS [3]. In the present work LC-ESI-MS/MS based on a linear ion trap mass analyzer (LTQ) has been applied to the characterization of SQDGs in plant leaf extracts. The chain length, degree of unsaturation and positional distribution of the fatty acids (FAs) attached to the primary (*sn*-1) and secondary (*sn*-2) hydroxyl groups of the glycerol moiety were established for 22 SQDGs, starting from negative ion CID-MS/MS spectra. In particular, ions corresponding to neutral losses of either free FA substituents ($[M\text{-}H\text{-}R_xCOOH]^-$) or of their ketenes ($[M\text{-}H\text{-}R_xCH{=}C{=}O]^-$) were exploited to identify the SQDGs acyl chains. Subsequently, their abundances were studied at different collisional energies. It was observed that the peak intensity of $[M\text{-}H\text{-}R_xCOOH]^-$ ions was higher than that of $[M\text{-}H\text{-}R_xCH{=}C{=}O]^-$ ones and this feature was attributed to the gas phase acidity of SQDG ions, making the neutral loss of the acid moieties easier than that of ketenes. Comparing the abundance of fragment ions, it was possible to establish the position of the *sn*-glycerol-bound FA chains (i.e., *sn*-1 vs. *sn*-2) [4,5], along with the fatty acyl composition within all SQDGs investigated.



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DSM-PO-04 Determination of Endocrine Disruptors in Water Samples with Mass Spectrometry - Liquid Chromatography Techniques

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Endocrine-disrupting compounds (EDs) [1] are chemicals that can mimic or block the actions of natural hormones in living organisms, incuding humans, and impair their normal functioning such as growth, metabolism and reproduction. Growing attention recently paid to safety of drinking water makes the development of sensitive and rapid analytical methods necessary to identify these micropollutants.

Different analytical methods [2], based on liquid chromatography - mass spectrometry techniques, were developed for the determination of six EDs: Nonylphenol (NP), Bisphenol A (BPA), estrone (E1), 17β-estradiolo (E2), estriol (E3) and 17α-etynilestradiol (EE2) in acqueos matrix (tap water, river water, waste water).

Both Ion Trap (Varian 500 MS) and Triple Quadrupole mass analyzer (Agilent 6430) were used and the chromatographic separation was optimized testing several commercial columns. A derivatizion reaction with dansyl chloride [3] was also developed to improve sensitivity for hormone molecules.

The analytical method were applied to real water samples, collected with traditional sampling and using passive samplers (POCIS).

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DSM-PO-05 Identification and Structural Characterization of Potentially Active Side Products in the Synthesis of 2-Arylbenzofuran Derivatives by GC/MS and Tandem Mass Spectrometry

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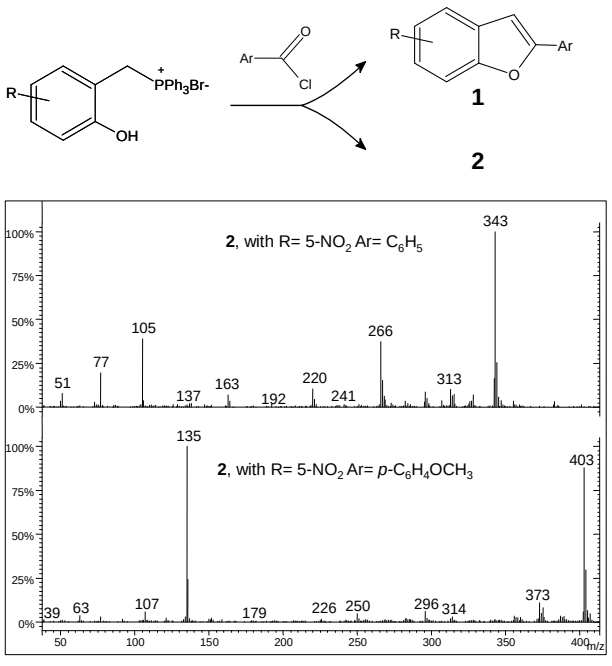
trans-Resveratrol is a natural phenolic component of *Vitis vinifera L.* (Vitaceae). It has shown a number of biological activities, including protection against coronary heart disease, as a result of different effects: significant antioxidant activity, modulation of lipoprotein metabolism, vasodilatatory and platelet antiaggregatory properties. Recently we have reported the synthesis of a series of resveratrolo-coumarine hybrids that showed interesting vasorelaxant and platelet antiaggregatory activities [1,2].

In an attempt to prepare more active heterocyclic derivatives which incorporate the nucleus of the resveratrol, we synthesised a new series of 2-arylbenzofurane derivatives by an intramolecular Wittig reaction starting from the appropriate triphenylphosphonium salt and the corresponding aroyl chloride. The desired Wittig reagent was readily prepared from the conveniently substituted 2-hydroxy-benzyl alcohol and triphenylphosphine hydrobromide (**Figure 1**) [3].

However, while developing our methodology, we observed, together with the desired 2-arylbenzofurane derivatives with the general stricture **1**, the formation of the side products **2** in a ratio ranging from 2:1 to 8:1.

To get insight on the structure and on the mechanism of formation of these side reaction products, we decided to undertake a mass spectrometric study using an ion trap mass spectrometer operating under both EI and CI conditions. In particularly, MS/MS experiments were performed on the molecular ions and on the diagnostic fragment ions generated by the side products **2** as well as by the side product synthesised from labelled starting aroyl chloride. The data so obtained were consistent with the aroyl-benzofurane structure, a scaffold of many pharmaceutical drug candidates [4], thus allowing us to discover and develop a new and convenient approach to the preparation of this class of active compounds.



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DSM-PO-06 Comparative analysis of plasma endogenous metabolite in mice exposed to smoke or air.

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The present study was aimed at investigating whether exposure of mice to cigarette smoke induces qualitative or quantitative changes in the endogenous metabolites. Plasma samples of four different mouse strains (C57BL/6J, CD1, BalbC and 129Sv) were analyzed by LC-MS/MS, FIA-MS/MS and GC-MS methods in order to monitor several metabolite classes such as amino acids, lipids, biogenic amines and energy metabolism intermediates.
The final purpose was the selection of the most suitable mouse model to be used for chronic obstructive pulmonary disease (COPD) studies. COPD is defined as a preventable and treatable disease, whose pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases. Current pharmacotherapy options are for treatment of symptoms only, and none of the existing medications is able to reverse the gradual decline in lung function that is characteristic for COPD. Therefore there is an urgent need for novel therapy for better manage the disease.
Mice were exposed to smoke (10 cigarettes/day, 3 days), using a nose-only inhalation system. Control animals followed the same procedures but were exposed to air. Plasma samples were collected 3 h or 24 h after the last exposure to smoke or air.
The most pronounced changes in term of metabolite concentration seen 3 h after exposure. Smoke caused systemic oxidative stress measured as an increase of methionine sulfoxidation. Furthermore, the energy metabolism (e.g. lactate production) was clearly affected by smoke in a strain-dependent way. Moreover, smoke influenced plasma amino acid levels and the metabolism of biogenic amines (putrescine) and lipids (acylcarnitines, free fatty acids).
Mice of strains BalbC and 129Sv seemed to be most susceptible to smoke exposure: these two strains might be used as animal models for smoke-related respiratory disorder studies.