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About the Cover: View of the famous rock named "il fungo", landmark of Ischia (NA), Italy, the venue of “ChimAlSi_2012” the IX° Italian Congress of Food Chemistry, "Food, Functional Foods and Nutraceuticals".
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2. Academic Keys
3. Academic Search Complete
4. Academic Resources
5. AFFRIT-ALIS
6. AgBiotech News and Information
7. AgBiotechNet
8. AGRICOLA
9. Agricultural Economics Database
10. AGRIS/ CARIS (FAO)
11. Agroforestry Abstracts
12. BASE
13. Biblioteca
14. Birmingham Public library
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16. CAB Abstracts
17. CAB Full Text Repository
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19. Crop Physiology Abstracts
20. Crop Science Database
21. Dairy Science Abstracts
22. DOAJ (Directory of Open Access Journals)
23. EBSCO Publishing, USA
24. E-Journal Consortium
25. Environmental Impact
26. Field Crop Abstracts
27. Forest Products Abstracts
28. Forest Science Database
29. Forestry Abstracts
30. FSTA (IFIS Publishing)
31. Genamics Journal Seek
32. GeonD
33. GFMER (Geneva Foundation for Medical Education and Research)
34. Global Health
35. Google Scholar
36. Grasslands and Forage Abstracts
37. Hinari (WHO)
38. HKU Libraries
39. Horticultural Science Abstracts
40. Horticultural Science Database
41. Index Copernicus
42. Index Veterinarius
43. Irrigation and Drainage Abstracts
44. iSEEK
45. Lupton Library
46. Maize Abstracts
47. Mathewson-IGT Knowledge Center
48. MU Libraries
49. NAAS
50. NewJour (Lauringer Library, USA)
51. Nutrition Abstracts A: Human and Experimental
52. Nutrition Abstracts B: Livestock and Feeding
53. Nutrition and Food Sciences Database
54. OAJSE
55. OhioLINK
56. OJS Database
57. Open J-Gate
58. Ornamental Horticulture
59. Peter Scott’s Library
60. Plant Breeding Abstracts
61. Plant Genetic Resources Abstracts
62. Plant Genetics and Breeding Database
63. Plant Growth Regulator Abstracts
64. Plant Protection Database
65. Postharvest News and Information
66. Poultry Abstracts
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75. Rice Abstracts
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77. SCOPUS
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82. Soybean Abstracts
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85. TROVE, National Library of Australia
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WATERS
Dear ChimAlSi_2012 Attendants:

The IX Italian Congress of Food Chemistry (ChimAlSi_2012) took place in Ischia, Italy, on 3-7 June 2012. The Congress was attended by 270 participants. Among them, 60% were from academia, 16% from Industry, and 24% were doctoral students.

On behalf of the Organizing Committee we would like to thank the invited speakers, oral presenters and poster presenters as well as all the attendants for your active participation during the sessions and the social events, which created a vibrant and motivating atmosphere. We would also like to express our gratitude to our sponsors who made this event possible through their generous contributions.

We hope that you enjoyed the conference, strengthened old friendships and collaborations, and made new ones. During five days we discussed and shared ideas on the latest scientific and technological advances in food chemistry and in new perspectives for the nutraceutical products formulation as tools for the discovery of new, safer and more efficacious natural health products. The impact of food characterization, food quality, new analytical techniques in food analysis, functional bioactive compounds, in achieving this goal, was evident throughout the meeting.

The scientific program included two inaugural and closing lectures, 10 plenary lectures, 64 oral presentations and 244 posters, divided in two poster sessions. Among the posters, ten were selected for short oral communication in a special young researchers’ forum, and were awarded prizes, generously provided by FederSalus.

Although major research advances have been made in food chemistry, until recently the focus have been almost exclusively on the safety, composition, quality, technology and analysis, both for the whole and processed foods. The ChimAlSi_2012 was therefore a hallmark event, signifying a considerable shift in research interest from food chemistry composition and analysis towards functional foods and nutraceuticals and their role in disease prevention and health promotion. The congress established a venue for scientists and practitioners to present new research and discusses the effectiveness and quality of nutraceuticals.

We would like to remind you that Journal Agricultural and Food Chemistry (ACS), Food Chemistry (Elsevier) and Emirates Journal of Food and Agriculture are preparing three Special Issue dedicated to the ChimAlSi_2012.

It was an honour and a privilege to host this meeting and to witness the exciting progress and tremendous diversity of research carried out by the members of our community. We noted with great joy the growing number of young researchers entering our field and making novel and significant contributions.

The Emirates Journal of Food and Agriculture is publishing as a supplementary issue, the Abstract Book of ChimAlSi_2012 that is, a Hot Topic entitled “Nutraceuticals and Functional Foods: Chemistry, Technology and Biological Activities”. 320 abstracts are included in this Book focusing in various aspects related to food chemistry and nutraceutics. In Western countries, disorders related to healthy lifestyles and not balanced diet is increasing sharply. Among these diseases are more and more space than those associated with aging and degenerative diseases. In the last years the concept of foods have been changed regarding their use and conception, currently the foodstuff doesn't have only to satisfy the energetic requirement of the organism, but also has to bring nourishing and substances of biological interest able to prevent illnesses and to promote the physical-mental comfort of the consumer. Nutraceuticals are food or food products that provide health and medical benefits. Nutraceutical food products are isolated or purified from foods, feed, or by products of food industry.

This Book will serve to stimulate the studies on these areas that are extremely important for academia and industry.

We would like to thank the contributors who gave so generously of their time and experience and who made this publication a valuable tool for scientists in the field of food chemistry. We are also grateful to components of Organizing Committee who lent their considerable talents to the project.

We are especially grateful to the Editorial Board of Emirates Journal of Food and Agriculture for embracing this project with interest and enthusiasm, and for the opportunity to publish this Abstract Book.

With kindest regards,

Ettore Novellino and Luca Rastrelli
OPENING AND CLOSING LECTURES

OPENING LECTURE

Comprehensive two-dimensional chromatography in lipid analysis: An overview

Giovanni Dugo
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Abstract
The improvement and development of suitable methods for thoroughly unravelling lipid profiles are of fundamental importance in academic, industrial, and clinical analysis. From a nutritional point of view, great importance has always been given to the human lipidic intake, as these dietary constituents maintain cell membrane integrity (mainly ω-3 and ω-6 essential fatty acids), encompass a wide range of health-promoting effects, and are necessary for the generation of cell mediators, e.g. prostaglandins. Furthermore, fats and oils are essential sources of vitamins, and greatly contribute towards the physical structure of foods, and the solubility of taste and aroma constituents. From the analytical standpoint, both liquid chromatography (LC) and gas-chromatography (GC) techniques have been successfully applied for the separation of lipids in real-world samples, before mass spectrometry (MS) detection which, on its side, allows for an additional level of characterization. To this regard, the development of comprehensive two-dimensional chromatography (GC×GC, LC×LC) techniques has been an important evolutionary step in the field of lipid analysis, given the enormous complexity of the matrices encountered; moreover, interpretation of MS data is easier and more reliable when high-quality spectra are obtained for completely resolved compounds. An overview of such different approaches is here provided, for the analysis of polar and non-polar lipids in foodstuffs of both plant (e.g., borage and olive oils) and animal (e.g., milk and fish extracts) origin. In all cases, the formation of group-type patterns provided by the two-dimensional approach enabled the identification and, sometimes, the quantification of both well-known and rather unexpected molecules contained in the lipid samples. Besides, novel ion liquid stationary phases of various dimensions were successfully employed for further discrimination of trans- and cis-fatty acid isomers in fish oil.

CLOSING LECTURE

Interaction of food polyphenols with gut microbiota: New prospects for nutritionists and food technologists

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Abstract
Polyphenols have received intensive attention from food and nutrition scientists over the last 20 years, mainly due to their antioxidant effects that were associated with health benefits. The current evidence shows that polyphenols, however, are only slightly absorbed in the gastrointestinal tract, and they reach the colon almost unaltered. There, they meet the microorganisms that colonize the large intestine. The colon microbiome has a dual relationship with polyphenols, as they modulate the gut microbiota population, and on its side the microbiota transforms polyphenols producing metabolites that differ substantially from the original dietary constituents. There is evidence that dietary polyphenols favor the development of some bacterial groups considered beneficial (particularly lactobacillus and bifidobacteria) while inhibit the growth of other bacterial groups (enterobacteria, bacteroides, etc.) and this could lead to benefits for gut health. The colon microbiome can metabolize polyphenols to produce metabolites that have an enhanced absorption when comparing with the original compounds, and that often extend and improve their biological effects. Therefore, depending on the composition of the gut microbiome, polyphenols can be metabolized to more bioavailable metabolites or with an improved biological effect. The identification of the bacteria responsible for the metabolic transformation of specific phenolics is an active area of research. This means that human volunteers can produce, absorb and excrete different metabolites, and enjoy different biological effects due to polyphenols intake, depending on their microbiome. This opens new opportunities for the development of functional foods. The recent discovery of the human enterotypes will have future implications in the nutritional treatments and in the development of specific food products for individuals with a specific enterotype.
PLENARY LECTURES

PL-1

A role for oleylethanolamide (OEA) in the regulation of fat-induced satiety

Daniele Piomelli

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Abstract

OEA, the naturally occurring amide of ethanolamine and oleic acid, is an endogenous lipid mediator that modulates feeding, body weight and lipid metabolism by binding with high-affinity to the ligand-activated transcription factor, peroxisome proliferator-activated receptor-alpha (PPAR-α)\(^1\)\(^2\). In my presentation, I will briefly describe the biochemical pathways responsible for the initiation and termination of OEA signaling, the pharmacological properties of this compound in relation to its ability to activate PPAR-α, and its impact on feeding behavior in rats and mice. I will then outline the role of dietary fat in the regulation of OEA biosynthesis in the rat small intestine, which suggests that activation of small-intestinal OEA mobilization serves as a molecular sensor linking fat ingestion to satiety\(^6\), and provide evidence that activation of oxytocinergic neurons of the hypothalamus is responsible for mediating the anorexic effects of OEA\(^7\). Finally, I will describe new data integrating the actions of OEA with those of the endocannabinoids, another class of lipid mediators involved in the sensing of dietary fat\(^8\).

PL-2

Foods and health claims

Rosangela Marchelli

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Abstract

In the last few years many different functions have been associated with foods besides the basic nutritional and organoleptic properties, such as conviviality, status symbol and often “health” properties, in the meaning of maintaining a healthy situation of the body or even of reducing the risk of developing different types of diseases. Novel names have been proposed to account for these new properties, such as “Functional Foods” and “Nutraceuticals”. In this lecture I will focus on the current European legislation regulating the field of Nutritional and Health Claims (Regulation (EC) N. 1924/2006 of the European Parliament and Council) and on the requirements necessary for a food in order to bear a “claim”. The European Food Safety Authority (EFSA) is encharged to provide opinions on the different issues, on the base of the scientific literature publicly available and/or on new scientific evidence provided by the applicant. Several examples of positive and negative opinions will be discussed together with the motivations for acceptance or refusal. Good positive examples are the opinions on the cholesterol lowering activity of phytosterols and phytostanols, on a water-soluble tomato concentrate which helps to maintain a healthy blood flow and benefits circulation, on the beneficial properties of unsaturated fatty acids and on the ability of olive oil polyphenols to reduce LDL- oxidation. Negative opinions were expressed for most antioxidants, whose activity was shown essentially in “in vitro” experiments. Characterization of the food/component is the preliminary requirement followed by the description of the production process. Safety is the other compulsory requirement (absence of contaminants, xenobiotics, solvents, pathogens, mycotoxins, etc). At this point, the “clou” is the demonstration that a cause-effect relationship exists between the food and a particular function of the organism, leading to the maintenance of a healthy state or to the reduction of the risk of developing a disease. The tests must be made on humans. Experiments on animals or “in vitro” are considered only supportive evidence for the claim. What type of research is needed to substantiate the claim will be discussed together with some guidelines, developed by EFSA.
Setting the real nature of the problem

Cosimo Piccinno
The Commander of the Carabinieri Headquarters for Healthcare NAS (Anti Fraud Squad), Carabinieri Military Police (IT).
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Abstract
Crime analysis results have confirmed that food crime is a trans-national phenomenon that has developed differently in countries with different legal systems. This is a barrier, sometimes insurmountable, for police forces and enforcement agencies. The presentation describes both the organization and the point of view of a specialized police force which deals with organized crime operating in food sector and reports briefly some recent cases carried out among different EU member states. Secondarily, the presentation will focus on some legal difficulties faced during international investigations where some enforcement agencies did not have police powers and could not use traditional investigative tools. Finally, improving international cooperation at all levels and implementing existing training initiatives for enforcement agencies should be considered necessary ingredients in this challenge to tackle effectively the phenomenon.

Advanced liquid chromatography techniques for the analysis of proteins of food concern

Luigi Mondello1,2,3
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Abstract
The analysis of proteome has always represented a challenging task for analytical chemistry; due to the enormous complexity and variability in dynamic range (up to 20,000 proteins may be present in a serum proteome, with a concentration range of 10^10). Additionally, proteins can undergo a variety of post-translational modifications (PTMs) which result in structural alterations, e.g. deamidation, oxidation, glycosilation and phosphorylation. The latter can in turn affect the role of proteins in determining nutritional and functional properties of food products; among these, casein exhibits several phosphorylation sites and represents the main cause of dietary milk intolerance (dephosphorylation can occur either chemically, or enzymatically). Within the wide host of hyphenated techniques, liquid chromatography-mass spectrometry (LC-MS) has recently emerged to a central role in modern proteomic research, addressing many of the limitations affecting traditional gel-based methods. As a front-end separation technique, enhanced chromatographic resolution can be achieved either by extending the stationary phase length (e.g., by serially coupling more LC columns), or by the combined use of orthogonal separation modes (in multidimensional or “comprehensive” LC systems) prior to MS or MS/MS analysis. An overview of such different approaches is here provided, for of albumin and casein isoforms tryptic mapping (protein unfolding, trypsin digestion, and chromatography of the peptide samples), followed by ESI-MS characterization and database search for sequence coverage. High efficiency at moderate backpressure was achieved through the use of fused-core (2.7 mm d.p.) ODS stationary phases (4.6 or 2.1 mm I.D. columns), operated under reversed-phase gradient conditions. Selectivity was further tuned by careful selection of the mobile phase pH (basic or acidic buffered solution), and column temperature (35-60 °C), and the analytical performances compared in terms of peak capacity.
NMR-metabolomics in food analysis

Luisa Mannina,1,2 Anatoly P. Sobolev,2 Donatella Capitani2
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Abstract
In metabolomics approach, NMR is recognized as one of the main analytical methodologies giving a complete view of the foodstuffs metabolites without chemical separation avoiding biases against certain classes of compounds. NMR methodology has shown to be a valuable tool for the qualitative and quantitative analysis of the metabolites in foodstuff such as olive oils (Mannina et al.), sea bass (Mannina et al.), truffles (Mannina et al.), kiwifruit (Capitani et al.) and lettuce (Sobolev et al.). The metabolite profiling along with the application of a suitable statistical analysis has allowed food characterization in terms of geographical origin, genetic origin and farming. Here, some significant applications of NMR metabolimics are discussed.

A holistic overview of modern ultra performance chromatography: Fundamentals, current revolution and applications

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Abstract
High-performance liquid chromatography has progressed dramatically in the past decade. Increased system pressures, reduced peak broadening and novel stationary phases have all contributed to the increment of the separation power (higher peak capacities) and to shorter analysis times (higher peak-elution rates). Considerable progress has been made in column technology over the last five years. The advent of monolithic columns in 2000 became a strong incentive for manufacturers of conventional particles to be more competitive in terms of analytical efficiency. Their efforts have led to the preparation of sub-2 \( \mu \)m totally porous particles as well as to the development of superficially porous or core-shell particles with a diameter of 2.6 and 1.7 \( \mu \)m. Columns packed with these new-generation particles are several times more efficient than conventional HPLC columns in production only five years ago. The state of the art of stationary phases available for Rapid-Speed-LC (RSLC) and Ultra-High-Pressure-LC (UHPLC) will be presented. Advances will be discussed and illustrated, with emphasis being placed on both experimental results and theoretical concepts. Moreover, up-to-date developments in the field of organic monolithic capillary columns will be shown concerning in particular their application to the high-performance separation of bio-macromolecules. Finally, the role of chiral chromatography in the separation of enantiomers will be described, both in compound screening and compound isolation on a lab-scale as well as on the biochemical, pharmaceutical and nutraceutical industry level. Research continuously promotes the design of new chiral selectors capable of separating a wide variety of chiral compounds. In order to enhance the kinetic performance of the separation process without loss in selectivity, efficiency and resolution, analytical chemists are turning towards Enantioselective-UHPLC (EUHPLC)\textsuperscript{[1]}, where shorter columns (50-100 mm length) with a smaller ID (3.2-2.1 mm) are being packed with smaller particles (sub-2 \( \mu \)m).
Effectiveness and quality of nutraceuticals
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Abstract
The term “Nutraceuticals” (NUT) refers to a large category of products, ranging from functional foods to dietary supplements, containing concentrated forms of natural compounds with presumed biological activity, originally derived from foods, but now present in non-food matrixes, and used to improve health in dosage largely exceeding those obtainable in food. Several studies report that in adequate doses NUT can be beneficial for prevention of some chronic diseases. The favorable metabolic effect of NUT, reported by recent research, can also yield improvement in cardiovascular risk profile and eventually outcome. In the last decade, multiple scientific research have been conducted in order to evaluate the potential benefits of nutraceuticals in different therapeutic areas. The use of effective natural substance in order to strengthen the preventive strategies of many chronic diseases is certainly attractive, especially for early prevention in the setting of a reduction of resources devoted to public health. Very interesting seems to be the use of nutraceuticals in CV prevention, for their potential benefit in controlling major risk factors such as dyslipidemia, hypertension, insulin resistance and abdominal adiposity. The current European Guidelines for the Management of Dyslipidemia, issued jointly by the European Society of Cardiology (ESC) and atherosclerosis (ESH), suggest the use of nutraceuticals to strengthen lifestyle changes strategy that are the basis of an efficient CV disease prevention. The nutraceutical market in Italy is expanding, due to the increasing interest for this product category. Sales of dietary supplements in Italian pharmacies grew up by almost 10% in 2011, reaching a total turnover of almost 1.7 billion euro. Against the increasing demand, also increases the number of companies that fill this market. In Italy there are over 1,100 manufacturers of dietary supplements that market their products in Italian Pharmacies An increasing number of pharmacologic companies, alongside the traditional ethical drug market, are now extending the development and production of nutraceuticals in different work areas. However, the lack of strict regulation on the production and marketing of nutraceuticals, compared to those existing for the other drugs, results in an easy entry into this market by companies with manufacturing standards and quality control not according to those that guarantee pharmaceutical companies operating in this sector, leading to dangerous presence of products without any demonstrated efficacy and safety. So the European Guidelines suggest the use of nutraceuticals as a preventive strategy in reducing cholesterol in primary prevention, emphasizing the need of demonstrated efficacy and safety reported by clinical studies on man. Nowadays the nutraceuticals may represent a new and valid option when the relation between risk-benefit and cost-benefit does not recommend or suggest the use of pharmacological treatment. However, as for the drug, even for nutraceuticals, it is necessary a demonstrated effectiveness and quality of the product in order to safely advising these products.

The proteome argonauts: Conquering the “golden fleece” of non-alcoholic beverages via combinatorial peptide ligands
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Abstract
Proteomic science has been vastly exploited in the past ten years for biomarker discovery in sera, in search of panels of proteins able to warn about the onset of various diseases. According to Nature Biotech. Editorial (28, 2010, 665-670), this has been the biggest “fiasco” in this arena, with billions of dollars wasted. Completely different results have been obtained by us when analyzing a “fiasco” (a 1 liter jug) of non-alcoholic beverages with the combinatorial peptide ligand library (CPLL) technology. The aim of this research has been to assess the quality of such soft drinks, in general claimed to be made with plant and herbal extracts, in order to verify their genuineness and distinguish them from artificial beverages, made only with synthetic additives and flavours (Coca Cola, classical example). The assumption being that, if such beverages had been produced with vegetable extracts, trace proteins (at the very least) should be present in the final product. Examples will be given on the proteome analysis of, e. g., almond milk, coconut milk, orgeat syrup and a Cola beverage. Also a ginger beverage, claimed to be made with a natural extract of ginger root, and sold in specialized shop at higher prices, has been evaluated, with results in conflict with the label claims. Considering the extreme sensitivity reached by the CPLL technology, able to detect as little as 1 micro-gram protein/L, regulatory agencies and customers will have a new, formidable tool for protection against adulterated and counterfeited foodstuff and beverages.
Plant-based alcohol beverages: The science behind the lore

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Abstract
The development of distillation and the availability of high degree alcohol solutions have led to the “upgrade” of medicinal wines into liqueurs, a trend that started in the 18th century and peaked in the second half of the following century. Medicinal wines and liqueurs have now disappeared from modern pharmacopoeias, but are still popular as social lubricants and/or digestive aids. Many liqueurs contain proprietary mixtures of plants, and are therefore difficult to study in a biomedical context. Others are produced from a single, or from closely related, botanical species, and are associated to specific beneficial effects. Thus, the consumption of genepy, a liqueur obtained from some closely related Alpine wormwoods, is traditionally associated to the management of cold and inflammation (Delahaye, 2008). Artemisia umbelliformis Lam., the only genepy that can be cultivated, has an interesting phytochemistry, containing structurally unique sesqui- and sesterpene lactones as well as flavonoids (Appendino et al, 2009). Some of these compounds show potent anti-inflammatory activity in vivo, targeting critical players of the inflammatory cascade like NF-κB, STAT3, and TRPA1. Research on the identification of the pharmacological and the gustatory targets of these compounds will be highlighted, discussing their interplay and critically evaluating their possible clinical translation.

Nutrition, aging and longevity: An opportunity and a challenge

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Abstract
The deep relationship between Nutrition, Aging and Longevity has to be traced back in the evolution of H. sapiens and is documented by a large number of investigations both in humans and in animal models. The topic is becoming hot and urgent for both basic science and public health owing to epidemics of diet-related diseases such as obesity, metabolic syndrome and type 2 diabetes (T2D), correlated with the rapid increase of lifespan and the changes in nutritional habits that have affected all populations in the planet as a consequence of economical development, cultural/anthropological transition and the acquisition of new lifestyle. The current hypothesis and theory suggest that the entire nutritional lifespan, from the period we spend in utero to the extreme ages, matters for healthy life and healthy aging. Within this integrated perspective and in order to pursue the scientific challenge of a personalized nutrition, new topics are emerging, such as the importance of the gut microbiome (GM) and of nutrition-driven epigenetic modifications, besides more classical but still poorly understood nutrigenomic approaches. Another challenge is the use of powerful technologies such as metabolomics - applied to urine, plasma and organ samples - and glycomics both potentially capable of discovering new biomarkers related to diet and nutrition. I will illustrate what we are doing on models of healthy aging (centenarians and their offspring), accelerated aging (Down syndrome) and T2D using the above-mentioned omics, in order to identify new biomarkers and pathways involved in human longevity and age-related pathologies. Finally, I'll also illustrate the rationale of the new EU project NU-AGE (2011-2016) I'm coordinating, which will investigate nutritional, immunological, epigenetic, metabolomic and GT changes related to 1 year ad hoc fortified Mediterranean diet in 1,250 subjects aged 65-79 years recruited in five EU countries (Italy, France UK, The Netherland and Poland).
ORAL COMMUNICATIONS

CO-1

The detection of allergens in food matrices by LC-MSMS

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Abstract
The number of adults and especially children in industrial nations suffering from food allergies is rising continously. Due to this fact there is a need amongst food producers and regulators for specific and sensitive methods to detect allergens at trace levels. Screening for these protein allergens in food matrices is traditionally performed using enzyme-linked immunosorbent assays (ELISAs), which in most cases only detect a single allergen per kit. Here we present the use of mass spectrometry for the detection of allergens. This work will discuss the development of a multi allergen LC-MSMS screening method as well as the application of this technique to the detection of milk and egg in baked goods. The results presented will show that when applying LCMSMS to processed food samples sensitivities achieved were equivalent to sensitivities of some currently available methods based on ELISA and real-time PCR.

CO-2

Masked mycotoxins in durum wheat: Occurrence, significance and metabolic fate

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Abstract
Recent studies have shown that, in mycotoxin-contaminated commodities, many structurally related compounds generated by plant metabolism or by food processing can co-exist together with the native toxins. These mycotoxin derivatives (conjugated or “masked” mycotoxins) show different chemical behaviour, thus easily escaping routine analyses, but potentially contributing to the overall toxicity, exerting specific toxic effects or releasing the native form of the toxin upon gastrointestinal digestion. This peculiar phenomenon is related to mycotoxins produced by Fusarium species (trichothecenes, zearalenone) and it has been attributed to a detoxifying mechanism exerted by plants to convert the relatively apolar trichothecenes and zearalenone in more polar derivatives via conjugation with sugars or sulphate groups, in order to compartmentalise them in vacuoles, thus enhancing the plant resistance to the infection. Zearalenone-4-glucoside and deoxynivalenol-3-glucoside were detected in wheat naturally contaminated by F. graminearum (Berthiller et al. 2005) up to 30% of the native form. Fusarium head blight is actually the most important pathology affecting durum wheat cultivation with several important consequences both for the safety of the consumers as well as for the market of derived products (Bergamini et al. 2010). The occurrence of these masked form in durum wheat samples grown in Italy was undertaken using LC/MS analysis and a deep investigation of the effect of different factors as well as of the wheat genetic lines on the accumulation of the mycotoxins was performed, both on naturally and artificially inoculated wheat plants, in order to evidentiate eventual correlations with resistance to the pathology. The stability of these masked forms to simulated gastrointestinal conditions as well as their toxicities towards different human cell lines were investigated. Also, in silico docking experiments were performed in order to clarify some aspects of the toxicological mechanisms.
The influence of in vitro simulated digestion process on α-dicarbonyl compound cytotoxicity

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Abstract
α-Dicarbonyl compounds are intermediate substances occurring in thermally treated foods as reaction products of caramelization, Maillard reaction, and lipid peroxidation, in fermented foods and beverages, where are produced by microorganism metabolisms, in plants, produced in response to stress conditions, in water, in tobacco smoke, and in medical products (peritoneal dialysis fluids). Glyoxal (G) and methylglyoxal (MG) can be produced also by many strains of bacteria present in the intestinal tract, and are endogenous compounds because are human physiological metabolites. α-Oxoaddehydes are highly reactive alkilating agents, that have been found to rapidly modify side chains of protein and form reversible Schiff’s base that after subsequent rearrangements, oxidations and dehydratations, yields relatively stable Amadori products, known as Maillard reaction products (MRPs) if occur in foods, or advanced glycation products (AGEs) if are endogenously formed. Recent publications reported that both α-dicarbonyl compounds and AGEs induced several cellular damages, mainly attributed to their interaction with essential cellular macromolecules, leading to the alteration in structure and function of some kind of cells. In recent years the role of exogenous α-dicarbonyl compounds in gastrointestinal tract is under investigation to understand whether excess consumption of such dietary compounds might be a risk for human health. Nevertheless, to our knowledge, the influence of the digestion process on food derived α-dicarbonyl compounds has not been investigated. Therefore, a mixture of α-dicarbonyl compounds, commonly occurring in foods (G, MG and diacetyl), before and after in vitro simulated gastrointestinal digestion process, was evaluated for its ability to induce cytotoxicity against three different cultured cell lines and to inhibit the function of selected enzymes responsible for DNA repair in human cells. Then these properties were correlated both to the α-dicarbonyl compound content (determined by a validated RP-HPLC-DAD method) before and after digestion and with the digestive enzyme carbonylation induced by α-dicarbonyl compounds. The digested α-dicarbonyl mixtures were found to contain compound(s) with apparent selective in vitro antiproliferative activity against colon cancer cells, but not other cell lines. Overall, the results showed that digested α-dicarbonyl compounds should not be cytotoxic towards normal cells, but may display specific anticancer activity.

Lipidomic approach to the analysis of goat and cow yogurts

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Abstract
The production of goat yogurt is becoming relevant for the agro-food industry of Sardinia (Italy). Up to date analytical methods must be employed to assess quality, authenticity, and traceability of goat and cow yogurts. Currently there are not studies that may clearly establish scientific criteria for discriminating among goat and cow’s yogurt. We conducted a study to evaluate if lipidomic analysis of commercial yogurt samples could represent a new tool for yogurt quality characterization and to protect consumers from fraud. Yogurt samples, collected in a local market, were extracted following the Rose-Gottlieb procedure (1) and methylated with methanol/KOH. Fatty acid methyl esters (FAMEs) were chemically analyzed by the means of GC/MS and GC/FID techniques (2). Data matrix generated by these procedures were analysed by means of PLS-DA models (Partial least squares discriminant analysis; SIMCA-P+ version 12.0, Umetrics, Sweden) to test the hypothesis of the discriminating power of yogurt lipids concentrations. The analysis on the variables of primary importance in this separation was applied to evaluate the set of discriminating lipids. PLS-DA models from both GC/MS and GC/FID analysis were able to discriminate goat yogurt (n=20) from cow’s yogurt (n=20) (PLS-DA_NMR [R2X=0.44; R2Y=0.788 Q2=0.550; P-value=0.002]; PLS-DA_GC_MS [R2X=0.3; R2Y=0.8; Q2=0.4; P-value=0.005]). A lipidic fingerprint based mainly on: butyric, caproic and caprilic acid, X03, pentadecanoic acid, X04, palmitic acid, 11-hexadecenoic acid, elaidic acid, (Z)-9-octadecenoic acid, linoleic and (E,E)-linoleic acid. Multivariate statistical analysis conducted on the GC/MS and GC/FID data, collected from yogurt samples, confirm that lipid fingerprint was affected by the species, type of feeding and can be used for discriminating between goat and cow yogurt.
**Application of the Oxitest® method to evaluate the oxidation stability of vegetable oils at different working temperatures under accelerated conditions**

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**Abstract**

The main cause of deterioration of lipids and lipid containing foodstuffs is lipid autoxidation. A number of accelerated methods have been developed to test the resistance of edible fats and oils to oxidation. Generally, these tests allow obtaining an oxidation curve, characterized by an Induction Period as the time required reaching an end point of oxidation corresponding to either a level of detectable rancidity or a sudden change in the rate of oxidation. In the field of accelerated tests, Oxitest, an instrument developed by Velp Scientifica, permits food oxidative stability to be investigated. This paper presents data on evaluating the oxidation stability in different vegetable oils such as sunflower seed oil, corn oil, soybean oil, mixed seed oil and extravergin olive oil obtained with the Velp Scientifica Oxitest oxidation test reactor using different working temperatures 90, 80 and 70°C to demonstrate the sensitivity of this method in discriminating the resistance to oxidation of oils of different botanical origin. The simultaneous use of two instruments allows the estimation of the repeatability of the response (expressed as the percent coefficient of variation) at each temperature tested. The lowest values, less than 5%, have been calculated for the analyzes carried out at 80 and 90 °C. Higher values, but still below 6%, have been estimated for the tests at 70° C. As expected, with increasing of the temperature the induction period of the oxidative reaction decreases. In particular, an increase in temperature of 10° C, brings to an induction period decreasing of about 2.5 times, this value is much closed to ones found out in literature for other edible oils.

**Authentication and traceability study on truffles from Piedmont**

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**Abstract**

Truffles are among the most renowned and appreciated foodstuffs in the world. Among the different species, a primary role is played by the *Tuber Magnatum pico* variety, also known as *Tartufo bianco di Alba* from the Piedmont town in which countryside it is mostly found. The main feature of *Tuber Magnatum pico* is its extremely high commercial value, due to its rarity and to the fact that it is not possible to cultivate it, unlike other less valuable truffle varieties such *Tuber melanosporum* or *Tuber borchii*. The *Tartufo bianco di Alba* is a DOP, namely a protected appellation of origin, which means that parts of it high commercial value comes also from its geographical origin that must be guaranteed. The *Tuber Magnatum pico* or white truffle, with a cost ranging from 1,000 to 2,000 €/Kg, is one of the most prized foodstuffs in the world and it is therefore an optimal subject for adulteration. Fraud is mostly perpetrated in two ways: by selling different varieties of truffles in place of *Tuber Magnatum pico*, or by selling white truffles coming from geographic areas located outside the DOP zone. In the first case bioanalytical techniques such as DNA sequencing can be used to reveal the frauds; for the second problem, nevertheless, there is no analytical technique to apply, nor is a technique suggested in the scientific literature that permits to distinguish among white truffles coming from Alba zone and from other areas. Scientific research on this argument, moreover, is hardly favoured by the high cost of samples. We have found that some useful information can be yielded by elemental analysis with particular reference to trace- and ultra-trace elements. In particular, rare earth elements or lanthanides have been successfully used in authentication and traceability studies of hazelnuts (Oddone et al.). In the present work, the distribution of lanthanides, as determined with ICP-MS, has been used to verify the link among the truffles and the soils where they have grown. This allowed to state that the original fingerprint, i.e. the lanthanides distribution in soil, is well maintained in truffles, so that it can be used to study the traceability of truffles themselves. On this basis, an authentication study has been performed by applying multivariate analysis to lanthanides concentrations determined in samples of truffles grown in different zones, allowing a clear discrimination among white truffles from Piedmont and truffles from foreign zones.
Flavonol content and agronomical traits as a tool for the characterization of ‘Cipolla di Giarratana’, a traditional onion landrace cultivated in Sicily

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Abstract
‘Cipolla di Giarratana’, a locally cultivated onion landrace, is listed as a item in the ‘List of Traditional Agro-food Products’ of the Italian Department for Agriculture and itemized as ‘slow food presidium’ by the Slow Food Foundation. In order to typify this landrace, we investigated agronomical, morphological and biochemical characteristics of nine onion accessions grown in five experimental fields: two located in the area where this population is traditionally cultivated, two in different areas with similar average altitude, and one in the South East coast of Sicily. Two onion cultivars were adopted as control. High-performance liquid chromatography coupled with diode array detection and electron spray-mass spectrometry (HPLC/DAD/ESI-MS) was used to identify the phenolic profile and quantify phenolic content in bulbs. Ten different flavonols (fig. 1) were identified in ‘Cipolla di Giarratana’, with quercetin (Q), quercetin 3,4’-O-glucoside (Q 3,4’DIGLC) and quercetin 4’-O-glucoside (Q 4’GLC) detected as major components. Differences in term of total and individual flavonol content were ascertained between ‘Cipolla di Giarratana’ accessions (not differentiated between them) and the two control cultivars, where a very low total phenolic content has been detected. The ‘Cipolla di Giarratana’ landrace showed a high values of bulb diameter (11.8±1.5 cm) and weight (502±155 g), and no statistical differences emerged within accessions. Assuming that the phenol profile could represent a powerful tool to assess genetic variability, this research allows to achieve useful data for the certification and protection of this typical product.

Genotype and chemotype profile analysis of Tropea red onion (Allium cepa)

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Abstract
Random amplified polymorphic DNA (RAPD) genomic analyses and chemical composition have been applied to the identification and authentication of ‘Tropea Red onion, a typical Italian product with Protected Geographical Indication. Amplification of genomic DNA using 60 random primers highlighted a good clustering differentiating Tropea Red onion from three commercial reference samples used in our study (Saratoga, a yellow onion; Crystal, a white onion; Primula Rossa, a red variety), underlining the existence of intervarietal genetic difference. The antioxidant properties of onions was also investigated. Antioxidant capacity, assessed by three different methods (DPPH, ABTS and photochemiluminescence both with ACL and ACW mode) showed a highest antioxidant activity of Primula Rossa. The antioxidant properties may be correlated to the large amount of polyphenols and flavonoids contained. The analysis of total phenolics content was performed by using the Folin Ciocalteu methods corroborated the same tends of antioxidant capacity. Quercetin and glucosides forms in onion varieties were characterized and quantified using high-performance liquid cromathography (HPLC) at λ=365 nm. On the basis of our results, flavonoid profiles seems to be variety specific, showing same differences between cultivars.
Environmentally lycopene purification from tomato peel waste: enzymatic assisted aqueous extraction

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Abstract
The antioxidant and anticancer properties of lycopene makes it an ideal components for daily supplement pills. For this reason we investigated the possibility of extracting lycopene from tomato waste peels using a green chemistry protocol devoid of organic solvent. Tomato peels, having an initial lycopene content of about 0.3-0.4% (w/w, dry basis), were first treated with sodium hydroxide at 70°C. This treatment do not open the cells wall (as confirmed by light microscope studies) but rather loosen the waxy layer responsible for cementing tomato peels cells and making them very hard to lyse. Tomato peels were subsequently treated with an enzymatic mixture of cellulase and pectinase at 50 °C and pH 5. The sodium hydroxide pre-treatment allows the enzymes to reach the cell wall. Cells are lysed and the lycopene containing chromoplasts are isolated by the addition of HCl, to further lower the pH, followed by a centrifugation step. At this stage the lycopene content of the isolated chromoplasts shows a tenfold increase (3-5% w/w, dry basis) with respect to untreated tomato peels. A further improvement in lycopene concentration is obtained by a second enzymatic treatment with a proteases cocktail at 50-60°C at basic pH for 3 hours. This catalytic step eliminates unwanted proteins that are bound to the chromoplasts, but are not essential for their stability, by hydrolysing the peptide bonds. Free amino acids and small peptides produced by this treatment are fully soluble and can be eliminated by acidification followed by a centrifugation step. The final product shows a lycopene content of about 8-10% (w/w, dry basis) which represent a 30-fold increase with respect to the lycopene concentration of the untreated peels.

Fatty acid composition of Arabica and Robusta coffee cultivars as a discriminative factor

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Abstract
The aim of the work represents a contribution to fatty acid (FA) discrimination and quantification in the two most representative commercial varieties, Coffee arabica and Coffea canephora var. robusta, to assess the authenticity of the commercial coffee varieties. The high resolution gas-chromatography (HRGC-FID) was applied to the study of FAs composition and triglycerides profile of fat extracted from green and toasted beans. The methodology was specifically developed for this purpose was simple, precise, accurate and appropriate for routine analysis. Analysis were made on the single cultivar, on different blend obtained in laboratory by mixing from 10 to 90 percentage of the two cultivar respectively, and on commercial blends. Green and toasted coffee bean samples were supplied by the coffee industry. Coffee beans were ground in a hammer mill to pass 0.8 mm. It was found that the FAs composition was able to discriminate Arabica and Robusta coffee. In agreement with other authors (J. Martin et. al., 2001; G. Lercker et. al.,1996), the percentage of stearic acid and linoleic acid were higher in arabica cultivar. Instead, the rousta cultivar differed for higher content of oleic acid. Among different FAs ratios identified, C18:1ω9c/C18:3ω3, C22:0/C22:6ω3 and C18:2ω6c/C18:3ω3 were positively correlated (R²>0,85) with the concentration of Coffee arabica contained in the blend. For commercial blends in which the producer had the care to indicate the formulation on the label, the average gap of 10% was found. The triglycerides profile did not seem to have provided useful information to differentiate the single cultivar and the blend that had a known composition.
Ambient mass spectrometry of food without sampling preparation using high resolution, exact mass tof detector

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Abstract
Ambient mass spectrometry is an innovative technique to record mass spectra directly from samples skipping preparative steps and front-end separations like LC and GC shortening analysis time from minutes to seconds. Ions generate outside the mass spectrometer from the sample “as it is” and transferred inside the spectrometer through the atmospheric pressure inlet. An optimized system is needed to achieve best results, coupling high analytical speed, sensitivity and intra-spectrum linearity as provided by modern Trap-Time of Flight spectrometer with a Direct Sample Analysis ion source able to generate ions from solid, liquid and gas samples. Food samples are suitable for direct analysis using ambient mass spectrometry and the speech, after an introduction explaining system design and operations, will report several examples of spectra from olive oil, fruit juices, vegetables and grain and other food products showing both natural products and trace contaminants.

New analytical methodologies for geographical and varietal traceability of oenological products

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Abstract
AGER Project Presentation (www.progettoager.it)

δ¹⁸O of wine ethanol for fraud detection

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Abstract
In the last 10 years the demand for alcoholic products (wine and distillates) has increased by around 8.6 % (source: VINEXPO 2011). Price increases and difficult access to raw materials have encouraged sophistication in the oenological field (source: “Alto Commissario per la lotta alla contraffazione” – 2008 Report). Since 1986, the European Union and the Organisation Internationale de la Vigna et du Vin (OIV) have established some official isotopic analytical methods in order to detect the illegal addition of sugar and water to wine and to enable geographical traceability (OIV MA-AS-311-05, OIV MA-AS2-12, OIV MA-AS-312-06). Recently a new isotopic method for improving the detection of water added to orange juice has been proposed (Jamin et al., 2003; Monsallier-Bitea et al., 2006). The method is based on determining the ¹⁸O/¹⁶O isotope ratio of ethanol derived from sugar fermentation using a pyrolyser coupled to an IRMS. In this study we apply this method in order to identify the origin of ethanol from grapes (N=60), cereals and fruit (N=60) and synthetic products (N=5). The δ¹⁸O values ranged from +26 to +36 ‰ in wine, +17 to +26 ‰ in cereal distillates and from -2 to +12 ‰ in synthetic ethanol.
Characterization of lambrusco wines by means of Sr isotope ratio as provenance marker

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Abstract
Recently, geographical origin and authenticity of food are topics of great interest for consumers as well as for producers in the food industry. Furthermore, in the oenological field, these concepts are strictly tied with the quality of food. For different reasons, people are more focused toward food characterized by a well-established geographical origin. In the course of time, the symbiosis of quality and the geographical origin of products is certainly related to ethics of the producers and the whole determines the reputation of the territory. The present research is part of a project dealing with the development of authenticity and geographical traceability models of Italian wines, with particular reference on Lambrusco wines, which are one of the main typical products of the Modena district. Among the different indicators used for traceability studies, 87Sr, and in particular the 87Sr/86Sr isotopic ratio (I.R.), has provided excellent results for different types of food matrices. Aim of this work is to obtain a reliable fingerprint for Lambrusco wine on the basis of its 87Sr/86Sr isotopic value, linking it to its territory of provenance. Moreover, different analytical methods for the elimination of matrix interferences, before the Sr/Rb separation on resin, are also tested. In particular, the first method, widely used in literature, consists of the following step: i) ethanol elimination by means of HNO3 addition and ii) sample digestion through microwave assisted in acidic media. While, the second one merely consists in the addition of an aliquot of HNO3. Finally, as a preliminary study, wine samples were pre-treated according to the best procedure and 87Sr/86Sr measurements were accomplished with an high resolution multicolonocator inductively coupled plasma mass spectrometry (HR-MC-ICP-MS). The isotopic data of wines were compared with the isotopic range of Modena soils, with the aim to determine a correlation between soil and food, obtaining promising results as far as the potentiality of 87Sr/86Sr as geographical tracer is concerned. To the authors’ knowledge, this is the first report developing an easy analytical methodology for the pretreatment of the studied matrix, obtaining high precision and accuracy measurements, in terms of repeatability, reproducibility and time variability of the monitored indicator.

NMR applications on oenological products

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Abstract
The present work, which is part of the extensive research project AGER - AGroalimentare E Ricerca (“New analytical methodologies for geographical and varietal traceability of oenological products”) coordinated by the University of Modena, aims to use the HR-NMR techniques as molecular fingerprints in order to serve as indirect indicators of authenticity and quality control. In this context, 110 PDO Protected Designation of Origin Lambrusco wines of Modena (34 Lambrusco di Sorbara, 38 Lambrusco Salamino di Santa Croce and 38 Lambrusco Grasparossa di Castelvetro) and several grape musts at different stage of winemaking, provided by local producers joined to the research project AGER - AGroalimentare E Ricerca, were analyzed. The data obtained were coupled with mono and multi variate chemometric analyses to effectively interpret the complex results collected from mono and bi-dimensional spectra (HR-1H-NMR, 1H-1H COSY, 1H-13C HMBC) acquired. Before statistical analysis, all mono-dimensional spectra were calibrated using the TMSP signal, whereas the bi-dimensional spectra were calibrated using one of the signals present in all the spectra analyzed. In this context, NMR spectroscopy represents an important tool for the authentication and quality control of foodstuff. The wine area, in particular, is one of those in which the NMR has proved most successful in recent years (Brescia et al.; Viggiani and Castiglione). High-resolution techniques were rather powerful tools for studying minor components of oenological products. Since the quality and characteristics of a product are not the simple sum of individual chemical characteristics, NMR analysis with chemometric data analysis certainly is a useful tool in this regard. To the best of our knowledge, only a few studies were focused on geographical and varietal traceability of PDO Lambrusco wines of Modena, using simple 1D and 2D high-resolution NMR coupled with chemometrics directly on samples with minimal pretreatment.
Effect of fining by conventional animal proteins and a vegetal nonallergenic protein on volatile composition and sensory characteristics of red wine

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Abstract
The fining of red wine by means of proteic materials is generally used to improve mouthfeel perception, namely to obtain a reduction of astringency by removing phenolic compounds. A wide range of animal proteins is used, however, in recent years, the safety of animal fining proteins has been discussed. After the emergence of the bovine spongiform encephalopathy (BSE) in the 1980s, some animal proteins were banned (e.g. blood albumins). Moreover, the problem of the allergenicity of animal proteins led to the introduction of specific regulations in European Union (Directive 2003/89/EC, Directive 2000/13/EC, Directive 2007/68/EC), Australia, and New Zealand (Food Standard Australia New Zealand, 2002), requiring the labeling of potential allergens, like milk and egg proteins, used for wine fining. Due to the negative impact that the labeling of potential allergenicity could have on the consumers, the demand for alternative nonallergenic fining products has increased and the use of plant-derived proteins has gained increased interest. A recent study showed the suitability of patatin (P), a family of glycoproteins that can be recovered from potato aqueous by-product, for red wine fining (Gambuti et al., 2011). In this study, the effect of patatin on volatile composition and sensory characteristics of red wine, compared to conventional protein fining agents, was evaluated. A comparative fining trial with 10, 20 and 30 g/HL of patatin, potassium caseinate, gelatin and egg albumin on an Aglianico (Vitis vinifera L.) red wine was performed. Free and bound volatile compounds were determined by Solid Phase Extraction (SPE) and GC/MS analysis. Treated and standard wine olfactory characteristics were compared by sensory analysis. Results showed that the loss of volatile compounds caused by the fining with patatin is comparable to those caused by the other conventional fining agents. All the finings caused only slight modifications of the olfactory profile of wine and no off-flavour was perceived after patatin fining.

Effect of post-fermentation extended maceration on phenolic compounds extraction of “Aglianico di Taurasi” wine

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Abstract
The quality of wine is strongly affected by the concentration of bioactive polyphenolic molecules. It is know that the agronomic factors (Ribéreau et al. 2006) and the application of oenological practices (Sacchi et al. 2005), can influence the polyphenolic concentration. The maceration is the main phase of the winemaking process which has a substantial effect on the kinetic of dissolution and/or extraction of phenols from skins and seeds of grape. The objective of this study was to explain how the post-fermentative extended maceration (EM) of “Aglianico di Taurasi” wine could influence the extraction of phenolic compounds and to evaluate the performance during aging wine in wood barrels. Wine were taken after 30, 60 and 90 days of EM and then was aging in tonneau for six month. Evolution of the major chemical/physical parameters, Folin Ciocalteau Index (TPI), total anthocyanins (TA), phenol and polyphenol detected by HPLC/DAD were followed during EM and aging in tonneau. Results shown that post-fermentative EM allowed to improve significantly the concentration of polyphenol extracted from skins. These differences were most evident when time of contact was prolonged for 60 days because was determined the highest values of TPI and TA and major ratio (+)-catechin/(-)-epicatechin. At the end of aging process, the content of polyphenol and anthocyanins were higher in wine where skin contact with wine was prolonged for 60 and 90 days. Results obtained suggested that maceration post-fermentative was suitable to obtain wine with a better qualitative content of bioactive molecules, particularly in the case of the “Aglianico di Taurasi” wine.
Fast screening of food contaminants by LCMS analysis

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Abstract
Depending on the application people are using more and more LCMS based techniques for the detection and quantification of contaminants in foods, like for example pesticides or mycotoxins. Based on European regulations and for intercompany quality control some of the food products need to be regular tested for these classes of compounds to avoid any health risk for human or animals. Very often large number of samples sets are analyzed, therefore the used method need to be fast, robust, reliable and cheap. Extracting the compounds out of the food matrix is the first step. A common way to extract pesticides is the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) approach which is being increasingly used for the development of multi-class pesticide residues methods in various sample matrices. After extraction the separation by HPLC in combination with LCMS/MS tandem mass spectrometry is the method of choice to achieve fast and reliable results for qualitative as well as quantitative analysis. There is a strong tendency to use very fast UHPLC separation methods with high resolution power resulting in narrow peak width. The different compounds will be in general analyzed by the mass spectrometer in the MRM mode, where they will be selected one by one in the first quadrupole, fragmented in the collision cell and confirmed as well as quantified by some of its fragment ions, called quantifier as well as quantifier, in the third quadrupole. To be able to detect in one run hundreds of different compounds it is necessary that the instrument can scan very fast for the different masses, has the ability to switch very fast between the positive and negative ionization mode in one run and also allows the scanning for a high number of MRMs per second in the collision cell. The presentation will show different application examples and highlight different instrument features useful for this kind of analysis.
Enantioselective multidimensional GC (MDGC) and carbon isotope ratio MS (GC-C-IRMS) for the authenticity assessment of Citrus essential oils and other food flavors

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Abstract
Enantiomeric ratios of chiral volatile components represent a useful parameter for the assessment of authenticity of essential oils and natural flavors. However seasonal variations and geographical origin generate quite wide ranges, not yet well assessed, thus rendering this tool not completely reliable by itself. Assessment of genuineness is thus often carried out by multiple analytical techniques (physico-chemical analyses, GC, es-GC, HPLC) in order to evaluate sufficient parameters to express a secure judgment. More over extraction procedures and distillation processes can drastically affect the enantiomeric ratios, due to the possible reactions, with consequent tendency to racemization of some chiral volatiles. The carbon isotope ratio on the other hand is strictly dependent on the plant biochemistry. It is not subject to seasonal variation nor to the extraction procedure used. This study has been carried out on numerous samples of different Citrus essential oils by Es-MDGC, Es-GC and GC-C-IRMS, to determine if the combination of enantiomeric ratios with the carbon isotope ratios can be effective to determine the genuineness of the samples studied. The isotope ratios of the oils extracted by distillation from the leaves were also compared with those determined from the fruit peel of the same plant, in order to determine possible similarities. In addition some food flavours were extracted by headspace SPME and analyzed by the same techniques, then the results were compared to commercially available flavoured food. In conclusion the synergy of the two analytical approaches results extremely useful for the quality assurance of the matrices investigated, drastically reducing the number of components to be investigated.

Chemical blueprint of food: Is GC×GC mature in this respect?

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Abstract
The concept of “blueprint” in the food chemistry field has been exploited by the Molecular Sensory Science defining the so-called key-aroma compounds that unequivocally evoke, if properly distributed in terms of quali-quantitative profile, the characteristic food aroma (Christlbauer). This concept can be extended to a wider group of sample attributes and fruitfully adopted for classification and comparative processes. In this perspective, for example, it is possible to recognize a sample “technological” blueprint that includes chemicals, strongly related to industrial or artisanal treatments a raw matrix undergoes to be transformed in a food-end product, or a “safety” blueprint that refers to the presence (above a certain threshold) of specific contaminants to be monitored. Multidimensional separation techniques, and in particular, comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC-MS), has demonstrated to be a powerful tool to deeply investigate complex mixtures of food interest due to the enhanced peak capacity compared to one-dimensional GC (Adahchour, Pierce, Cordero). In particular, non-targeted fingerprint analysis has demonstrated to be successfully in revealing qualitative/quantitative differences in chemical compositions facilitating the identification of potential marker compounds (Almstetter), and grouping and classification of samples (Cordero, Cordero). Current research is mainly focused in the development of non-targeted, peak-based fingerprinting methods able to exploit the informative content of three dimensional GC×GC-MS data sets to maximize the information extractable from each single analysis (Reichenbach). Moreover, GC×GC can be considered a mature technique, thanks also to the technological advancements recently introduced that have simplified the instrumental set-up making it easier to adopt multidimensional GC as a routine control technique. In this perspective the chemical “blueprint”, embodied in the GC×GC sample fingerprint, should be easily revealed and subsequently adopted ad a discriminating tool. In particular, becomes of crucial importance the development of suitable informative tools able to act as Analytical Decision Makers(Sandra) enabling the analyst to decide whether or not a sample necessitates of a further detailed analysis to clarify its composition. Complex food samples of vegetable origin have been analyzed by GC×GC-MS and GC×GC-FID and sample “blueprints” revealed and adopted, as analytical probe, to categorize samples on the basis of their aroma quality, process impact and safety compliance.
Evaluation of thermal treatment markers in flours and related products by capillary electrophoresis coupled to tandem mass spectrometry

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Abstract
Thermal processes have been employed since a long time for food sanification to improve quality and safety, and also with the aim of conferring to product peculiar characteristics due to the generation of color and flavor compounds. On the other hand these processes can produce a damage regarding the nutritional value of a food. Maillard reaction is the principal complex of reactions responsible for the formation of compounds deriving from the heating process of food, and furosine has been the most studied compound along more than 40 years. Furosine has been demonstrated to be an index of heat damage in many food products, and its content in flour may depend on the drying step overcome by the seeds before milling, and on milling process. We optimized and validated a new method for furosine determination by capillary electrophoresis coupled to tandem mass spectrometry (CE-MS-MS), and proposed it as a valid alternative to the traditional HPLC-UV methods. An additional potentiality of our method involves the chance to study other molecules reported to be sensitive markers of graduated heat treatment. The aim of this work is the investigation of the amount of furosine and its related markers in different flour samples, with great attention at those formulations proposed as baby-food to be administered in the first step of weaning. The study has been extended to the analysis of bakery products cooked at different temperatures and cooking times, and proposing the cook index value as a system to evaluate the entity of thermal treatment.

Orbitrap technology: quantitative and untarget analysis

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Abstract
Qualitative confirmation and quantitative determinations of metabolites in biological matrices is one of the challenging analytical fields. Various methodologies are employed to meet the challenge. Orbitrap LC/MS technology is the recognized standard for accurate mass and high-resolution measurement. The platform of choice for the most confident protein and metabolite identification, characterization and quantitation. We will discuss some of the basic concepts that have led to the design of the Fourier Transformed Mass spectrometry known as Orbitrap. The Orbitrap analyzer as well can be hyphenated with Tandem Mass spectrometers, like the Linear trap, and new developments in this field will be presented as well. Both techniques are the most powerful tool for mass spectrometry researchers in the field of large molecules and small molecules. Some applications will be shown enhancing the need for higher resolution in the analysers in the field of proteins, peptides and even small molecules.
Alpha-dicarbonyl compounds reactivity in presence of digestive enzymes and biological activities of reaction products

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Abstract
Alpha-dicarbonyl compounds such as glyoxal (G), methyglyoxal (MG), and diacetyl (D), are \( \alpha \)-oxoaldehydes, highly reactive intermediates that can be formed in different types of foods, especially after heat treatment and fermentation. The presence of these compounds in foods raises important questions because of their toxicological profile. In fact, G and MG have shown mutagenic and carcinogenic properties in vitro. In addition, these compounds are known to participate in vivo in the processes of protein glycation involved in the development of various chronic-degenerative diseases. In view of the above mentioned, we performed in vitro studies to verify a possible influence of digestive enzymes on free \( \alpha \)-dicarbonyl compound concentration. At this purpose standard solutions of each compound and their mixture and three different foods were submitted to digestive enzymes treatment quantifying G, MG, and D before and after digestion, using validated HPLC-DAD methods. The obtained results have shown as the digestive process may affect the detectable concentrations of these compounds even though in a very different way depending on the specific considered compound but also, and above all, on the food matrix in which the \( \alpha \)-dicarbonyl compounds occur. The research continues in order to study both the reactivity of these compounds in presence of other food matrices and the biological activity of the adducts formed in \( \alpha \)-dicarbonyl compounds and digestive enzymes reactions.

CO-24

NMR spectrometers as “magnetic tongues”: Predition of sensory descriptors in food

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Abstract
It is crucial to know consumers’ expectations, habits and preferences in order to ensure product success on the market. Brand, label information (such as geographic origin, technology, etc.), price, packaging, factory image, product concept, and effective communication are all critical factors. However, when the consumer decides whether to buy the product again or not, success is tightly connected to the products’ features. It is therefore extremely important to understand how much consumers’ preferences are driven by differences in sensory features between products. Traditional consumer research helps determining acceptable versus unacceptable. It is helpful when an overall, synthetic understanding of the products acceptance is needed. However, it is not of any help when an explanation, in terms of sensory descriptors, is needed in order to provide R&D with technical information useful to enhance product features. Such information can only be provided through analytical products evaluation, of which consumers are not capable. A detailed sensory description, in fact, requires the ability to decompose each sensory feature, requires selective attention, and thus requires people specifically trained to the application of sensory analysis (quantitative descriptive analysis – QDA). Sensory analysis is a discipline through which the sensory analyst evokes, measures, analyzes and interprets human responses to stimuli as perceived through the senses. Human sensory tests are regularly employed in the food and beverages industries and they are sometimes integrated by a number of techniques, including the electronic nose2 and the electronic tongue2. Here, we demonstrate the utility of ¹H-NMR as a tool to analyze the taste of food without any other chemical analysis.
Direct plant tissue analysis by leaf spray mass spectrometry

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Abstract

Highly sensitive techniques such as LC-MS/MS and GC-MS/MS, traditionally used to perform qualitative and accurate quantitative analysis of foodstuff samples, require time- and chemical-consuming steps for sample preparation prior to the instrumental analysis step. Recently, a number of different ambient ionization methods (e.g. DESI, DART, LTP) have been introduced to address the problem of MS analysis of untreated samples in their native state, i.e. without prior sample preparation or chromatographic separation. Now, we report the use of leaf spray (LS)¹ ², a form of paper spray³ as a new ambient ionization method for direct mass spectrometric analysis of untreated samples. In leaf spray experiments, the plant material without a natural tip is cut to a sharp triangular point (typically ~0.3-1 cm base width, ~1-2 cm long, and <3 mm in thickness), a high voltage (usually 2-5 kV) is applied to the base of the sample triangle and the tip, from which gas phase ions are generated, is held 0.3-1 cm from the MS inlet. No other ionization device or support is needed since the triangle of plant tissue, used without any further treatment, acts simultaneously as the sample and as the source of ions. In the present communication we report the application of LS to mass spectrometric determination of: i) agrochemicals in fruit and vegetable tissues and ii) flavonoids in citrus albedo. In the former experiments, the performance of LS-MS will be shown for rapid screening of “organic” from non-organic fruit and vegetable tissues without sample pre-treatment. This rapid method allowed (in approximately 100 seconds) the identification and quantitative or semi-quantitative determination of a number of typical pesticides (acetamiprid, diphenylamine, imazalil, linuron, thiabendazole) from a variety of different fruits and vegetables (apple, pear, lemon, orange, carrot, cucumber, eggplant, potato). In the latter experiments (ii) will be shown the application of LS-MS approach for determination of several secondary plant metabolites (e. g. hesperidin, naringin, diosmin, eriocitrin, melitidin, brutieridin, amongst others) from citrus albedo.

Discrimination and characterization of the metabolic profile of the "Igp Pachino" tomato by high-resolution proton NMR

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Abstract

As is well known, metabolism represents the physical and chemical reactions that occur in a living organism (or part thereof), and consists essentially in transformations of matter caused by energetic variations. The scientific discipline able to identify and quantify the different metabolites that determine and characterize the studied bio-system in its particular state is called “Metabolomics” [Lindon et al.]. Essentially, one can create a precise molecular fingerprint in order to typify a particular foodstuff. In this work we present an experimental study aimed to discriminate and characterize the metabolic profile of the "IGP Pachino" tomato. We used the nuclear magnetic resonance (NMR) technique at 700 MHz in the condition known as Magic Angle Spinning in order to canceling the contribution due to the dipolar interactions so obtaining very high resolution NMR spectra even on few amount of sample (in the order of 50 µl). Our study concerns different samples of tomato (Lycoperscion esculentum, part of the Solanaceae family), sure and “certified” as Pachino (P) and of dubious origin (NP). The obtained high resolution NMR spectra allowed us to perform a very good assignment of all metabolites with respect to those present in the actual literature [Sobolev et al.]. Then, using a multivariate statistical analysis in terms of the Principal Components Analysis (PCA) the spectra of two different categories of samples were compared and analyzed to highlight the differences distinguishing them. In doing so, it was possible to quantify the concentrations of some important metabolites which help to determine the difference between the two species.
The glucosinolate profile of capers by high resolution FTICR MS

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Abstract
An important group of bioactive components occurring in capers are glucosinolates (GLSs) which are sulfur-rich plant secondary metabolites [1]. It is believed that the typical flavour of capers, as most cruciferous vegetables, is largely due to the presence of GLSs [2]. All Capparis species are sources of these compounds with the structurally simplest representative, glucocapparin as the most widely distributed one [3]. In this communication we describe a very selective and sensitive approach using LC-ESI-FTICR MS and tandem MS fragmentation performed by infrared multiphoton dissociation (IRMPD), successfully applied to identify targeted and untargeted GLSs of flower-bud extracts of capers (C. spinosa). Along with most common GLSs already found in capers (Capparis species) such as glucocapparin, isopropyl/n-propyl-GLS, mercapto-glucocapparin, and two indolic GLSs, namely 4-hydroxyglucobrassicin and glucobrassicin, the occurrence of the uncommon glycinyl-glucocapparin and of more sulfur-rich GLSs in caper extracts was discovered. On the basis of their fragmentation behavior all compounds were successfully identified including disulfanyl-glucocapparin [C8H14NO9S4]− (mass error, -0.23 ppm) and trisulfanyl-glucocapparin [C8H14NO9S5]− (-1.50 ppm). All GLSs were characterized by several product ions, including the [HSO4]− ion at m/z 96.96012, being a good clue of a compound belonging to the GLS family. Moreover, typical fragments derived from the thioglucoside moiety and from the side-chain were observed and will be described in great details [4]. Interestingly, IRMPD showed an increased selectivity towards disulfide bond cleavages with thiol migration, suggesting the side chain structure of both sulphur-rich GLSs [5].

Solid-phase extraction of phenolic compounds from food matrices by molecularly imprinted polymers

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Abstract
Determination of phenolic compounds in vegetables was performed using a molecularly imprinted polymer (MIP) as sorbent material in a solid phase extraction (MISPE). MIPs are functional polymers generated by molecular imprinting, an efficient method for producing functional materials equipped with selective identification characteristics. The technique consists of self-assembly of a functional monomer and a template molecule in solution followed by co-polymerization of the functional monomer with an excess of an appropriate cross-linking monomer. After removal of the template, the resulting polymer exhibits high affinity for the molecule used as template and structural analogues. In the recent years MISPE has been successfully applied to solve several challenging issue in food, biological and environmental analysis. Components of Propolis and olive oil, as well as other food matrices have been of considerable interest in recent years because of their potential utility as pharmaceutical agents for their antioxidant and anticarcinogenic activity 1,2,3. Synthesis of a selective MIP was achieved using (E)-resveratrol as the template, 4-vinyl piridine (4-VP) as the monomer, ethyleneglycol dimethacrylate (EGDMA) as the crosslinker and 2,2’azobisisobutyronitrile (AIBN) as the iniziator4. The solid phase extraction protocol consists of three steps: aqueous sample loading, washing by mean of acetonitrile and elution with methanol-acetic acid (9:1 % v/v). The phenolic compounds such as quercetin, apigenin, luteolin and caffeic acid were selectively extracted from these matrices because of their structures similar to (E)-resveratrol. Separation of phenolic compounds was performed by reversed phase high-performance liquid chromatography (RP-HPLC) using Luna 2.5 u C18 (2)-HST (100 x 2.0 mm) as analytical column. Quantitative analysis was conducted by UV DAD and ESI-MS detection.
Analysis of plasticizers in coffee capsules by SPE-GC-MS
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Abstract
In recent years great importance has been directed towards the monitoring of alimentary products for the presence of organic contaminants including plasticizer. Among these, phthalic acid esters (phthalates, PAEs), adipic acid esters (adipates, AAEs) and sebacic acid esters (sebacates, SAEs) were today the most notable groups. They have been used in food contact materials and are generally allowed for this use. The negative health impacts from these compounds (e.g. potential for reproductive and developmental harm) have been documented and discussed extensively. There are no specific restrictions in the EU, and the phthalates, adipates and sebacate are allowed in any concentration in all foodstuffs. Nowadays, is increasingly widespread use to make coffee with coffee machines that use coffee pods and capsules. In the present study, solid phase extraction followed by capillary gas chromatography coupled to mass spectrometry (SPE-GC-MS) was used for quantitative analysis of 27 plasticizers in coffee capsules, which consist in a single dose of ground coffee pre-packaged into their own plastic or aluminium packaging. The performance of the method was evaluated in terms of sensibility, linearity, accuracy and precision, achieving satisfactory results. Di-methyl phthalate (DMP), di-isobutyl phthalate (BiBP), di-butyl phthalate (DBP) and bis-(2-ethylhexyl) phthalate (DEHP) residues were found in all analyzed samples, while di-ethyl adipate (DEA), bis-(2-ethylhexyl) adipate (DEHA) and bis-(2-ethylhexyl) sebacate (DEHS) in only a few. All other plasticizers were below their quantification limit. Anyway, the determinate levels should not represent a risk for the consumer.

Double dispersive liquid-liquid microextraction for the analysis of ionizable compounds in complex matrices: Case study of ochratoxin a in cereals
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Abstract
Dispersive liquid-liquid microextraction (DLLME) is an emerging miniaturized sample-preparation technique that is becoming increasingly popular due to simplicity of operation, rapidity, low cost, environmental benignity, high recoveries and enrichment factors. DLLME has been largely used for the analysis of environmental water samples, demonstrating excellent analytical performance for different types of analytes. By contrast, not many applications were devoted to the analysis of organic compounds in complex matrices such as food, soil and biological samples, owing to the presence of potential matrix interferences. In order to improve the DLLME selectivity, equilibrium distribution of target analytes between water and extractant could be affected. In the case of ionizable compounds, the DLLME efficiency could be modulated by aqueous phase pH adjustment. Thus, it should be advantageous to perform a first DLLME process in order to eliminate matrix interferences, while the ionized analyte remains into aqueous phase. Subsequently, the analyte could be recovered with a second DLLME by exchange of pH to shift the dissociation equilibrium from ionized to unionized form. In present paper, a new use of DLLME, termed double DLLME, was evaluated as sample pre-treatment method for analysis of ochratoxin A (OTA) in cereals. OTA is a mycotoxin classified as possibly carcinogenic for humans and cereals and related products are the major source of OTA intake and also the main foods for human and livestock consumption throughout the world. Thus, the mycotoxicological safety of these commodities is of significant concern to human health.
A rapid method to assess authenticity of “100% pure” pomegranate juices by UV-visible spectroscopy and multivariate analysis

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Abstract
Scientific research employing in vitro, animal, and human models has found that pomegranate juice (PJ) consumption promotes cardiovascular health and inhibits the proliferation of many types of cancer [1, 2]. These activities have been largely attributed to the phenolic fraction of PJ. Indeed it contains a significantly high level of powerful antioxidant ellagitannins, such as ellagic acid, punicalagin and punicalin, as well as anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3,5-diglucosides) [3]. Several studies have even shown that PJ contains much more antioxidant compounds than other common fruit juices and beverages [4]. Thus consumer demand for pomegranate juice has considerably grown, during the last years, for its health benefits. Since it is an expensive functional food, cheaper fruit juices addition (i.e. grape and apple juices) or its simple dilution are deceptively used. At present, the quality control of this product is often based on modern and sophisticated instruments that are time-consuming and/or involve high costs and/or require well-trained analysts [5]. The purpose of this study was to propose a high-speed and easy-to-use shortcut. Based on UV–Visible spectroscopy and chemometrics, a screening method is proposed to quickly detect the dilution or the adulteration of pomegranate juice that decrease the antiradical scavenging capacity of pure products. The analytical method was applied to representative experimental mixtures at different levels of water and filler juices. The outcomes were evaluated by means of multivariate exploratory analysis. The results indicate that the proposed strategy can be a useful screening tool to assess authenticity of “100% pure” pomegranate juices.

Electronic nose to detect peach quality changes during cold storage in relation to fruit optical properties measured by time-resolved reflectance spectroscopy

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Abstract
The absorption coefficient measured at 670 nm (µa670) in fruit pulp at harvest by time-resolved reflectance spectroscopy (TRS) is linked to fruit biological age and has been successfully used to predict softening rate and market destinations in nectarines (Tjiskens et al., 2007). Peaches and nectarines quickly ripen at ambient temperature and cold storage is used to slow down both ripening process and decay development. The aim of this research was to study the relationships between electronic nose pattern, maturity of peaches at harvest time measured by TRS, and quality evolution during a 4 week cold storage. Peaches, cultivar ‘Spring Belle’ harvested on June 22, 2010 (n=270), were measured for µa670 by TRS, ranked according to decreasing µa value, divided into 3 TRS maturity classes (less (LeM), medium and more (MoM) mature) and randomized into nine samples of 30 fruit each, so that fruit from the whole µa670 range were present in each sample. At harvest and after 1, 2, 3 and 4 weeks of storage at either 0°C or 4°C, all fruit of each sample were evaluated for firmness and µa670. LeM and MoM peaches of each sample were analysed for ethylene production by GC, aroma pattern by elecronic nose and static HS-GC, and sugar (glucose, fructose, sucrose, sorbitol) and organic acid (quinic, malic and citric acids) compositions by HPLC. Data were analysed by PCA statistical analysis. Four functions were extracted explaining 67% of the total variance. PC1 decreased with storage time and in LeM class and grouped low molecular weight volatile compounds, which positively related to W5S, W1S and W2W sensors, and negatively with W1W, W1C, W3C and W5C sensors. PC2 opposed total sugar/total acid ratio to methyl and ethyl acetate, (E)-2-hexenal and γ-decalactone, was not influenced by storage temperature and showed the minimum score after 2 weeks’ storage and was lower in LeM class. PC3 was bound to sugar and acid compositions and showed the highest score at harvest, for fruit stored at 4°C and in MoM class. PC4, relating sensors to acid composition, decreased with storage time and was not influenced by storage temperature and TRS maturity class.
Palytoxin in seafood: Development of an electrochemical screen-printed electrode coupled with a haemolytic assay for the measurement of LDH activity

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Abstract

Blooms of Ostreopsis spp. have been recently reported along the Mediterranean coasts of Spain, France, Italy and Greece, posing serious risks to human health. Occurrence of Ostreopsis spp. may result in palytoxin (PITX) contamination of seafood (250 µg/Kg proposed regulatory limit) and, in order to prevent sanitary risks, the need exists to develop rapid and sensitive monitoring methods of PITX-like compounds (PITXs) in seafood, coupled with an efficient extraction procedure. The effect of PITXs is due to their ability to bind the sodium-potassium pump membrane (Na⁺/K⁺-ATPase), causing an ionic imbalance with consequent haemolysis of mammal erythrocytes and alteration of the functioning of excitable cells. The aim of this work is the development of an electrochemical sensor based on screen-printed electrodes (SPEs) for the detection of PITX and its related compounds. Our method is based on the amperometric measurement of lactic dehydrogenase (LDH) released into the medium when sheep erythrocytes are lysed after incubation with PITXs. The degree of haemolysis, and therefore the amount of LDH measured, using NADH and appropriate mediators (PMS⁺, Fe(CN)₆³⁻), is correlated to the concentration of these toxins. A feasibility study, about the choice of the working potential (+260 mV), the optimization of NADH, PMS and Fe(CN)₆³⁻ concentrations (0.5 mM, 0.05 mM and 2 mM respectively), the measurement of LDH activity and the blood dilution (1:40), were carried out. Two different incubation times (24 h and 4 h), between blood sheep and PITX standard solutions were tested obtaining a working range of 0.007-0.02 ng/ml and 0.15-2 ng/ml, respectively. The specificity of the test for palytoxin was confirmed by the fact that the haemolysis was not detected after exposition to high concentrations (200 ng/ml) of: saxitoxin, brevetoxin, tetrodotoxin, okadaic acid and yessotoxin. Experiments to evaluate the matrix effect and recovery on mussel samples, using different extraction procedures, are in progress and will be presented.
Isothermal breakdown and kinetic study using biosensor for peanut oil heated to 180°C and triglycerides contained using thermal analysis: Comparison with results obtained for extra virgin olive oil

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Abstract
In recent years an in-depth research has been carried out in our laboratory on the isothermal rancidification of extra-virgin olive oil (EVOO) performed at 98°C in an air stream. The kinetic rate of this process was also calculated. The aim of the present research was instead to study the kinetic rancidification of peanut oil (PO) when a sample of this oil was artificially rancidified isothermally at 180°C in air; in practice the oil sample was heated to a high temperature in order to determine the kinetic rate value and therefore the time span over which the product can effectively and safely be used for cooking and frying. However, as reported in literature, the process of heating the oil samples leads to chemical changes and causes the formation of radical species, which results in an appreciable increase in oil toxicity. In the present study radical species formation due to heating was evaluated using a radical index. This was detected using a biosensor method based on a superoxide dismutase enzyme (SOD), while the increasing toxicity was monitored using a suitable toxicity measuring device. Two different kinetic methods for processing data derived from biosensor measurements exploited during the isothermal breakdown of peanut oil at 180°C were used. The corresponding data for an extra virgin olive oil were used for the purpose of comparison. The first method is called model-fitting, while the second method is denoted as traditional kinetic. Both methods, based on very different approaches, adopted the same mechanism, really F1 and first-order reaction, as experimentally evidenced. From the slopes of the regression lines adopted in both methods the kinetic rate constant values at 180°C were obtained. The values, when referred to the same oil and the most suitable mechanism, are of the same order of magnitude (10⁻⁴ s⁻¹) for both methods. Lastly, to complete the information regarding the principal decomposition processes affecting the triglycerides during the heating of the two food oils tested, the latter were subjected to TG and DTG thermoanalysis. The data obtained, processed according to Kissinger’s method, allowed the activation energy of the thermoxidation processes occurring in the triglycerides present to be assessed.

The role of the light scattering phenomenon in FT-NIR quantitative calibrations of milk fat and protein contents

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Abstract
In dairy farms and quality control laboratories, milk macro-constituents are usually determined by off-line analysis performed with appropriately calibrated MIR spectrometers after a homogenisation step. NIR spectrometers, thanks to longer optical pathlengths and quartz optical fibres, are suitable for on-line measurements. NIR measurements on raw milk are affected by the scattering phenomenon, i.e. radiation redistribution without loss of energy in the medium, due mainly to fat globules causing fluctuations in medium density. Such a phenomenon is often undesirable and usually reduced by operating at the level of both sample preparation and optical geometry, and also by applying chemometric techniques for data pre-processing. However, the scattering can bring useful information, since it’s closely related to the properties of the particles that determine it. The aim of this work was to verify, if and in which measure, NIR spectrometers can replace MIR spectrometers in the analysis of raw milk. For this purpose, individual milk samples were analyzed with two spectrometers: a Milkoscan FT2 (FOSS Italia, Italy) in the spectral range 926-5000 cm⁻¹, and a NIRflex N-500 (Buchi Italia, Italy) in the spectral range 4000-10000 cm⁻¹. NIR calibrations for fat and proteins were performed using Milkoscan data. NIR calibration for fat gave good results (R²_val=0.97) comparable with that obtained with MIR spectroscopy, while a lower sensitivity for proteins quantification was observed (R²_val=0.86). The best performances of fat calibration are due to a secondary phenomenon: the spectral contribution of the scattering produced by fat globules in milk that plays a primary role during the processing of spectral data. NIR spectral data were also correlated with laser light scattering granulometer data (Mastersizer 2000, UK) in order to develop a predictive model for the estimation of milk fat globule distribution.
Metabolomic approach and projection methods in food science
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Abstract
The metabolomic approach can be defined as the analysis and interpretation of the global metabolic data expressing the response of living systems to genetic modification, pathophysiological stimuli and environmental influences. In this presentation will be introduced and discussed the most important and used multivariate data analysis techniques useful to approach discrimination and data integration, two typical problems in food science. Two studies will be considered to illustrate the projection methods. The aim of the first study will be to characterize, by means of an NMR-based metabolomic approach, honey samples having different botanical origins[1]. We considered 7 different kinds of honey. Specific markers were identified for each monofloral origin and a classifier based on a hierarchical modeling strategy was build. The objective of the second study will be to integrate data sets obtained by complex experiments. In particular, the metabolic content of basil plant was analyzed under different growing conditions by LC-MS technique[2]. Mechanical stress and light exposure were modified to simulate different natural environmental factors. By analysing the relationships between growing conditions and metabolic content we were able to highlight the role played by the different factors under investigation. Projection methods such as Bidirectional Orthogonal Projections to Latent Structures (O2PLS)[3] and Principal Components Analysis (PCA)[4] will be applied and useful visualization tools will allow us a clear understanding of the statistical models in terms of measured variables.

Functional bioactive compounds from hazelnut (C. avellana L.) seeds and skins:
New perspectives for the nutraceutical products formulation
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Abstract
Hazelnut (Corylus avellana L., Betulaceae) is one of the most cultivated and consumed nuts worldwide. Turkey is the world’s largest hazelnut producer (65% of the total world global production), followed by Italy (16%), USA (4.0%), and Azerbaijan (3.4%) (FAOSTAT, 2009). Hazelnut seeds, raw or roasted, are particularly rich in fat (up to 65%) and in protein (up to 22%); also polyphenols and fibers are major components (Alasalvar et al., 2003; Locatelli et al., 2011). Recently, FDA highlighted that a regular intake of nuts should be correlated to a reduction of the risk of certain type of cancer, coronary heart disease (CVD), stroke, atherosclerosis, inflammation and other neurodegenerative disease correlated with oxidative stress. Hazelnut skins, a by-product spontaneously released from the seeds during or after the roasting process, was recently suggested as new functional ingredient, source of antioxidants (Shahidi et al., 2007). In this work, we aimed to study the total radical scavenging capacity (RSC) of polyphenols (from seeds/skins) and the prebiotic properties of soluble/insoluble fibers (from skins), focusing on their oligosaccharides composition. RSC of defatted hazelnut pellicles revealed high ABTS+" scavenging properties (particularly for roasted samples) (Locatelli et al., 2010). Both soluble and insoluble fibers stimulated significantly the growth of probiotic bacteria (L. plantarum and L. crispatus; range: 0.11- 0.03 % w/V), showing cryoprotectant capacity during freeze-drying step. MALDI-FTICR and Micro-Chip LC-high-performance MS (Accurate-Mass-Quadrupole-TOF) were used to characterize free oligosaccharides in skins (constituent monosaccharides: Hex, HexNac, Fuc, NeuAc, Pentoses, GalNAC and GlcNAC). Concluding, we suggest new findings about the bioactivity of both polyphenols and fiber fraction isolated from hazelnut seeds and skins, suggesting their use for the formulation of new functional ingredients, functional foods and nutraceuticals.
Carnosine and related histidine dipeptides as breakers of the damage axis AGES-ALES/RAGE

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Abstract
Reactive carbonyl species (RCS) are cytotoxic molecules generated by lipid and sugar oxidation, leading to the formation of protein adducts termed AGEs (advanced glycoxidation end products) and ALEs (advanced lipoxidation end products). AGEs and ALEs are involved in oxidative cellular damage through different mechanisms including direct protein dysfunction, protein oligomerization and deposition, signal transduction, immune response and by the activation of the AGE receptors (RAGE). The damaging AGEs/ALEs-RAGE axis and the downstream pathways leading to injurious effects is believed to be involved in different oxidative based diseases including diabetes, atherosclerosis and some neurological disorders and hence its suppression is now a promising target for therapeutic and/or nutraceutical interventions. Carnosine (beta-alanyl-L-histidine) and other histidine-containing dipeptides (HCD) are particularly abundant in excitable tissues such as nervous system and skeletal muscle and are contained in high amount in the diet (red and white meat). HCD have proven to be beneficial in a variety of disease models, in which chronic oxidative or glycative stress is a characteristic feature. We propose that HCD mediate their health-promoting effects by decreasing the levels of AGEs and ALEs and hence acting by interrupting the damaging axis AGEs-ALEs/RAGE. Such a hypothesis is based on the following findings obtained in our laboratories over the last decade: 1) HCD are selective detoxifying agents of RCS including alfa,beta-unsaturated aldehydes (acrolein, 4-hydroxynonenal), di-aldehydes (glyoxal, malondialdehyde) and cheto-aldehydes (methylglyoxal), as demonstrated in in vitro and in vivo experiments; 2) HCD and derivatives were found effective in different animal models where AGEs/ALEs-RAGE axis is involved as damaging mechanism (Zucker rats, db/db mice, apo E null mice) and the biological responses were paralleled to a significant inhibition of AGEs/ALEs and RAGE activation and up-regulation and by an increased excretion of the unreactive adducts between HCD and RCS. Based on the promising animal studies and in view of human intervention studies based on HCD supplementation, the absorption kinetics and bioavailability of HCD were then investigated in humans after ingesting pure carnosine or HCD rich foods.

Antioxidant activity of Mediterranean food: The case of cannonau wine, myrtle berries liqueur and strawberry-tree honey

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Abstract
A diet rich in antioxidant compounds is essential in preventing oxidative damage in humans. The Mediterranean diet is famous worldwide for being rich in antioxidants. Among these antioxidants, polyphenols are a group of molecules with proved antioxidant properties and protective effects against LDL oxidation and platelets aggregation. The aim of this work was to use different assays to evaluate the antioxidant properties of three typical food products from the Mediterranean area and to correlate these activities with their phenolic content. For this purpose, a red wine (cv. Cannonau), a liqueur obtained by cold maceration of myrtle (Myrtus communis L.) berries and a bitter honey obtained from strawberry-tree flowers (Arbutus unedo L.) were analyzed. The total phenols (TP) content was measured by spectrophotometric determination with a modified Folin-Ciocalteau method and phenolic compounds were identified and dosed by LC-DAD-MS (Rosa et al., 2011; Tuberoso et al., 2010). Antioxidant activities were evaluated with the DPPH, FRAP and ABTS assays and the direct vasodilatory effects were assessed using norepinephrine precontracted rat aortic rings. Cannonau wine and myrtle liqueur showed high levels of TP (1732.0±98.7 and 1840.9±10.8 mg GAE/kg, respectively), linearly correlated to FRAP, ABTS and DPPH values. Their maximal vasodilatory activity was 62±4% and 53±3%, respectively. Although strawberry-tree honey contained relatively high levels of phenolic compounds (930.8 mg GAE/kg), it did not induce significant vasodilation, and even at the highest dose tested (0.206 g/L). These results indicate that foods with high levels of phenolics should be studied using several antioxidant assays before being recommended to the public as functional foods.
Effects of novel sirt activators in wound healing

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Abstract
NAD+-dependent histone deacetylases (sirtuins, SIRT1-7) emerged as potential therapeutic targets for treatment of human illnesses such as cancer, metabolic, cardiovascular and neurodegenerative diseases. In particular, SIRT1 is able to deacetylate a number of both histone and non-histone targets involved in transcription, metabolism, and energy homeostasis.1 Among others, SIRT1 deacetylates the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ) and its transcriptional coactivator PPARγ coactivator-α (PGC-1α), which regulates a wide range of metabolic activities in muscle, adipose tissue, heart, and liver.2 In addition, through eNOS deacetylation SIRT1 improves endothelial cell survival and functions.3 Compared to the number of SIRT inhibitors (SIRTi) described in the literature, only few SIRT activators (SIRTa) have been disclosed so far. Among these, resveratrol and some imidazo[1,2-b]thiazoles are being studied in clinical trials for diabetes mellitus, inflammatory diseases, and muscular atrophy. Searching new putative SIRTi based on the 1,4-dihydropyridine (DHP) scaffold, we identified some novel SIRTa, able to increase SIRT1 as well as SIRT2 activity in both enzyme and cellular functional assays, and showing anti-senescence effect, activation of PGC-1α, and increased mitochondrial activity, similarly to resveratrol.4 In vitro experiments performed on DHPs revealed that SIRT activation stimulated proliferation of endothelial cell, keratynocytes and skin fibroblasts paralleled by the induction of eNOS phosphorylation and nitric oxide (NO) production. Investigating the effect of these novel SIRTa during angiogenesis and wound healing following skin damage, a series of studies performed in mice showed a strong induction of proliferation and a significant increase in neo-angiogenesis.

NMR screening in the quality control of food materials and nutraceuticals

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Abstract
NMR since several years is one of the two leading analytical technologies used in Metabolomics. Due to its highest reproducibility, NMR is suited to visualize many small changes in the composition of a mixture simultaneously. Therefore NMR is the technology for nontargeted screening, comparing new samples to established models of normality and to detect deviations be they unknown or known. At the same time with the same experiments, NMR can deliver targeted analysis to quantify known compounds in the mixture spectra. A first NMR based product has been introduced to investigate fruit juice quality and safety, which already had influence on improved detection of deviations from normality. Examples for the procedures described are given for various food matrices and for nutraceuticals, where NMR Metabolomics methods can be used for quality control of incoming extract batches for production, this is exemplified on St. John’s worth. The advantage of the NMR approach in all examples given, is based on minimized preparation needs, targeted and non-targeted results in one experiment and complete push-button operation, freeing the users for other important tasks.
Synthesis and evaluation of the antioxidant properties of 5-S-lipoylhydroxytyrosol and polysulfides thereof

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Abstract
Hydroxytyrosol occupies a prominent position among natural polyphenols because of its antioxidant potency and the wide range of biological properties. Several efforts have therefore been directed toward the preparation of hydroxytyrosol derivatives with improved antioxidant and pharmacological activities and different solubility properties, particularly enhanced lipophilicity. Along this line, 5-S-lipoylhydroxytyrosol (1) was prepared by conjugation of hydroxytyrosol with dihydrolipoic acid. The expedite synthetic procedure involves regioselective oxidation of tyrosol with 2-iodoxybenzoic acid to hydroxytyrosol o-quinone, followed by addition of dihydrolipoic acid. Further aim of the study was the preparation of polysulfide derivatives of 1, as polyfunctional compounds combining the potential of the catechol moiety with that of the polysulfide functionality, typically associated to remarkable chemical, biological, and pharmacological properties. Specific conditions to obtain each polysulfide, namely the disulfide 2, the trisulfide 3 and the tetraysulfide 4, were developed relying on a fine tuning of the reaction parameters such as the absence or presence of sulfur in different solvents. All the polysulfides 2-4 were found to have stronger hydrogen donor ability than Trolox in the DPPH assay. In the FRAP assay, 1 exhibited the best reducing activity. All compounds 1-4 acted as efficient hydroxyl radical scavengers at concentration as low as 10 μM in a Fenton reaction inhibition assay. The antioxidant activity of compound 1, disulfide 2 and tetraysulfide 4 was also tested in human hepatocarcinoma cell line (HepG2). Direct treatment of cells with the compounds induced significant changes in cellular intrinsic antioxidant status, reducing ROS generation. Moreover, pretreatment of cells with the compounds counteracted cell damage induced by t-BOOH by decreasing ROS generation. All the compounds proved more active than the parent hydroxytyrosol.

Unripened grapes from the "green harvesting" of table and wine cultivars: An evaluation of the procyanidins content in this waste over three years

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Abstract
The procyanidins, known as condensed tannins, represent an ubiquitous group of plant phenolics. In grape seeds procyanidins in which constitute the major part of the total polyphenol extract and are present with various degrees of polymerization. They derived by the condensation of flavan-3 ols units, (+) catechin and (-) epicatechin usually linked through C4-C6 and C4-C8 inter flavanoid bonds and acylated by one of more galloyl groups. The procyanidins are partially responsible of the organoleptic characteristics of grapes and wines (e.g. astringency and bitterness) as a result of their tanning properties which depend on the procyanidin structures and increase with their degree of polymerization. The green harvesting is a common procedure applied to the table grapes to guarantee an optimal grown of the grapes left on the plant until a complete ripening. Although this practice may be applied also to some selected wine cultivars. In this work a study over three years, monitoring the phenolic and procyanidins content in seven green table and seven wine grapes harvested from the same cultivars was carried out. The derived findings from table grapes indicated the flavonoids as almost completely absent, while the main metabolites resulted the procyanidins. To date, no quali-quantitative data are available on these matrices. The study was conducted both on laboratory extracts and on some samples obtained applying a scale-up of the extractive procedure. All the analyses were performed applying an HPLC/DAD/ESI-MS method an selecting a core-shell RP 18 column. The results within the same years, highlighted consistent differences between the table grapes and those from the wine cultivars. Moreover a preliminary correlation with the climatic condition was achieved. From the quantitative point of view it was possible to detect those cultivars with the highest content in procyanindis and to open the door to a possible future utilization and consequently to valorize these wastes widely produced all over the world.
CO-45

**Nutraceutical potential of polyphenolic fractions from Annurca apple (M. pumila miller cv annurca)**

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**Abstract**

Polyphenols from flesh and peels of Annurca apple (M. pumila Miller cv Annurca) fruit were quantified by employing an extract sub-fractionation procedure applied for the first time to this fruit. Fractions were submitted to DPPH and FRAP tests and good radical-scavenging capacity and reducing property were detected. Antimicrobial assays revealed for both flesh and peel a broad inhibition spectrum, highlighting, particularly, an appreciable antifungal activity which is less common than the antibacterial one generally expressed by food and food polyphenolic extracts. In vitro experiments on cardiomyocyte cell culture indicated a significant capacity to confer cardioprotection both against physiological reactive oxygen species and induced oxidant injury. Our data confirmed previous knowledge on the healthy effects of Annurca apple and clarified, for the first time, the contribution given by each polyphenolic class to the fruit biological properties tested.

CO-46

**Extraction, LC-ESI-MS charachterization and HPLC-DAD-fluorescence quantification of procyanidins and phenolic derivatives in cranberry extracts**

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**Abstract**

Cranberry extracts are used as active ingredients in many nutraceutical and functional foods because of the potential health benefits linked to phytoconstituents present in the fruits as proanthocyanidins (PAC) and flavonols (1, 2). PAC containing at least one A type linkage are considered the active compounds of Cranberry and this compounds are present in the fruits at different degree of polimerization ranging from DP2 to DP 16 (3). PAC have a high structural diversity due to the presence of different monomers and their stereoisomers. Cranberry extracts are characterized by most predominant (2R, 3S) catechin and (2R, 3R) epicatechin (3, 4). Different methods have been used for the standardization of cranberry PACs (5) and difficulties in the evaluation of results obtained with spectrophotometric and HPLC methods have been reported (1); recently a colorimetric method, using 4 dimethylaminocinnamaldehyde (DMAC), was validated for quantification of cranberry PACs (6). On the other hand HPLC analysis is favorable due to the observation of a chromatographic pattern and due to the opportunity to have structural information on the PAC if using a MS detector. HPLC separation of PAC are performed in normal phase HPLC using diol or silica stationary phase. In this paper we present a HPLC-HILIC method using an amide stationary phase. DAD, fluorescence and ESI-MS detectors were used and quali-quantitative information about different cranberry extracts were obtained. Comparison between Bate Smith, BL-DMAC and HPLC were also obtained. Several extracts were analyzed showing differences in qualitative and quantitative compositions.
Activity of natural hydroxytyrosol versus a synthetic sample and its chiral analogues: Hypothesis on mechanism of action

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Abstract

Hydroxytyrosol is a potent phenolic antioxidant present in olive oil and olive mill waste water (Manna). It exhibits a wide range of biological effects such as scavenging of superoxide anion and hydroxyl radical (Visioli), antimicrobial, and immunostimulatory action (Furneri, Pinelli).

The aims of the present research are to obtain:
– natural extracts of hydroxytyrosol from olive mill waste waters;
– the synthetic standard of hydroxytyrosol;
– analogues of hydroxytyrosol both in racemic form and in their corresponding optically active forms.
– evaluation of the effects of the above compounds on human peripheral blood mononuclear cell cytokine production in order to verify the contribution of this activity to the overall pharmacological profile of hydroxytyrosol. Data on chemical and enantiomeric purity obtained by the use of capillary electrophoresis as well as on biological activities, even including regulation of the immune networks, of both synthetic and extract derived catechols will be presented.

Novel packaging films based on chitosan nanoparticles loading vitamin E for food shelf life improvement

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Abstract

Vitamin E is known to be unsoluble in aqueous media due to its lipophilic nature. Nevertheless, its antioxidant activity could be conveniently adopted for increasing food shelf life. Among packaging materials, chitosan (CS) has been well-known for its excellent film-forming property, antimicrobial activity, and unique coagulating ability with metal and other lipid and protein complexes. Its high binding ability and antimicrobial properties are both beneficial in developing new applications of this natural polymer in food preservation [1]. Moving from this background, the aim of the present work is to characterize in vitro new CS based NPs encapsulating vitamin E and potentially useful for food packaging. CS NPs were prepared according to a modified procedure of the ionic gelation technique [2], at appropriate polycation/polyanion mass ratios under magnetic stirring at room temperature. How the features of the nanocarrier system can affect in vitro the availability of the vitamin E was also investigated. In fact, the NPs were characterized in terms of light scattering, zeta-potential, vitamin E content and by X-Ray Photoelectron Spectroscopy (XPS) measurements. In vitro release studies of vitamin E from NPs were also performed in order to gain insight into the possible applications of the nanodevices for novel food packaging materials. The following step will be the evaluation of the potential of the new coating materials after incubation with food products.
Quali-quantitative evaluation of chemopreventive coumarins in citrus fruits by HPLC-UV/F/MS: Comparison of original analytical methods

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Abstract
The Citrus genus includes fruits belonging to several species and hybrids commonly used for food and industrial purposes (lemon, sweet and bitter orange, tangerine, grapefruit, lime, bergamot orange, kumquat). In addition to being rich in vitamin C, folate and fibre, some Citrus fruits contain aurapten and umbelliferone, coumarin-based molecules that can increase the potential of these fruits as functional foods. Aurapteni (7-((E)-3,7-dimethylocta-2,6-dienyloxy)-2H-chromen-2-one) seems to be promising as an adjuvant for cancer prevention: in particular against melanoma, gastro-intestinal, liver and mammary cancers. Due to its strong UV absorbance, umbelliferone (7-hydroxycromen-2-one) is employed in cosmetic and sunscreen preparations. Both aurapten and umbelliferone are also reported to have antioxidant properties. Therefore it is very useful to develop innovative analytical methods able to provide a reliable measurement of the content of these molecules in Citrus fruits. Three methods have been developed for this purpose based on HPLC with spectrophotometric (UV), spectrofluorimetric (F) and tandem-mass (MS/MS) detection. The sample pre-treatment is carried out by means of original miniaturized extraction procedures. The HPLC-UV system has the great advantage of being simple and inexpensive, thus a good choice for preliminary assays. At the same time, HPLC-F takes advantage of the native fluorescence possessed by the analytes to achieve very high sensitivity. Finally, LC-MS/MS system allows analyte identification through mass spectra and provides a very accurate and selective quantification. The comparison of the developed analytical methods gives an useful overview about the content of chemopreventive coumarins in different types of Citrus, as well as in the same fruits coming from several cultivars and at different ripening levels. Moreover, coumarin levels are being also investigated in various parts of the fruits (exocarp, mesocarp, endocarp and seeds) to gather information both from a nutraceutical point of view, and for the possibility of use as raw materials for nutritional supplements.

Catechin stability in commercial teas during their shelf-life

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Abstract
Tea, second only to water, is the most widely consumed beverage in the world and is obtained from the leaves of Camellia sinensis L. (family Theaceae). Tea leaves, in particular those of the non-fermented green tea, are very rich in catechins (representing up to 30% of dry weight) and other polyphenols, which show significant antioxidant activities in vitro. More recently, some in vivo scientific studies have been performed in scientific papers, where some convincing evidences supporting healthy properties of green tea are described. These benefits include: improving blood pressure, serum cholesterol reduction, prevention of low density lipoprotein oxidation and, as a consequence, decreased risk for cardiovascular disease and cancer. The healthy effects are mainly associated with catechins, whose activity strongly depends from the amount intaken and bioavailability. Numerous studies have been published on catechin bioavailability, but results are widely variable. Several factors could affect the amount of catechins reaching the target tissues/organs after consumption; among others: medium pH, temperature, oxygen availability, the presence of metal ions, concentration of other active ingredients and storage condition. The aim of this study was to investigate the main catechin (epicatechin, epigallocatechin gallate and epicatechin gallate) relative abundance and total polyphenol content in different tea samples commercially available in Italy (green and black, including tea-based beverages). The second objective was the assessment of catechin stability during the shelf-life. The stability of catechins is important to guarantee the potential healthy effects of tea for the whole period of commercialization. Catechins during tea shelf-life were analysed by RP-HPLC (Phase A: water: formic acid 99.5+0.5 v/v); phase B acetonitrile: formic acid 99.5: 0.5 v/v). Profile of total polyphenol content and antioxidant activity were also assayed by using Folin-Ciocalteu’s colorimetric method and DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay, respectively, using gallic acid as a standard. In green tea, the average content of total catechins decreased significantly by 2.44% to 40.72% in the first nine months. The correlation between catechin content and tea expiration period will be considered and discussed.
Effect of Colombian potato on lipid oxidation in plasma and broiler breast meat

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Abstract
Reported benefits of long-chain polyunsaturated fatty acids on human health have increased the interest in animal products with high amounts of these acids. Poultry meat has high concentrations of polyunsaturated fatty acids which can be further increased by dietary strategies such as supplementation with fish oil. However, increasing the degree of unsaturation increases the susceptibility of chicken meat lipids to oxidative deterioration during storage. Recent studies demonstrate that Colombian potatoes contain high natural intrinsic antioxidant capacity. A total of 456 1-d broiler chicks were randomly assigned to one of the 19 treatment group under a factorial designed: 4 Colombian potatoes (Tocarreñ a, Pastusa, Criolla Colomb, Clon 67), 4 levels of inclusion according to the DPPH activity of the potatoes (36, 144, 252, 360 ug ET/g), a negative control group without potato, a group with synthetic antioxidant (BHT), and a group with natural antioxidant (vitamin E). Results showed that FRAP activity on plasma of broilers fed potato diets with 360 ug ET/g of DPPH activity (56.8 mg ET/100g) was similar to BHT group (54.9 mg ET/100g) (P>0.05) and greater than vitamin E group (44.9 mg ET/100g) (P<0.01). In breast meat, BHT group showed the lowest lipid oxidation (P<0.001), however birds fed diets with potato at 252 ug ET/g of DPPH level (3.14 mg MDA/g) showed similar lipid oxidation compare to vitamin E group (3.34 mg MDA/g) (P>0.05). This study concluded that Colombian potato has a potential to be used as a natural alternative to the use of synthetic antioxidants in broiler diets.

Characterization of the aromatic profile of Italian Malvasia wines by HS-SPME/GC-MS approach

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Abstract
Wine, from a chemical point of view, is a very complex matrix containing a high number of components those can influence its sensory properties. Thus, the wine aromatic profile is composed by several volatile substances such as alcohols, esters, aldehydes, terpenes and organic acids, all present at different percentages and able to distinguish a wine from another (García-Jares et al. 1995). White wine volatile fraction is mainly represented by monoterpenes, such as linalool, geraniol, nerol, α-terpineol, β-citronellol, hotrienol and limonene. These molecules play an important role in characterization of wine flavor, because their amount strictly depends on the specific grapes used for the winemaking process (Dziadas and Jelen 2010). “Malvasia di Candia Aromatica” white wine derives from a typical Italian grape, harvested in Emilia Romagna region, in particular in the hilly areas of Parma and Piacenza territories. This grape is particularly rich in aromatic compounds, those make it unique among the 17 “Malvasia” cultivars existing in Italy. The aim of this study was to characterize the volatile profile of “Malvasia di Candia Aromatica” white wine utilizing the HS-SPME/GC-MS technique. Several samples from different wineries of Parma province were analyzed and a statistical analysis of data was applied in order to distinguish the different products from each others on the basis of their typical volatile composition, in a brand-dependent fingerprinting approach. Moreover, for a single winery, some wines were collected and analyzed maintaining samples coming from different cultivation areas separated from each others. In this way, it was possible to characterize the single contribution of the so called terroir to final wine flavour.
Composition, antioxidant and antigenotoxic activities of Dendrobium speciosum extracts

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Abstract
Dendrobium speciosum is widely distributed in Australia where it is known as Wara-gal-darra or Rock Lily. The Cadigal people used to eat the starchy stems raw or roasted on hot stones. No scientific studies have been reported for this species. The aim of the present work was to evaluate fatty acid profile, total soluble sugars, total proteins, total flavonoids and total polyphenols and to evaluate the radical scavenging properties and the genotoxicity and the antigenotoxicity potential of Dendrobium speciosum. The analyses were performed on the methanolic extracts of dried leaves and stems. Results are reported. Genotoxicity/antigenotoxicity activities of the extracts have been studied using Comet Assay. The methanolic stem extract neither affected cell viability nor induced DNA damage at tested doses and was able to diminish the DNA damage induced by 4NQO. The extract obtained from the leaves did not show any significant DNA damage reduction induced by 4NQO.

Table 1: Chemical compositiona of D. speciosum leaves and stems, antiradical and genotoxic/antigenotoxic activities of extracts.

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Stems</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>6.90±0.30</td>
<td>6.10±0.25</td>
<td>1.13</td>
</tr>
<tr>
<td>Total lipids</td>
<td>3.60±0.28</td>
<td>2.20±0.19</td>
<td>1.64</td>
</tr>
<tr>
<td>Total soluble carbohydrates</td>
<td>0.80±0.11</td>
<td>0.45±0.09</td>
<td>1.78</td>
</tr>
<tr>
<td>Total polyphenols</td>
<td>1.15±0.10</td>
<td>1.06±0.12</td>
<td>1.08</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>0.21±0.08</td>
<td>0.12±0.07</td>
<td>1.75</td>
</tr>
<tr>
<td>DPPHb</td>
<td>0.026±0.004</td>
<td>1.054±0.047</td>
<td>0.02</td>
</tr>
<tr>
<td>Antigenotoxicity (GIR%)c</td>
<td>16.84±9.04d</td>
<td>42.70±5.04g</td>
<td></td>
</tr>
</tbody>
</table>

aData are given as mean ± SD (n=3) and are expressed as g/100g dry weight. bIC50 are expressed in mg/ml; Trolox was used as reference compound (IC50 0.020±0.003 and 0.014±0.001 mg/ml for leaves and stems, respectively). cTested dose: 50 µg/ml. dTested dose: 5.0 µg/ml. eGIR%: genotoxicity inhibition rate vs. 4NQO (3 µM) induced DNA damage (tail intensity 14.26±1.67 and 17.24±0.24 for leaves and stems, respectively). fTested dose: 25 µg/ml. gTested dose: 2.5 µg/ml.
Antioxidant properties and lignans content of legume and sweet chestnut flours

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Abstract
The food industry is always interested in finding healthy food ingredients and food formulations. In this context, our research aims at evaluating the beneficial properties of four unconventional flours derived from chick-pea, green and red lentils and sweet chestnuts that could be conveniently used in processed foods. Total phenols (PFT) and antioxidant properties of flours have been determined in both aqueous-organic extracts and their residues by the Folin Ciocalteau method and by the FRAP assay, respectively. In addition, lignans (secoisolariciresinol, lariciresinol, isolariciresinol, pinoresinol, matairesinol) were quantified in the same flours by means of HPLC. Regarding antioxidant properties, FRAP values ranged from 3.73±0.30 µmol/g d.w. to 140.32±6.49 µmol/g d.w. in aqueous-organic extracts and from 45.97±5.30 µmol/g d.w. to 116.44±7.12 µmol/g d.w. in residues. The highest FRAP and PFT values were reached by green lentils in both aqueous-organic extract and residue. The total lignans content was significantly higher in green lentils than in red ones: this could be due to the highest concentration of secoisolariciresinol and pinoresinol in the green lentils flour. In chick-pea flour the major contribution to the total lignans content was given by lariciresinol, while isolariciresinol and secoisolariciresinol were the major contributors for sweet chestnut flour. Our findings show that lentils and sweet chestnut flours are a good source of antioxidants and lignans and they could potentially contribute to the formulation of functional foods.

A third generation lactose biosensor based on cellobiose dehydrogenase and aryl diazonium modified single wall carbon nanotubes electrode

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Abstract
Food control analyses require robust, sensitive, and selective detection methods. The most commonly used methods such as chromatography and mass spectrometry are very reliable but at the same time they require expensive instrumentations, time-consuming sample preparation steps, and skilled technicians. Enzyme biosensors are a good alternative because they incorporate robustness, selectivity, and sensitivity to low cost of equipment and easiness of sample preparation (Kubota et al.). Biosensors are successfully applied to the control of ethanol, lactate, glutamate, and pesticides content in wine, milk, and fruit juice (Kubota et al.). The monitoring of lactose is of great importance to the dairy industry because of the considerable market segment constituted by lactose-free products. Stoica et al. proposed a lactose biosensor already in 2006. This biosensor was based on the direct electron transfer between cellobiose dehydrogenase from \textit{Trametes villosa} (\textit{TmCDH}) and from \textit{Phanerochaete sordida} (\textit{PsCDH}), and spectrographic graphite electrodes (Stoica et al.). CDHs contain a larger catalytically active flavor domain and a flexible smaller haem domain, which acts as an electron transfer mediator between the flavo domain and an electrode. Here we show how we could improve the electron transfer efficiency between the haem domain of \textit{PsCDH} and the electrode, and the stability of the \textit{PsCDH} biosensor through the modification of glassy carbon electrodes with single wall carbon nanotubes and in situ generated diazonium salts bearing carboxylic acid or amine groups (i.e \textit{p}-amino benzoic acid and \textit{p}-phenylenediamine). Especially after \textit{p}-phenylenediamine modification DET current densities up to 500 µAcm\textsuperscript{-2} were obtained for oxidation of 5 mM lactose. Moreover, the onset of the electrocatalytic current for lactose oxidation started at a 50 mV lower potential than when only SWCNTs were applied. The stability of the electrode was increased up to 3.5 times with respect to modification with only SWCNTs and only a 15% loss of the initial activity was recorded during constant measurements for 50 h.
Antithrombotic activity of twelve table grape varieties relationship with polyphenolic profile

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Abstract

The synthesis of tissue factor (TF) by monocytes/macrophages activated by inflammatory agents is of utmost importance in the pathogenesis of thrombotic diseases. The appearance of TF on the surface of these cells leads to the activation of coagulation and, eventually, to fibrin accumulation and thrombosis. We have previously shown that two Italian table grapes were able to down-regulate the synthesis of TF, but the relationship with their phenolic content was rather elusive. In this study we investigated the effect of 12 table grape varieties on TF synthesis and how the phenolic profile contributed to their antithrombotic activity. Five white (Italia, Baresa, Regal, Autumn Seedless, Beogradska), four red (Red Globe, Apulia Rose, Crimson Seedless and Supernova) and three black table grapes (Palieri, Autumn Royal and Summer Royal) were studied. Grape extracts (GSE) were prepared by overnight incubation of grape skins in 30% ethanol-1% hydrochloric acid. Phenolic content was measured by Folin-Ciocalteu’s method and flavonoids were identified by HPLC-DAD-MS system. Antithrombotic activity was assessed as the ability of GSEs to inhibit TF expression in human whole blood or in purified monocytes challenged with endotoxin (as TF inducer). All grape varieties inhibited TF synthesis in whole blood in a concentration-dependent manner, but with a different efficiency. On the whole, red grapes were the most active (up to 95% inhibition at 24ug/ml Supernova GSE), followed by white and black grapes (P<0.0001). Experiments on purified monocytes, performed with selected grapes (Supernova, Autumn Royal, Autumn Seedless), confirmed the ability of GSEs to reduce TF synthesis, thus suggesting a direct effect on the target cell. As anticipated, there were striking differences in phenolic composition among grape varieties. In multivariate stepwise regression analysis the compounds showing an independent association with TF synthesis were cyanidin and myricetin, which together explained 45% of the variability in TF response. However, when these substances were tested in purified form their efficacy in inhibiting TF expression was markedly lower as compared to an equivalent amount of whole extracts (P<0.01), suggesting that different compounds may synergize with each other. These findings may help generating new grape varieties with improved antithrombotic potential.

Intercalation of long chain fatty acids with different unsaturation degree into layered double hydroxides

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Abstract

Epidemiologic studies provide important insight into the relationship between dietary fatty acid (FA) intake and the risk of disease development, in particular the scientific community agrees on the importance of polyunsaturated FA in human nutrition and disease prevention. For these reason production of FA nutritional supplements is an important topic for pharmaceutical and food industries (Shahidi and Wanasundara, 1998). Hybridization of biomolecules in inorganic systems lead to interesting properties distinguished from those of each component in simple physical mixtures (Carretero and Lagaly, 2007). In this regard, layered double hydroxides (LDH), a class of anionic clays with high anionic exchange capacity and biocompatibility, have been used as host for many molecular anions of biological interest as aminoacids, anti-inflammatory, antibiotic and UV-filters (Costantino et al., 2009). The intercalation of saturated FA with different chain length has been widely studied (Saber and Tagaka, 2003), while only few researches investigated the intercalation of monounsaturated FA (Kameshima et al., 2009). The aim of this research was to design and to prepare LDH with unsaturated FA to be applied in pharmaceutical and health food industries. FA with different chain length and unsaturation degree have been used as guests of MgAl LDH in order to optimize the intercalation conditions and to study the LDH selectivity.
Functional foods as carriers for Synbio®, a probiotic bacteria combination

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Abstract

The popularity of functional foods continues to increase as consumers desire flavourful foods that will fulfil their health needs. Among these foods, probiotics may exert positive effects on the composition of gut microbiota and overall health. However, in order to be beneficial, the bacterial cultures have to remain live and active at the time of consumption, so food products formulation can be manipulated to aid probiotic efficacy (Ranadheera et al., 2010). Since dairy food products are considered as an ideal vehicle for delivering probiotic bacteria to the human GI, the development of new non-dairy probiotic food products turns out to be increasingly challenging, as it has to fulfill the consumer’s expectancy for products that are simultaneously relish and healthy. The aim of this study was to develop new probiotic food products, such as seasoned cheeses, salami, chocolate and ice-cream with a final probiotic concentration of approximately 10^9 CFU/daily dose of Lactobacillus rhamnosus IMC 501® and Lactobacillus paracasei IMC 502® (SYNBIO®) (Verdenelli et al., 2009 and Verdenelli et al., 2011). The survival and viability of probiotics were determined during and after the foods shelf-life. The values of viable probiotic bacteria of all dairy and non-dairy foods were between 10^7 and 10^9 CFU/g of food certainly at the end of the shelf-life and for some of them the values were maintained also after the expiry date. All the dairy (“Caciotta” cheese, “Pecorino” cheese, “Büscion” Swiss cheese and “Fiordilatte” ice-cream) and non-dairy (“Ciauscolo” salami, Larded salami, Swiss small salami, milk chocolate, dark chocolate, organic jam and chocolate mousse) foods studied would be excellent vehicles to deliver the probiotic health effects because of the good viability of probiotics in this products. The sensory profiles reported by the assessors’ panel showed no significant differences between the probiotic-enriched foods and the control foods.

Study and characterization of innovative active food packaging materials

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Abstract

Many food products are perishable by nature, and require protection from spoilage during their preparation, storage and distribution to deliver a satisfactory shelf life and to ensure that the finally-consumed product is of high quality and safe. The variables that influence shelf life properties of packaged food are: product quality, gas mixture, package and headspace, packaging equipment, storage temperature, and eventually added additives. Active packaging is a mode of packaging in which the package, the product, and the environment interact to prolong shelf-life or enhance safety or sensory properties, while maintaining food quality. It is usually designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food. In this work preliminary studies on the antimicrobial activity of films are reported. These coatings, deposited on PET, low density PE, PP and PVC substrates, were obtained by sol-gel technique. All the sols prepared were water-based and were obtained using polyvinyl alcohol (PVA), polyethylene glycol (PEG), tetraethoxysilane (TEOS) and glycidoxypropyl-trimethoxysilane (GLYMO) as reagents. Different parameters, as concentration, viscosity, pH, were optimized to obtain stable and homogeneous sols. Cold plasma technology was used to improve the adhesion of the coatings to PET: in this way the compatibility of the hydrophobic substrate and water-based sols is increased. Antimicrobial agent, such as lysozyme was added to the film during the synthesis. The films obtained were characterized by FTIR and AFM and the amount of antimicrobial agent released in time has been determined by HPLC analysis using the buffer phosphate as soaking medium. Furthermore the obtained active packaging materials were put in a culture plate containing a suspension of M. lysodeikticus to evaluate their bacterial inhibition activity. Results will be presented and discussed.
Soyasaponins I and $\beta$g in raw and cooked legumes: Determination by SPE-HPLC-MS and its bioaccessibility by an in vitro digestion model

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Abstract
Legumes represent an important component of human diet in several areas of the world, especially in the developing countries where they complement the lack of proteins from cereals (Kalogeropoulos et al., 2010). Legumes include lentil (Lens culinaris L.), bean (Phaseolus vulgaris L.), pea (Pisum sativum L.), chickpea (Cicer arietinum L.), lupin (Lupinus albus) and others. Legumes are low in fat and rich in proteins, exhibiting lower glycaemic index compared to other starchy foods. In addition, legumes contain a rich variety of phytochemicals, including phytosterols, natural antioxidants and bioactive molecules such as soyasaponins (SSs). SSs are triterpenoidal glycosides (group A, monodesmosidic and group B, bidesmosidic) that possess multiple health-promoting properties such as lowering of cholesterol. Although the mechanism is not completely clarified, a proposed theory is based on the formation of non-absorbable complexes between SSs and cholesterol in the gut lumen. Notably, the hypocholesterolemic action was referred to SSs belonging to group B, such as SS I and SS $\beta$g, that are particularly abundant in legumes (Sagratini et al., 2009). The aim of this work was the quantification of SS I and SS $\beta$g in raw and cooked legumes of different provenience and variety (60 samples) by a SPE-HPLC-MS method developed in our lab. The total level of SS I and $\beta$g in raw seeds was in the range 1200-2500 mg kg$^{-1}$. The cooking process produces a small loss of SSs in water, i.e. 5.6-7.6%, depending on time cooking and legume type. The bioaccessibility of SS I and $\beta$g contained in lentils was determined by using an in vitro digestion model.

Carotenoids composition in some tropical fruits

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Abstract
In tropical regions there is a great diversity of fruits that are consumed by wild animals, indigenous people or farmers. Many tropical fruits can be considered a reservoir of bioactive substances with a special interest due to their possible nutraceutical properties. The interest in carotenoids from a nutritional standpoint has recently greatly increased, because of their important health properties. Carotenoids esters with fatty acids are reported to enhance the stability of the xanthophylls. Here we report the native carotenoids composition in six tropical fruits (Corozo, Sastra, Sapote, Maracuya chino, Maméy rojo and Frutita) from Panama, which is considered a region of great biodiversity. The native carotenoid composition was directly investigated by an HPLC-DAD-APCI-MS methodology, for the first time. In Corozo (Aiphanes aculeate) 32 different carotenoids were detected, including a high content of $\beta$-carotene (10.9%), lycopene (12.7%) and esters of zeaxanthin and $\beta$-cryptoxanthin; lutein, $\zeta$-carotene and $\alpha$-carotene were also detected. Sapote (Garcinia intermedia) showed the highest content of zeaxanthin (26.5%) among the fruit investigated; $\beta$-carotene, lutein, $\alpha$-carotene and esters of lutein, zeaxanthin and $\beta$-cryptoxanthin were also present in good amounts. In Sapote (Quararibea cordata) 22 different carotenoid were detected, including $\beta$-carotene (23.3%), $\alpha$-cryptoxanthin (2.6%), $\beta$-cryptoxanthin-5,6-epoxide (4.1%); moreover it showed the presence of 10 different di-esters of zeaxanthin, including esters with unsaturated fatty acids. The present LC-MS method allowed for the most detailed analysis of zeaxanthin esters than reported previously. Frutita (Allophylus psilospermus) showed a very high content of the apo-carotenoid $\beta$-citraurin (29.8%) and of a number of its esters with fatty acids from C6:0 to C16:0. In Maracuyá chino (Cionocyscos macranthus) 14 carotenoids were detected, including neoaxanthin, $\beta$-cryptoxanthin-5,6-epoxide, $\beta$-cryptoxanthin-5,8-epoxide, and a high amounts of $\beta$-cryptoxanthin esters, in particular the mono-ester of C12:0 (16.2%); moreover, it also contained a high amount of the mono-ester with C12:0 of cryptocapsin, a ketocarotenoid bearing a $\beta$ and $\kappa$ ring. Maméy rojo (Pouteria sapota) was characterized by ketocarotenoids with $\kappa$ ring, both hydroxylated and not hydroxylated; it also showed a number of ester of $\kappa$ ring ketocarotenoids which were also reported here for the first time.
Oxidative stability of unconjugated and conjugated linoleic acid

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Abstract
Despite the wide interest in conjugated linoleic acid (CLA) over the recent years, little is known about CLA oxidation products. The initial stage of CLA autoxidation has been studied and the primary oxidation products have been identified (Hämäläinen et al., 2001). The oxidative stability of CLA is a controversial and unresolved issue due to contradicting reports regarding CLA oxidative stability and antioxidative capacity. Most researchers agree that CLA oxidizes faster than linoleic acid (LA), and there is evidence that CLA has a greater oxidation rate than linolenic and arachidonic acids (Yettella et al., 2011). Moreover only few studies have reported the isomer composition modifications of CLA isomers; generally cis,cis isomers were most susceptible to oxidative degradation in respect to trans,trans isomers (Yang et al., 2000). Since CLA has potential health benefits (Park, 2009) and is naturally found in small amounts in foods, there is much interest in CLA enriched products and in the investigation on CLA oxidative stability after heat treatments. The present research was undertaken to examine the oxidative stability of LA, unconjugated and conjugated, in different forms: as free fatty acids, as methyl esters and as homogeneous triacylglycerols. These products were subjected to heat treatments at 180 °C for different times. The primary oxidation products were analyzed by the determination of the hydroperoxide value, while the secondary oxidation products were determined by solid phase micro-extraction, coupled with high resolution gas chromatography and mass spectrometric detection. Moreover it was interesting to investigate the isomer composition modifications of the different products by silver-ion high performance liquid chromatography analysis.

Diagnostic methods to detect allergen residues in food

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Abstract
Food Safety is needed to ensure that foods do not injure consumers, in particular that there are no consequences to their health. Public Health problems with regard to food allergies and the existing European Regulations will be addressed. The technology platforms and diagnostic methods (ELISA, lateral flow, PCR) for monitoring allergens, with specific examples of commercial kits will also be presented. The advantages and disadvantages of the systems currently in use and innovative systems (microarrays) will be discussed.
The genus *Lupinus* is an efficient source of functional ingredients with low environmental impact

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Abstract

Today the consumer is more and more aware of the environmental impact of food production and consumption. In particular, it is well known that the use of arable land, fresh water and energy for the production of animal proteins is much higher than for the production of vegetable proteins. Since grain legumes are nitrogen fixing plants, they provide the protein-richest seeds. In Asia numerous populations have used soybean as the main protein source for centuries. In Europe, instead, similar characteristics are encountered in lupin, which may contain up to 40% protein, with a satisfactory nutritional value. There are now on the market different lupin ingredients suitable for numerous food applications. From some years our group is investigating different issues related to the utilization of *Lupinus albus* and *Lupinus angustifolius* in human nutrition. Specific aspects considered are related to the presence of particular nutrients, such as polyunsaturated fatty acid and tocoferols (Boschin et al, 2011), or anti-nutrients, such as the quinolizidine alkaloids (Boschin et, 2008); to the allergenicity (Sirtori et al, 2011), which depends mostly on the cross reactivity with peanut proteins; to the traceability; and to the nutraceutical properties (Sirtori et al, 2012). In this area we have demonstrated that lupin foods may be useful for decreasing total and LDL-cholesterol and for controlling blood pressure. These are relevant results, considering the growing demand for innovative ingredients for the formulation of functional foods and dietary supplements.
POSTERS

P-1

Nutritional characteristics and functional properties of new Italian rice varieties
(Oryza sativa)
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Abstract
Recently released Italian rice varieties interesting for some characteristics have been considered in this study. Samples selected belong to different classes (round, long A long B) and each of them are peculiar for a specific characteristic (high amylose content, pericarp pigmented, scent, suitable for the parboiling process, resistant to fungal disease etc). Preliminary, all samples were characterized from the agronomic and biometric point of view (length, width, 1,000 kernel weight, color index). After cooking, samples were analyzed for hardness, resistance to extrusion, stickiness, gelatinization time and organic matter leached into cooking water. Main chemical characteristics were evaluated through the determination of the moisture, ash, protein, lipid and starch content. In addition, raw samples were analyzed for the damaged starch while the resistant starch was determined both on raw and cooked samples. Pasting properties were determined by means of a Rapid Visco Analyser (RVA) under standardized operating conditions. The main parameters such as the maximum peak viscosity, the holding strength and the final viscosity were determined by the viscogram while the breakdown and the setback were calculated by these three basic parameters. Starch granule organization in raw and cooked samples was evaluated by means of Scanning Electron Microscopy (SEM). Samples examined showed starch granules tightly packed which form cells of different size and shape depending on the rice sample investigated. The pasting properties during the heating and cooking also resulted different as a consequence of chains rigidity and the strength of the internal structure of starch. In addition, also other components in the matrix such the amylose, lipid or protein content were able to affect the starch behavior.

P-2

Effects of glycosilated polyphenols from Olea europea of heart tissue: Morphological and immunohistochemical study
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Abstract
Polyphenols are a large and diverse family of organic molecules characterized by a marked structural and functional variability, but also all powerful antioxidants. Among the polyphenols, especially flavonoids are able to protect cells from oxygen free radicals, which cause progressive structural and functional alterations of cellular aging and chronic diseases. Several studies have shown that regular intake of phenolic compounds in the diet plays a preventive action against several diseases, due to the multiple properties: antioxidant, antitumor, anti-atherogenic, anti-inflammatory, anti-bacterial and lipid-lowering. The research aims to evaluate the beneficial effects of polyphenols in a hypercholesterolemic diet using C. auratus as an experimental model. The samples were divided into three groups: the first was fed with feed enriched with cholesterol to 10% for 30 days, the second group was fed with hypercholesterolemic diet and treated with polyphenols during the last 15 days (curative treatment) and the third group was used as control (normal fed). Tissue preparations coming from the heart from both control and experimental fish are stained with routinary histological techniques, and serial sections are immunolabelled with α-SMA antibody. The results show, in the hypercholesterolemic group, a general disorganization of the pericardium and cardiac chambers including the sinus venosus. In the latter chamber the lipid substances cause degenerative changes of both the myocardial and connective tissue components. In the curative group, by contrast, morphology tends to normal with the restoration of the pericardium and better cellular organization. It is noted more α-SMA positivity in the hypercholesterolemic group, reflecting the alteration of the endothelium of blood vessels due to the accumulation of cholesterol. These results suggest the use of polyphenols as carriers of cell-mediated immune reactions involved in inflammation and atherosclerosis.
Biological effects of Astaxanthin on hepatopancreas with alcoholic damage: Morphological evaluation

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Abstract
Alcohol abuse is a major cause of diseases of the digestive and excretory systems. This study proposes to make an assessment of the effect of astaxanthin on morphological changes in alcohol-induced experimental model of Carassius auratus. Astaxanthin is a carotenoid pigment that occurs naturally mainly in microorganisms. The yeast Phaffia rhodozyma has been used as a source of astaxanthin. The animals, normal-fed, were divided, after acclimation, in 2-liter aquariums each in 3 groups: one group received a daily administration of EtOH at a dose 1.5% (vol/vol %), while the second group received a daily dose of EtOH 1.5% (vol/vol %) and was fed, in addition, with Phaffia rhodozyma, suitably freeze-dried, and the third group was used as control. After 15 days, the samples were processed by usual techniques for light microscopy and then evaluated by histomorphological and histochemical staining. The alcohol group shows vacuolated hepatocytes and pancreocytes with a heterogeneous parenchyma. As evidence of alcohol-induced oxidative stress are melanomacrofagic aggregates. In the curative group it can be seen abundant aggregates of Rodlet-cells and macrophages, and eosinophils circulating in response to the restoration of cellular damage. The results show the beneficial effect of the carotenoid pigment astaxanthin in the treatment of alcohol-induced oxidative damage.

Vitis vinifera cv. Uvalino, a neglected grape vine as a source of nutraceutical lipids

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Abstract
Vitis vinifera cv. uvalino is an old native grape vine from Piedmont which has been recently rediscovered for the production of wine. Chemical studies have shown that the cultivar has the highest content of resveratrol (Borsa D. et al 2003) which is consistent with the high antioxidant activity displayed in in vitro experiments (Bertelli A. et al 2004). As a continuation of the chemical study on Vitis vinifera cv. uvalino we aimed to characterize the composition of the oil from its seeds. Grape seeds are considered to be a left-over product from the wine making process whose utilization is however of economic relevance for waste reduction. Grape seed oil, “olio di vinaccioli”, is a high-quality edible oil with beneficial health properties mainly due to its content of unsaturated fatty acids. In our study, chemical profiling of the seed oil from V. vinifera cv uvalino has been obtained by a combination of spectrometric and spectroscopic techniques such as GC, GC-MS, NMR and ESI-MS/MS. Uvalino’s grape seed oil was found to consist of triacylglycerols (TAG) as in the case of seed oils from other grape cultivars (Bail S. et al 2008). Analysis of TAG fatty acid composition indicated that the two unsaturated linoleic acid (72.87 %) and oleic acid (13.20%) are dominant, whereas palmitic acid (9.59%) is the most abundant saturated fatty acid. Moreover, ESI-MS/MS allowed to disclose the most abundant TAG species which include components at m/z 901 (OLO), 898 (LLL) and 877 (LSP). In addition to the above, an analytical method based on HPLC-ELSD detection has been developed for identification of TAG species in grape seed oils and it will be presented here.
**Astaxanthin treatment of alcohol induced hypoxia on kidney of *Carassius auratus***

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**Abstract**

Hypoxia is a pathological condition caused by a deficiency of oxygen throughout the body (generalized hypoxia) or in a region (tissue hypoxia). Alcohol abuse is a major cause of the digestive and excretory systems diseases. This study proposes to make an assessment of the effect of astaxanthin on hypoxia alcohol experimentally-induced on *Carassius auratus* kidney. Astaxanthin is a carotenoid pigment that occurs mainly in microorganisms, it is also poorly present in some vegetables and fruits. The yeast *Phaffia rhodozyma* has been used as source of astaxanthin. The animals, normal-fed, were divided, after acclimation in aquariums 2-liter each, in three groups: one group received, mixed in water, a daily administration of EtOH at a dose 1.5% (vol/vol %), while the second group received a daily dose of EtOH 1.5% (vol/vol %) and was fed, in addition, with *Phaffia rhodozyma* (suitably freeze-dried) and the third group was used as control. The experiment lasted for 15 days, then the samples were processed using the usual techniques for light microscopy and so evaluated by histomorphological and immunohistochemical staining. In the alcoholic group the results show that the renal parenchyma presents hypoxic vacuolated cells and disorganized cell nuclei with fibrotic elements, that assume the typical hypoxic, dark and spherical shape. In contrast the curative group shows the presence of intrarenal cells appointed to restore the cellular elements of the parenchyma. The morphology tending to be normal supported by an increased presence of laminin and collagen. It is evident the positive effect of tissue regeneration of astaxanthin in the treatment of alcohol-induced hypoxic conditions.

**Luminescence methods to identify irradiated ingredients used in plant food supplements**

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**Abstract**

There is an increasing interest by both consumers and industry for the development of plant origin food products with health benefits. The market for these health products, including plant food supplements, in fact, is growing rapidly. In EU the treatment with ionizing radiation is allowed for dried aromatic herbs, spices and seasonings, but not for plant food supplements and their ingredients. Nevertheless, controls carried out in EU at the product marketing stage and in the survey carried out in Italy on imported herbs and spices, showed a large number of irradiated plant food supplements and their ingredients. Among the methods to identify irradiated foods, luminescence methods (photostimulated luminescence and thermoluminescence) appear to be particularly reliable to detect irradiation in raw materials as well as plant extracts. However, preliminary studies carried out on this kind of products highlighted limits in the application of the standardized detection methods, due to the low content of irradiation markers (silicates minerals contaminating foods). This work reports the preliminary results of a study performed on a group of herbal ingredients selected among those more used for plant food supplement preparation. The aim of this study was to test the applicability of the luminescence-based methods for the identification of irradiation in this kind of products. This research was partially funded by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 245199. It has been carried out within the PlantLIBRA project (website: www.plantlibra.eu). This report does not necessarily reflect the Commission views or its future policy on this area.
Exploring the application of a universal method for pesticide screening in foods using a high data acquisition speed MS/MS

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Abstract
Effective management, use, and disposal of agrochemicals, particularly pesticides, is an increasing important health and environment issue in developing countries where economies may be heavily reliant on agriculture. The conventional approach is to develop highly optimized triple quad MRM methods to achieve the required levels of sensitivity and selectivity whilst still providing confidence in pesticide identification. The technology developed for fast scanning MRM analysis creates the advantage of developing multi-screening approaches for environmental target analysis and allows the possibility of a single generic ‘universal’ method. In this present study, high speed MRM analysis and a generic parameter set were used for screening 176 pesticides (352 SRM transitions) with 5msec dwell and 1msec pause times in different food matrices. Compounds were analysed with a triple quadruple mass spectrometer (LCMS-8030, Shimadzu Corporation, Japan) with modified ion optics. Separation was achieved with UHPLC (Nexera, Shimadzu Corporation, Japan). 352 MRM transitions were monitored over the entire chromatographic run, 0 minutes to 20 minutes; therefore retention time data was not required for each compound. The dwell and pause time for each SRM transition was 5 msec and 1 msec, respectively, including one polarity switch during the loop. The total loop time was 2.058 seconds which allowed approximately 10 data points to be collected for each peak, with a peak width for studied compounds of about 20 seconds.

Regolamenti di profilassi alimentare nella UE, in seguito all’incidente alla centrale nucleare di Fukushima dell’11.03.2011

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Abstract
In questo lavoro vengono prese in considerazione le norme emanate in ambito UE volte a tutelare i consumatori da possibili rischi alimentari connessi con l’incidente alla centrale nucleare di Fukushima, dell’11 marzo 2011, per seguire come si è evoluta la percezione del rischio alimentare in relazione a tale evento ed alla luce delle più precise informazioni acquisite nell’ultimo anno. Infatti, a seguito dell’incidente alla centrale di Fukushima, causato dal maremoto dell’11 marzo 2011 l’Unione Europea aveva provveduto ad emanare un Regolamento che imponeva condizioni speciali per l’importazione di alimenti per animali e prodotti alimentari, originari del Giappone, o da esso provenienti. Questo regolamento, nella versione originale il 297/11 del 25 marzo 2011, è stato aggiornato periodicamente, tenendo conto dell’evolversi della situazione, alla luce degli aggiornamenti delle notizie provenienti dalla zona interessata, e dei risultati delle analisi condotte nel frattempo. L’ultimo di questi aggiornamenti successivi risale al 21 dicembre 2011 ed ha validità fino al 31 marzo 2012. A differenza di quanto avveniva già per il controllo dell’importazione di alimenti sensibili alla radiocontaminazione da paesi extra UE, il regolamento riguardante i prodotti alimentari giapponesi interessati non prevede solo il dosaggio dei radioisotopi del Cesio, ma impone anche dei limiti restrittivi per gli isotopi dello stronzio, in particolare Sr-90, ecalabretti per la somma degli isotopi del plutonio e di elementi transplutonici, in particolare Pu -239 e Am-241.
Determinaton of phospholipids in various milk samples by means of HILIC-ELSD/MS

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Abstract
Phospholipids (PLs) comprise an important kind of amphiphilic molecules, with lipophilic acyl chains and a hydrophilic head. These compounds possess important physiological functions, as well as positive nutritional properties. Recent studies have in fact given considerable evidence that PLs can exert beneficial effects on human health, such as anti-inflammatory activity and reduction of the risk of cardiovascular disease. PLs are also used as emulsifiers or emulsion stabilisers in the food industry, in the form of complex with proteins. Five major classes of phospholipids are usually found in milk fat, and they are: phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylinositol (PI) and phosphatidylserine (PS). Phospholipids are located on the milk fat globule membrane (MFGM), where they contribute significantly to the emulsification role of the membrane by virtue of both lipophilic and hydrophilic properties [1]. The aim of this study was to determine the PLs content of Folch and SPE extracted milk samples from different animals such as cow and donkey. Hydrophilic interaction liquid chromatography (HILIC) was used to achieve baseline separation of major PL classes in various milk samples. Evaporative light scattering detection (ELSD) was further employed to attain the quantitative evaluation of major PL classes identified, achieved by external calibration. By interpolation of the calibration curves, major classes of PLs identified the Folch and SPE-extracted samples were quantified. ESI-MS detection was used to identify PLs classes and to determine the presence of single PL within each class. Extraction and chromatographic methods were validated.

Dietary and medicinal significance of wild vegetables from Calabria region (Italy)

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Abstract
Plant drugs have a long history in both traditional and modern societies both as herbal remedies or crude drugs and as purified compounds approved by Food and Drug Administration. The active principles are also used as starting materials for further medicinal chemistry modifications (Koehn and Carter, 2005; Jones et al., 2006). The present study deals with a screening of sixteen Mediterranean plants. They are spontaneous edible plants present in the area of Alto Ionio Cosentino, a territory characterized by different vegetation area from the sea level to the highest peak of Pollino Mt. (about 2000 m a.s.l.), mostly rich in Mediterranean elements. Plants selected for this study were chosen because of their use in local traditional cuisine. Information on the traditional culinary purposes of these plants was collected through structured interviews. The study shows that twelve species are eaten boiled which are namely: B. officinalis, C. sicula, C. pyrethrum, C. intybus, C. cardunculus subsp. cardunculus, E. vulgare, M. sylvestris, P. rheas ssp. rheas, R. raphanistrum subsp. raphanistrum and S. oleracea; seven are eaten fried: B. officinalis, C. sicula, C. pyrethrum, C. vitalba, C. cardunculus subsp. cardunculus, F. vulgare subsp. piperitum, R. raphanistrum subsp. raphanistrum and S. oleracea; three are eaten raw or in salads: C. intybus, L. sativum and S. oleracea; two are eaten roasted and are used to prepare stews: C. cardunculus subsp. cardunculus and R. raphanistrum subsp. raphanistrum; two are pickled or conserved in oil: C. sicula and F. vulgare subsp. piperitum; four are eaten mixed in soups: B. officinalis, F. vulgare subsp. piperitum, R. raphanistrum subsp. raphanistrum and S. oleracea; two are used as spices and in the preparation of liqueurs: F. vulgare subsp. piperitum and M. aquatica. The hydroalcoholic extracts obtained from the sixteen Italian plants above listed were studied to assess the lipophilic composition and their potential to inhibition the pancreatic lipase. Pancreatic lipase (triacylglycerol acylhydrolase) is a key enzyme for the absorption of dietary triglycerides. Interference with fat hydrolysis results in the reduced utilization of ingested lipids, therefore inhibition of lipases decreases fat absorption. Agents that inhibit fat digestion are of theoretical benefit in the treatment of obesity (Roh and Jung, 2012).
Separation of γ-oryzanol components and its synthetic p-coumarate and caffeate derivatives by NP-HPLC

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Abstract
Phytosterols occur in plants either as free or conjugated forms, the latter comprising steryl esters of fatty or phenolic acids and steryl glycosides. A mixture of esters, between hydroxycinnamic acids (CAD) and sterols based on the cholestane or the parent cycloartane skeletons, is collectively termed as "γ-oryzanol". Main CAD is ferulic acid; coumarate and caffeate esters of γ-oryzanol steroidal moieties have been reported in the literature and we prepared them by syntheses. γ-oryzanol appears to lower the plasmatic level of low-density cholesterol and has anti-oxidant and free-radical scavenging properties. Several analytical methods have been proposed, most efforts have been devoted to RP-HPLC after pre-treatment of the samples. The analysis of γ-oryzanol revealed some experimental drawbacks in the RP-HPLC methods, therefore we decided to explore the potential application of NP-HPLC in separating γ-oryzanol components. Our analysis was performed on cyanopropyl bonded column using a hexane/MTBE gradient system. A sample of standard γ-oryzanol was irradiated at UV light then subjected to NP-HPLC at semi-preparative scale; sample components were separated in two pairs of peaks. Each peak was collected and investigated by NMR spectroscopy and RP-LC-ESI-MS technique. The elucidation of 1H-NMR spectra of those fractions proved that our chromatographic method allows the separation of cis- from trans-ferulates (pairs eluting at shorter or longer tR respectively). The sensitive MS technique demonstrated that the peaks being eluted later in each pair contain steroid moieties which possess a double bond at the side chain whereas the peaks being eluted sooner in each pair contain compounds which are saturated at the side chain and, possibly, also at the polycyclic ring system. Natural γ-oryzanol contains saturated and unsaturated steroids which basically possess cholestane and cycloartane skeletons respectively. In conclusion, our method is suitable for quality assurance and determination of origin of γ-oryzanol and for its quantification as well.

Development of a HS-SPME-GC-MS method for characterizing volatile components of the DOCG wine “Vernaccia di Serrapetrona”

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Abstract
“Vernaccia di Serrapetrona” is a DOCG (Denominazione di Origine Controllata e Garantita) Italian red wine produced in Marche Region (Italy), in a small area of the hinterland. Its peculiar production process involves three fermentations leading to a “spumante” red wine having a high content of sugars (Potentini, R., 2007), obtained by using at least 85 % of grapes “Vernaccia Nera”. Its volatile composition has never been studied and together with other chemical and sensory characteristics (Boselli, E. et al., 2004), it could afford a fingerprint of this wine, depending both on the grape cultivar and on the production process. Thus, we are involved in the development of a method to characterize the volatile composition of “Vernaccia di Serrapetrona” by HS-SPME-GC-MS. Fiber selection, extraction time, extraction temperature and salt effect are being evaluated in order to find out the best conditions to achieve the highest sensitivity and precision. The main compounds extracted are esters, alcohols, phenols, hydrocarbons, organic acids. Among the SPME coatings evaluated, DVB/CAR/PDMS 50/30 μm afforded the highest sensitivity and precision followed by the PDMS 100 μm. Extraction times, temperature and type of salt are under investigation and results will be presented and discussed.
Antioxidant capacity of Colombian native potato (*Solanum phureja*)

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**Abstract**

Recent studies demonstrate that the native potato *Solanum Phureja* contains high intrinsic natural nutritional antioxidant capacity. The objective of this study was to evaluate the antioxidant capacity *in vitro* and *in vivo* of 22 promising cultivars of the native potato *S. Phureja*. The results showed that chlorogenic acid range from 126,20 to 627,49 µg/100g DW, ascorbic acid from 44,90 to 114,93 mg/100g DW and total phenols from 238,34 to 426, 96 mg EAG/100g DW. In vitro antioxidant capacity for FRAP varied between 75,14 to 198,14 mg ET/100g DW, ABTS from 70,54 to 101,55 ug ET /g DW, DPPH from 21,137 to 178,50 ug ET/g DW y ORAC from 21,68 to 47,49 µmol ET/g DW. The captation of peroxyl radicals varied between 11 and 28%, hydroxyl radicals between 14 and 74% (P <0.01), and superoxide radicals from 34 to 45%. In vivo test with rats, showed a protective effect against oxidative stress and acute liver damage, as revealed by the restoration of the enzymatic activities of SOD (65%), decreased the number of groups cabonyl, as well as decreased lipid oxidation (62%). Also, there was a reduction in serum levels of liver AST enzyme (77%), and improved the histopathological changes. This study allowed confirm the antioxidant capacity of colombian native potato, according to the concentration of compounds present and their hepatoprotective effect and inhibitor of lipid peroxidation.

Three years monitoring survey of pesticide residues in *Sardinia* wines following integrate pest management strategies

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**Abstract**

The list of pesticides used in integrated pest management (IPM) in grape growing and their annual application rates have been limited worldwide, despite this we are still confronted with the problem of pesticide residues in grapes and their by-products. This paper reports the results of a pesticide monitoring survey of wine grapes (*Vitis vinifera* L.) from the 2008-2010 vintage from vineyards included in IPM programs. Grape samples were analysed for the presence of 29 pesticides. A multi-residue gas chromatography-mass spectrometry (GC-ITMS) method in EI and CI mode has been used for the determination and quantification of the active compounds. The analytical method showed good recoveries between 75 and 116%, and allowed a good separation of the selected compounds. Repeatability and intermediate precision showed good results with CV < 20%. The instrumental method limits of determination (LOD) and of quantification (LOQ) were far below the MRLs set by EU for these fungicides in grapes and wine (when available). The analysis of pesticide residues showed that all analyzed wines had pesticide residues under the instrumental LOQ, and most of them, even if applied on grapes, were undetectable (< LOD). Only the 38% of the pesticide applied has been detected in at least one cultivar. Metalaxyl, myclobutanil and penconazole were the pesticides most frequently found in different cultivars, while carignano and vermentino were the cultivars with the higher number of residues.
Differences in the volatile profile composition of Sardinia red wines from Carignano cv subjected to different aging technologies

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Abstract
The overall aroma of wine results from a complex association of many different volatile compounds originated within the grapes and the fermentation process. In addition, flavour characteristics are influenced by storage and aging conditions. Aging represent a fundamental step to obtain high quality wines. During aging, wines mature and a large number of aromas emerge, many of which belongs directly from odourless precursor present in grapes, while other belongs from the aging technology used. The present paper reports the composition of the aroma profile of wines obtained from the cv Carignano before aging and subjected to four different aging technologies, stainless-steel tank, plastic vat, concrete vat, and oak barrel. Analysis have been performed by GC/FID and GC/MS and allowed the identification of 85 major compounds belonging to different chemical classes. The study of the differences in composition was assessed at two scales: chemical classes and individual compounds. The analysis of the classes showed an increase of the compounds of the class of alcohols after storage in oak barrel, concrete vat, and stainless-steel tank, while it did not showed changes after storage in plastic vat. Esters increase after storage in all containers, even if the total variations were slight. The acids do not show any significant changes in plastic vat, and concrete vat, while were almost double in oak barrel and of one third in stainless-steel tank. The mixed chemical class of "others" brought together many different compounds, on the whole showed similar values in plastic vat, higher values in concretevat and the lowest in oak barrel and stainless-steel tank. The composition within the individual classes showed the presence of many different compounds, both in types and in quantity, allowing the differentiation of the products obtained from the different containers after aging.

A carbon nanotube paste osmium polymer-mediated biosensor for fructose determination in food analysis

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Abstract
D-fructose is an important sugar used as a low-cost sweetener by the food and beverage manufacturers. Quality control is of particular importance in food and beverage industries. Since food is complex mixtures of chemically diverse compounds, highly specific and reliable methods are needed for their analysis. Most conventional methods for fructose determination, based on chromatography, titration and spectrophotometry are time-consuming, expensive and require highly skilled labour. Biosensors offer attractive alternatives to existing methods. They can be used in field and require no sample pre-treatment. For this reason they represent a fast, simple, low cost, accurate and reproducible alternative method which can assure a food high quality control. Furthermore, the coupling of nanotechnology to biosensors allows a large enhance in sensitivity. In this work, a new amperometric biosensor for fructose monitoring in food analysis has been developed and optimized. The biosensor uses a single-wall carbon nanotube paste (SWCNTP) electrode modified with an osmium functionalized polymer and fructose dehydrogenase (FDH) as recognition element. The osmium polymer was promising as electrochemical mediator to shuttle the electron transfer between the immobilized enzyme and the SWCNTP electrode [Antiochia et al., 2007]. The FDH enzyme was immobilized onto the osmium modified SWCNTP paste electrode by cross-linking with PEDGE or in an albumin hydrogel matrix fixed to the SWCNTP electrode surface. The dependence of the biosensor response for fructose was investigated in terms of pH, temperature, FDH amounts, kind of immobilization and applied potential. Also the reproducibility and the storage stability of the FDH based biosensor was studied and optimized. The designed fructose biosensor has been found as electrochemical mediator to shuttle the electron transfer between the immobilized enzyme and the SWCNTP electrode [Antiochia et al., 2007]. The FDH enzyme was immobilized onto the osmium modified SWCNTP paste electrode by cross-linking with PEDGE or in an albumin hydrogel matrix fixed to the SWCNTP electrode surface. The dependence of the biosensor response for fructose was investigated in terms of pH, temperature, FDH amounts, kind of immobilization and applied potential. Also the reproducibility and the storage stability of the FDH based biosensor was studied and optimized. The designed fructose biosensor has been found as electrochemical mediator to shuttle the electron transfer between the immobilized enzyme and the SWCNTP electrode [Antiochia et al., 2007]. The FDH enzyme was immobilized onto the osmium modified SWCNTP paste electrode by cross-linking with PEDGE or in an albumin hydrogel matrix fixed to the SWCNTP electrode surface. The dependence of the biosensor response for fructose was investigated in terms of pH, temperature, FDH amounts, kind of immobilization and applied potential. Also the reproducibility and the storage stability of the FDH based biosensor was studied and optimized. The designed fructose biosensor has been found as electrochemical mediator to shuttle the electron transfer between the immobilized enzyme and the SWCNTP electrode [Antiochia et al., 2007]. The FDH enzyme was immobilized onto the osmium modified SWCNTP paste electrode by cross-linking with PEDGE or in an albumin hydrogel matrix fixed to the SWCNTP electrode surface. The dependence of the biosensor response for fructose was investigated in terms of pH, temperature, FDH amounts, kind of immobilization and applied potential. Also the reproducibility and the storage stability of the FDH based biosensor was studied and optimized. The designed fructose biosensor has been found as electrochemical mediator to shuttle the electron transfer between the immobilized enzyme and the SWCNTP electrode [Antiochia et al., 2007]. The FDH enzyme was immobilized onto the osmium modified SWCNTP paste electrode by cross-linking with PEDGE or in an albumin hydrogel matrix fixed to the SWCNTP electrode surface. The dependence of the biosensor response for fructose was investigated in terms of pH, temperature, FDH amounts, kind of immobilization and applied potential. Also the reproducibility and the storage stability of the FDH based biosensor was studied and optimized. The designed fructose biosensor has been found as electrochemical mediator to shuttle the electron transfer between the immobilized enzyme and the SWCNTP electrode [Antiochia et al., 2007].
Biogenic amines content as a measure of the quality of wines of Abruzzo (Italy)

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Abstract

Aim of this research was to study the effect of some agronomic and oenological factors on the content of biogenic amines as quality index of wines from 14 wineries of the Abruzzo (Italy). Out of 66 samples of wine (bottled and ready to trade) the following amines have been researched, identified and quantified: ethylamine (ETY), 2-phenylethylamine (2-PHE), putrescine (PUT), cadaverine (CAD), isoamylamine (ISO), histamine (HIS), tyramine (TYR), spermidine (SPD) and spermine (SPM). Sum of amines was found to be decreasing in the order: red wine (19.3 ± 12.8 mg L⁻¹), rose wine (9.20 ± 6.34 mg L⁻¹), white wine (7.67 ± 3.84 mg L⁻¹). The single amines significantly correlated with their sum were the PUT (β = 0.94), the HIS (β = 0.91) and TYR (β = 0.89). The production of PUT was significantly associated (β = 0.69) with activity of malolactic bacteria (Mangani et al., 2005). The principal component analysis (PCA), as a result of autoscaling the data, explained about 50% of the total variability of the samples. The first component (LV1) was positively associated with PUT, HIS, TYR, and pH, negatively with malic acid content, discriminating red wines from white ones significantly. The second component (LV2) was negatively associated with ethanol and positively with CAD and total SO₂ amount, discriminating white wines from the rest of the samples. The differences among the types of wine are due to different biotechnological process: microbial strains involved in the fermentation of white winemaking, have characteristics different from those involved in the red one, the environmental conditions that are created during the red winemaking favor an increased availability of substrates and optimal parameters (pH, T, etc. ..) for the amino acid decarboxylase activity. Besides the vintage, influential seems to be the effect of the winery, regardless of the geographic area in which it is situated. However, in all analyzed samples the maximum amounts of TYR and HIS were below the levels of toxicity (Konakovskv et al., 2011), demonstrating a good quality of the wines of Abruzzo, whose consumption is no risk to the health of the consumer following the rules of proper nutrition.

Authors are very grateful for the co-operation of the Association of Enologists of Abruzzo-Molise.

Molecular modeling and docking of food-derived Angiotensin I-converting enzyme inhibiting peptides

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Abstract

Currently, the most successful strategy for controlling blood pressure is the inhibition of the angiotensin-converting enzyme (ACE). Chemically synthesized ACE inhibitors are widely used in the clinics to reduce mortality in patients with high pressure and heart failure. In addition to therapeutic drugs, increased attention has been paid to the identification of ACE inhibitory peptides from different food proteins, as sources of health-enhancing components. These peptides are inactive within the original protein but, once released during gastrointestinal digestion in the body or during food processing, they function as regulatory compounds with hormone-like activity (Vermeirssen et al, 2004). It has been hypothesized that the peptides bind tightly to ACE at its active site and compete with angiotensin I for occupancy. As for captopril and lisinopril, the identification of specific structural moiety and amino acid involved in the binding is important for the ACE inhibitory potency. In this study, three-dimensional models of ACE have been used to predict the biological orientation of inhibitors to ACE active site (C-domain), using the molecular docking software AutoDock4.2. The ACE model crystalized with captopril (1UZF) has been initially refined coupling Modeller with NAMD softwares (Eswar et al., 2006; Phillips et al., 2005) and hence used to setup and validate the docking parameters. Inhibitor peptides were designed using PyMol and afterwards docked against the ACE crystal. The docking models obtained provide valuable information for the design of ACE inhibitors with potent activity towards C-domain of ACE.
Composition and volatile aromatic fraction of “fine amber” Marsala wines

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Abstract
Marsala is a fortified wine, like Port and Sherry, added of wine alcohol, in the late XVIII century John Woodhouse, an English trader, started its wine marketing (Antelmo, 2004). This strong and aromatic product was soon appreciated by the Admiral Nelson. Woodhouse established his winery at Marsala in 1796, followed by Benjamin Ingham in 1812, and by Vincenzo Florio in 1833. The Marsala is a wine of Controlled Denomination of Origins (CDO) from 1969 (DPR 2 April 1969) and the wine-making procedure is regulated by the law (L 851/1984; DPR 17 November 1986). Marsala wine is a very typical Sicilian product, in fact: grape cultivars are autochthonous (Grillo, Catarratto, Inzolia and Damaschino), geological and climatic condition, cultivation, pruning, vinification and ageing techniques are particular. It is produced in three different colours Golden (oro), Amber (ambra), and Ruby (rubino). The wine can be sweet, semi dry and dry, relative to its sugar contents. For ageing time in wooden barrels, it is classified: "Fine", "Superiore" "Superiore Riserva", and "Vergine" (aged more than five years). “Fine Amber” Marsala, the most widespread on the market, is vinified using a base wine, cooked must and wine alcohol with 17% v/v minimum value of final alcohol content, as provided by law. This blending (concia), originally made to preserve the wine during sea travel, is an important and critical phase of production because several chemical and sensorial characters of final product, as sweetness and alcohol content, depend on it. Lenght of ageing in wood cask is also a decisive factor for wine final organoleptic characteristics. At present there are few researches on Marsala, the aim of this work is to study chemical composition and aromatic profile of all production of “Fine Amber” Marsala wines. Chemical and physical parameters and the aromatic fraction by SPME-HS-GC/MS techniques were determined on thirteen samples of Fine Amber Marsala wines. The results show that the wines have different chemical and aromatic composition because the DOC disciplinary does not standardize the blending (concia) process.

Phytochemicals content in Italian garlic bulb (Allium sativum L.) varieties

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Abstract
Garlic (Allium sativum L.) possesses several phytochemicals including sulfur-containing compounds, vitamins, saponins, flavonoids and moderate levels of carotenoids. The synergistic interactions between these components contribute to provide the observed health benefits from garlic as well as antibacterial, antifungal, hypolipidemic, antihypertensive, antiatherosclerotic, anticoagulant, hypoglycemic and chemopreventive effects. Several factors could impact upon the composition of any plant food, including differences between cultivars and growing conditions. This study evaluates the phytochemicals content (ascorbic acid, flavonoids and carotenoids) and antioxidant capacity in four Italian “typical varieties” (Aglio rosso di Castelliri, Aglio bianco Piacentino, Aglio rosso di Sulmona, Aglio rosso di Proceno). Samples were grown in two different geographic areas of Lazio (Viterbo and Alvito), using the same technical/agronomic trail. The flavonoids content varied amongst different varieties and growing locations. Myricetin and apigenin were found as the most abundant flavonoid in four varieties. The ascorbic acid content ranged from 11.01 to 21.59 mg/100g and Alvito bulbs ascorbic acid levels were higher than Viterbo bulbs. Same picture was present regarding β-carotene content ranged from 5.68 to 7.41 μg/100g and 6.36 to 7.46 μg/100g for Viterbo and Alvito bulbs respectively. Our findings have revealed that environmental interactions such as production areas and pedoclimatic factors could influence the bioactive molecules content and the antioxidant properties in Italian garlic bulb varieties.
Effects of anthocians extracts from different sources on antinflammatory and antioxidant pathways

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Abstract
Among Functional Foods there are many different "traditional" foods rich in specific compounds shown to produce an effect or modulate a function in our organism. However, in most cases, e.g. in tomato, the evidence has not been sufficient to obtain an official health claim. Nevertheless, it is important to investigate further the effects of vegetables in our diet and to communicate correctly their advantages for health. Nutritional genomics reflects gene/nutrient interactions, utilising high-throughput genomic tools in nutrition research. Nutrigenomic approaches, especially transcriptomics, enable simultaneous study of various signalling pathways and networks. We test the activity of extract from *Sambucus ebulus* L. berries and by-products from winery, very rich in anthocians (R.J. Kruger, V.L. 2008, Singleton et al. 1969), on hepatocyte cell line (HepG2) to evaluate their potential activity on two important metabolic pathways: inflammation and antioxidant mechanisms. We define the maximum concentration not cytotoxic for any extract and any cell line and then expose the cells for 24 h to the treatment and then we extract the RNA and perform the real-time PCR analysis. We used two Applied Biosystem Gene expression array every one with 96 different assays: TaqMan® Array Human Inflammation and TaqMan® Array Human Antioxidant Mechanisms. Our results demonstrate that every extract show a significative influence on the expression of different genes belonging to the two pathways.

Real-time PCR detection of bovine, equine and ovine DNA in milk and cheese

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Abstract
Animal species authentication in food products plays a major role in traceability, quality and safety purposes. Species substitution or cross-contamination can indeed lead to regulatory non-compliance, religious concerns, or allergic issues. Milk species identification in milks and cheeses has received great attention in recent years because of the possibility of detecting fraudulent procedures such as the substitution of ovine milk for bovine milk in cheeses or of equine milk for bovine in special milks. Recently biomolecular techniques such as real-time polymerase chain reaction have received particular attention. It is possible to use milk as a source of DNA and as a substrate for PCR (Lipkin et al., 1993). Certified DNA references from cow (Bos Taurus), goat (Capra hircus), sheep (Ovis aries), horse (Equus caballus), pig (Sus scrofa) were purchased and supplied lyophilized. Once reconstituted in TE buffer 0.1% DNA solutions were stored at -20°C. We designed TaqMan specific assays on mitochondrial 16S rRNA for bovine, ovine (Bottero et al., 2003), and equine species, we selected region of species-specific sequence. In total 5 samples of each kind of milk and 5 samples of cheese (only cow milk, only ovine milk or produced with a mixture) were analyzed. To obtain the relative concentration of cow milk in equine milk and cow milk in ovine milk we built standard curves prepared by serial dilution in water of cow, ovine or equine references DNA at known concentrations. We test our real-time PCR system using mixes of cow and equine DNA or cow and ovine DNA at defined proportions. The assay was shown to be specific against other animal species, and fast and sensitive enough for authenticity purposes. Moreover our RT-PCR systems are able to identify specifically cow, ovine or equine DNA with a discrimination limit of 1-2%.
Effect of wine inhibitors on two different cysteine proteases: a comparison between papain from *Carica papaya* latex and pineapple stem bromelain

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\textbf{Abstract}

The influence of potential inhibitors, naturally present in wine, on the activity of papain from *C. papaya* latex was investigated. The results were compared with those of a previous research, carried out in our laboratory, on bromelain from pineapple stem, in order to evaluate the applicability of these cysteine proteases for protein stabilization of white wine. Proteolytic activity was tested against a synthetic substrate (Bz-Phe-Val-Arg-pNA) in a model wine system (tartaric buffer, pH 3.2) after adding ethanol, sulphur dioxide (SO\textsubscript{2}), skin and seed grape tannins at the average ranges of their concentration in wine. Kinetic parameters (k\textsubscript{cat}, K\textsubscript{M}, K\textsubscript{i}) of papain and stem bromelain were determined according to Michaelis-Menten equation using a nonlinear regression procedure. For each substance tested, the inhibition constant (K\textsubscript{i}), reflecting the concentration of an inhibitor that decreases the rate of an enzyme-catalyzed reaction by 50\%, was evaluated. The examined wine constituents turned out to be all reversible inhibitors for both proteases. Papain activity resulted to be more affected respect to bromelain one by all tested substances. Ethanol showed to be a competitive inhibitor with a rather limited effect; K\textsubscript{i} values, determined by a secondary plot were 11.4(±1.0)% v/v and 4.6(±1.4)% v/v for stem bromelain and papain, respectively. The strongest inhibition was exerted by free sulphur dioxide (K\textsubscript{i} 4.55±0.17 mg/l\textsuperscript{-1}, K\textsubscript{i}' 0.40±0.09 mg/l\textsuperscript{-1} for stem bromelain and K\textsubscript{i} 0.0033 ± 0.0002 mg/l\textsuperscript{-1}, K\textsubscript{i}' 0.065±0.007 mg/l\textsuperscript{-1} for papain), which acted as a mixed-type inhibitor for both proteases. Finally, seed and skin grape tannins revealed to be uncompetitive and mixed-type inhibitors for stem bromelain and papain, respectively. The findings of study suggest useful information for a future biotechnological application of two proteases in winemaking process.

Development of an extraction method for the determination and the speciation of selenium in different vegetables matrices

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\textbf{Abstract}

A lot of features must be considered when a study is planned about possible health problems caused by the micronutrients that are essential to our life at certain concentrations, but toxic at other concentrations. This is the case of Selenium. Thanks to a diet or use of food supplements is possible to reward any possible deficiency of this fundamental element, but it’s very important understand the different mechanisms of assimilation. In fact, for all micronutrients, the mechanisms of assimilation are closely related to the speciation of metal species. The natural Selenium intake is essentially based on the consumption of cereals and vegetables; their content of Selenium is in turn a function of the mechanisms of absorption from the soil. The aim of this work is the study, the evaluation and the improving of a method for determining the content and speciation of Selenium in different vegetable matrices. The obtained information can be usefully used to investigate the correlation between the amount of Selenium that humans intake by vegetable consumption with the amount of Selenium in the soil where these vegetable grown up. In order to obtain speciation data it was thoroughly studied the extraction phase that can be useful in the determination of the Selenium species by adopting a sequential extractions procedure that permits the by pass of the chromatographic separations, procedures expensive and time consuming). Therefore it was studied and tested a sequential procedure of extraction that was then applied to discriminated vegetables collected in different areas characterised by different content of Selenium. The concentration of Selenium in the different fractions extracted was determined by ICP-MS technique. Since the extractive method presented some improvable points, two changes were evaluated. The first one was the replacement of the step of sonication by water with an extraction by ASE (Accelerated Solvent Extraction). The second was the replacement of the protease enzyme in the step of enzymatic extraction. In the phase of evaluation and validation of the procedure, samples of mushrooms and apples were considered because their very high and low concentration of Selenium, respectively. Afterwards the entire method has been successfully applied to garlic samples coming from an area near Alessandria (North West of Italy) in which has been reported low levels of Selenium in the soil, from an area with normal Selenium levels and, finally, a sample of unknown origin purchased at the supermarket.
P-25

Use of X-ray diffraction technique and chemometrics to aid soil sampling strategies in traceability studies

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Abstract

The present study is part of a main long term research supported by the Ager – Agroalimentare e Ricerca – cooperative project between grant making foundations, under the section ‘wine growing and producing’. Main aim of the work is the development of geographical traceability models for food, based on the use of direct indicators such as isotopic ratios of stable and radiogenic elements. Particular attention is focused on the PDO oenological products of Modena district, namely the Lambrusco wines. It is worth to note that in traceability studies, an extremely important step is the planning of soil sampling, because of its great influence on the significance of the final models. For this purpose, in this case, a pilot study has been developed for investigating the influence of sampling depth, field homogeneity, seasonal and time variability on the monitored traceability indicators. Four geographical areas, covering the different Lambrusco production according to the EU Regulations were identified and each of them has been sampled in several points splitted in five depths, in order to study the inter- and intra- areas variability. Furthermore, the sampling procedure was repeated in three different periods of the year, i.e. spring, summer and winter. The collected soil samples were characterized by means of X-Ray diffraction of powder and then the obtained signals were considered as fingerprints and analyzed by chemometric techniques. As processing step, the diffractograms were first aligned and then noise reduction and background correction were achieved in wavelet domain. Furthermore a blockscaling procedure was applied in order to allow minor components to contribute to the model without altering the relative scales of variables belonging to the same block. Finally, PCA and PARAFAC analysis were applied to the pretreated data as explorative data analysis tools in order to investigate the possible inter- and intra-site differences/similarities and to determine the impact of sampling depth and seasonal variability on metal content and isotope ratio.

P-26

Use of HR-NMR for functional properties estimation of balsamic vinegars of Modena

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Abstract

Over the past few years, many works based on proton NMR coupled with multivariate data analysis were been conducted concerning the study of the suitability of HR-NMR as fingerprint analysis tool in the study of vinegars. Classical studies on vinegars are normally based on composition data obtained by various analytical techniques, however, since the quality and characteristics of a product are not the simple sum of individual chemical characteristics, NMR analysis with chemometrics certainly is a useful tool in this regard. In this context, the aim of the present work was to select and optimize a NMR sequences to characterize the Balsamic vinegar of Modena and the Traditional Balsamic vinegar of Modena and to study the possibility to obtain by NMR spectra useful informations about the biologically active compounds and the functional properties. The application of HR-NMR techniques to the samples has generated very complicated spectra that needed to be previously processed and subsequently analyzed by chemometric methods. The NMR spectra will be correlated with some analytical parameters such as total polyphenols, total condensed tannins, total flavonoid and total antioxidant activity determined by several methods (ABTS, DPPH, photochemiluminescence). The different methods selected for the determination of the antioxidant activity allow the assessment of different aspects of the oxidation process: the DPPH and ABTS assays are based on both HAT (hydrogen-atom abstraction) and SET (electron transfer reaction) mechanisms; the photochemiluminescence (PCL) method is based on the photo induced autoxidation inhibition of luminol by antioxidants mediated from the radical superoxide. In this way, the measured antioxidant activity can be compared to antioxidant activity of the balsamic vinegar in biological systems.
**Protein extraction and identification as a honey adulteration detection method**

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**Abstract**

Honey is a nutritious and healthy natural food produced by honey bees from plant nectars: it consists mostly of the sugars glucose and fructose, as well as maltose, sucrose, water, pollen and other minor components. Food authentication is an international issue in quality control and food safety. Regulatory authorities, food processors, retailers, and consumers are interested in knowing the origin and quality of foods; for these reasons the deliberate mislabeling and adulteration of foods, particularly honeys, are matters of increasing global concern. Many studies have reported on the chemical constituents of honey such as sugars and flavonoids, and the analysis of many of those components is applicable to adulteration detection. Another approach is based on analysis of proteins, the honey contains many different proteins in minute quantities and several enzymes, such as amylase, α-glucosidase, β-glucosidase and glucose oxidase. Since they originate from Apis mellifera, the identification of a protein or an enzyme of a different origin could be interpreted as a proof of adulteration. We have focused on the detection of an α-amylase from Aspergillus niger, an enzyme that can be employed to produce glucose syrup, used for honey adulteration. This enzyme was added to honey samples in different concentration, ranging from 0,1 to 1 nmol/ml, and different isolation and analysis techniques were tested. Honey samples were dialyzed against ultrapure water for 3 days using a cellulose membrane with a 12KDa cutoff, then lyophilized to concentrate the residue protein. Passivation of the membrane to avoid loss of protein during the dialysis was also tested, but no significative difference was found after determination with Bradford assay. A separation using disposable C18 SPE column was also tested. Samples obtained after isolation were analyzed using both MALDI-TOF MS and HPLC CHIP Q-TOF MS. The sensitivity of MALDI-TOF resulted much lower than the one achieved with HPLC-MS, which was able to detect concentration as low as 0,1 nmol/ml for dialyzed samples. The separation obtained with the SPE columns was not satisfactory, probably because amylase was strongly bound to the C18 column and elution was not efficient.
**Application of flow cytometry to assess the viability of stressed Lactobacillus sakei**

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**Abstract**

The aim of this study was to develop a rapid assay based on a nucleic acid double-staining by using two fluorochromes [SYBR Green II RNA gel stain (SYBR-II) and propidium iodide (PI)] (Gregori et al., 2001) to distinguish viable from damaged and dead cells of *Lactobacillus sakei* strains after exposure to heat and acid stresses. Cells were discriminated in density plots of green fluorescence versus red fluorescence. After single labeling firstly with SYBR-II and then with PI, cytograms showed two bacterial clusters, live and dead cells. Moreover, dual staining differentiated cell populations in different physiological states, alive cells with intact membranes, an intermediate physiological state that corresponded to damaged cells that are still alive but with injured membranes, so with even a recovery ability, and dead cells killed because of a permanent membrane damage. This application was validated against the classical plate-counting method and the comparison of two methods produced interesting results. In the traditional method, the removal of the stress and the incubation of the cells in favourable growth conditions caused an increase of colony counts that corresponding to the recovery ability, attributed to damaged cells of the cytofluorimetric analyses. This study evidenced that, due to the complexity of the physiological status and heterogeneity of bacterial cells in a culture, especially after stress, multiparameter analysis is preferable. This approach allowed to achieve information about the dynamics and physiological heterogeneity of microbial populations and to monitoring changes in bacterial behaviour after exposure to different stresses. This is the first report about the use of double staining cytofluorimetric analysis on an important food bacteria as *Lactobacillus sakei*. This novel assay has potential for physiological research on lactic acid bacteria and for application in the food industry.

**Dietary and medicinal significance of wild vegetables from Calabria region (Italy)**

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**Abstract**

Plant drugs have a long history in both traditional and modern societies both as herbal remedies or crude drugs and as purified compounds approved by Food and Drug Administration. The active principles are also used as starting materials for further medicinal chemistry modifications (Koehn and Carter, 2005; Jones et al., 2006). The present study deals with a screening of sixteen Mediterranean plants. They are spontaneous edible plants present in the area of Alto Ionio Cosentino, a territory characterized by different vegetation area from the sea level to the highest peak of Pollino Mt. (about 2000 m a.s.l.), mostly rich in Mediterranean elements. Plants selected for this study were chosen because of their use in local traditional cuisine. Information on the traditional culinary purposes of these plants was collected through structured interviews. The study shows that twelve species are eaten boiled which are namely: *B. officinalis*, *C. sicula*, *C. pycrenochephalus*, *C. intybus*, *C. cardunculus* subsp. *cardunculus*, *E. vulgare*, *M. sylvestris*, *P. rhoeas* ssp. *rhoneas*, *R. raphanistrum* subsp. *raphanistrum* and *S. oleraceus*; seven are eaten fried: *B. officinalis*, *C. sicula*, *C. pycrenochephalus*, *C. vitalba*, *C. cardunculus* subsp. *cardunculus*, *F. vulgare* subsp. *piperitum*, *R. raphanistrum* subsp. *raphanistrum* and *S. oleraceus*; three are eaten raw or in salads: *C. intybus*, *L. sativum* and *S. oleraceus*; two are eaten roasted and are used to prepare stews: *C. cardunculus* subsp. *cardunculus* and *R. raphanistrum* subsp. *raphanistrum*; two are pickled or conserved in oil: *C. sicula* and *F. vulgare* subsp. *piperitum*; four are eaten mixed in soups: *B. officinalis*, *F. vulgare* subsp. *piperitum*, *R. raphanistrum* subsp. *raphanistrum* and *S. oleraceus*; two are used as spices and in the preparation of liqueurs: *F. vulgare* subsp. *piperitum* and *M. aquatica*. The hydroalcoholic extracts obtained from the sixteen Italian plants above listed were studied to assess the lipophilic composition and their potential to inhibition the pancreatic lipase. Pancreatic lipase (triacylglycerol acylhydrolase) is a key enzyme for the absorption of dietary triglycerides. Interference with fat hydrolysis results in the reduced utilization of ingested lipids, therefore inhibition of lipases decreases fat absorption. Agents that inhibit fat digestion are of theoretical benefit in the treatment of obesity (Roh and Jung, 2012).
Evaluation of cooked ham quality by assessment of its protein content during the entire process of transformation

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Abstract

Cooked ham is one of the most popular processed meat products, and constitutes an important sector of the meat industry. The final quality of cooked ham products depend both on the raw material used and on processing which can be influenced by many factors. One of these factors is technology – the type of meat cut, the composition and quantity of brine injected, the rate and extent of tumbling or massaging, and the cooking time and temperature. Injection of brine ensures a uniform distribution of sodium chloride, and other possible ingredients (i.e. sugars, spices, polyphosphates, etc.). The brine injection level and the ingredients used are characteristic of each product and determine the cooked ham quality. In particular, products of higher quality are generally made without polyphosphates and with a low level of brine injection. Tumbling is a mechanical operation which distributes the brine evenly inside the leg and induces tissue structure softening and rupture, which causes an increase of brine sorption and protein extraction from muscle fibres, and consequently an increment of cooking yield. Cooking denatures the extracted proteins, welding the muscles and making the ham slice compact. It gives rise to the distinctive texture, the sensorial properties and a suitable hygienic quality of the final product. The meat proteins, approximately 20% of a muscle’s weight, represent the main constituents that make up the structure of the meat product. The aim of this study was to classify whole-leg cooked hams, made without polyphosphates, by evaluation of protein content in raw meat and cooked ham. The protein content in raw meat and in meat during whole cooked ham process was determined using a Velp® protein analyzer based on the Dumas method. The practical application of this study is the possibility to use a protein content approach to verify meat traceability of the whole cooked ham process. The obtained results are presented and discussed.

Multidisciplinary ecosustainable approach for Grape marc microwave-mediated extraction of bioactive compounds with potential nutraceutical and cosmeceutical properties

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Abstract

The wine industry produces a large amount of organic residues that are both highly polluting and quite expensive to treat. Grape marc is one of the most abundant organic residues. It is rich in polyphenols (i.e. anthocyanins, flavonols and phenolic acids) and several authors reported antiradical and antioxidant activity of its extracts, suggesting the winery by-product for an eco-sustainable production of bioactive compounds. Regarding the extraction of antioxidants, the influence of process and extraction parameters were investigated, but some authors remarked the lack of systematic approaches to optimise the different kinds of extractions. The aim of this work was the development of an eco-sustainable microwave mediated extractive method to obtain grape marc extracts with potential nutraceutical and cosmeceutical properties. In order to optimise process efficiency together with purity and antiradical activity of the obtained extracts, a statistical experimental design, was implemented. A face-centred design was applied, considering the extraction temperature and time as relevant factors and studying them at three different levels. The extraction efficiency, the extracts purity and their free radical scavenging activities (DPPH method) were taken into account as experimental responses to be maximized. Since the final goal was to obtain an eco-sustainable process, water was used as extraction solvent. The results were compared to those obtained both using methanol and using a conventional extraction method (hot oil bath) previously optimised by means of a similar experimental design. Taking into account several points of Green Chemistry principles (natural and renewable sources, use of industrial wastes, alternative energetic sources, mild processes, safe products), this study represents a useful tool for industries involved in a global Responsible Care Program.
Carbon isotopic ratios and enantiomeric distributions of selected volatiles as parameters for the quality assessment of Citrus liqueurs determined by HS-SPME coupled to GC-C-IRMS, and ES-GC.

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Abstract

Liqueurs derived from Citrus fruits, generally obtained from maceration of the peel or the whole fruits of lemon, mandarin and bergamot in ethanol, water and sugar, are a category of spirit drinks in which the addition of nature-identical flavouring substances and preparations is forbidden. The traditional production methods and the protection of geographical indications of spirit drinks are governed by the Regulation (EC) No 110/2008 of the European Parliament and of the Council. Authenticity assessment of homemade and commercial Citrus liqueurs was performed using Headspace-Solid Phase Microextraction (HS-SPME) coupled to Gas Chromatography–Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS). Additional analyses were performed on all the samples, by means of enantioselective Gas Chromatography (Es-GC), measuring the enantiomeric distribution of the chiral volatile components, extracted by the same HS-SPME technique. Moreover, Gas Chromatography–Mass Spectrometry (GC-MS) analyses were also conducted by using HS-SPME, in order to obtain information on the qualitative aspects of the samples. Additional analyses also revealed the lack of the monoterpene fraction in some commercial samples. The results obtained by GC-C-IRMS on the liqueurs were compared with the carbon isotopic ratio ranges determined on authentic cold-pressed lemon, mandarin and bergamot essential oils. In particular, it emerged that the carbon isotope ratio of the volatile compounds of all the home-made drinks fell into the correspondent authenticity range of the cold-pressed essential oil, while some commercial products did not match the correspondent ranges of authenticity. GC-C-IRMS, ES-GC and GC-MS coupled with HS-SPME have shown to be a complete and rapid tool for the quality control investigation of Citrus liqueurs. The results obtained by these techniques were in good agreement, revealing the non-natural Citrus aromas in some commercial liqueurs, as well as assessing the genuineness of the home-made ones.

Passata di pomodoro authenticity checks using δ¹⁸O analysis

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Abstract

Tomato puree (passata di pomodoro) represents a classic and emblematic Italian product around the world and is of considerable importance for the Italian economy. It has recently been estimated that 15% of industrial tomato comes from China in paste form (http://affaritaliani.libero.it/green/coldiretti_pomodoro_cina280311.html). For these reasons Italian law (Ministerial Decree of 23 September 2005) provides a very strict definition of ‘passata di pomodoro’ and establishes that the use of tomato concentrates over 12° degrees Brix and subsequent dilution to obtain ‘reconstituted passata’ is not permitted. Furthermore it indicates δ¹⁸O measurement in vegetal water (UNI ENV 12141 method) as the official method for detecting fraudulent dilution, but does not report any reference values for authentic passata. Trifirò et al. (2007) verified that passata effectively shows δ¹⁸O values significantly higher than products obtained by diluting tomato paste and proposed a minimum threshold value of -1.6‰ for authentic passata with Brix values between 7.5 and 10°. In this work we analysed authentic samples of passata, paste and diluted concentrate in order to check the validity of this threshold value and to establish a δ¹⁸O limit value for passata produced by diluting products with a Brix value lower than 12°. A large number of samples of Italian ‘passata di pomodoro’ and paste from Italy, Greece, Turkey and China have been officially collected by the Italian Ministry of Agricultural, Food and Forestry Policy since 2009. Analysis of the δ¹⁸O of vegetal water was performed on these samples using an Isotope Ratio Mass Spectrometer (IRMS), interfaced with a CO₂ equilibration system, according to the ENV 12141 method. The results essentially confirmed that -1.6‰ can be considered as the limit δ¹⁸O value for authentic passata with a Brix of over 7.5°. Furthermore a δ¹⁸O of -2.5‰ can be considered as a limit value for passata coming from the dilution of products with a Brix level of less than 12°. All the diluted pastes showed δ¹⁸O values far below these limits. This research was funded by the Italian Ministry of Agricultural, Food and Forestry Policy.
HPLC method validation for the determination of fucoxanthin

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Abstract
The Fucoxanthin is a carotenoid present primarily in brown seaweeds (Feoficee) of the genus Undaria pinnatifida (commonly called Wakame), Sargassum fulvellum, Laminaria japonica (better known as Kombu) and Hizikia fusiformis, which gives them the peculiar dark color, overlapping the typical greenish color of the common algae. Fucoxanthin is a characteristic xanthophyll present in brown seaweed and the most abundant amongst aquatic carotenoids, accounting for more than 10% of estimated total natural production of carotenoids (Prabhasankar et al., 2009). The fucoxanthinol, primary active metabolite of this molecule, has been recently found to have anti-obesity effects through the mobilization of adipose deposits for energy purposes, as well in the production of DHA (docosahexaenoic acid) which is claimed to provide a variety of different health benefits (Maeda et al., 2006). In this work a HPLC-Vis validation method for determination and quantification of all-trans-fucoxanthin (CAS number 3351-86-8) in dry extracts of Wakame, used as raw material for the formulation of dietary supplements and in several commercial samples (fucoxanthin-based), was reported. The first step of this research was the optimization of the chromatographic conditions and its validation in accordance with the international guidelines ICH (International Conference on Harmonization), also applying common statistical tests suitable for validation of a chromatographic method. The second step included the optimization of a protocol for extraction of fucoxanthin and its quantification in dry extracts of Wakame seaweed and several commercial products.

Aroma characterization of Italian Muscat-based wines by HS-SPME/GCxGC/TOF-MS

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Abstract
White muscat (Muscat Blanc à Petits Grains) grape (Vitis vinifera L.) is used for the production of Asti and Moscato d'Asti wines from Piedmont. The evolution of the volatile aroma in wine is correlated to many parameters as grape variety, winemaking practices, microbial starters and ageing). The oxidation of some compounds was reported to be of fundamental importance in Muscat-type wines aroma (Ribereau-Gayon et al., 1975), but the unexpected development of off-flavors is a common problem (Lizarraga et al., 2004). First aim of our work was to develop a multi-dimensional comprehensive capillary gas chromatographic method for the analysis of Muscat wines volatile compounds. Second aim was the application of this method to the study of the evolution of the volatile aroma during ageing of these wines. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre was used in HS-SPME extraction from all wine samples. The volatile aroma was analysed using GCxGC coupled with a Time of Flight Mass Detector (Arlorio et al., 2005). In one-year aged wine samples, linalool, nerol and geraniol decreased respect to the young Muscat wine to become less important in the aroma composition. Therefore, alpha-terpineol, hotrienol, nerol oxide, furanic linalool oxides A/B and rose oxide concentration significantly increased, confirming an oxidative evolution of the aroma. We also identify some off-flavors, responsible of wine imperfections described as cork tones, plastic, mushroom. The presences of 2,4,6-trichloroanisole and 2,4,6-tribromoanisole have been highlighted in some samples.
Evaluation of quinolizidine alkaloids in four wild Mexican lupin species

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Abstract
Quinolizidine alkaloids (QAs) are an important class of secondary metabolites of plants belonging to Lupinus and other genera of the Fabaceae family, having interesting biological activities (Erdemoglu et al., 2007; Garcia-Lopez et al., 2004). Since it is known that QAs profiles are species specific, a high species biodiversity represents also a high diversity of QA structures. As a consequence, Mexican Lupinus seeds may be a relevant source of potentially interesting QA. In this work the QA composition of four wild Mexican lupins (Lupinus campestris, L. hintonii, L. montanus and L. aschenbornii) collected from volcanoes Iztaccihuatl Popocatepetl region was investigated with GC-MS (Boschin et al., 2008). The QA contents are in the range from 20.9 to 30.2 mg/g and three main QA profiles were observed. L. montanus contains mainly QAs with short retention times, being sparteine the main QA, followed by lupanine. L. campestris shows QAs with medium retention times, being lupanine the principal peak, followed by epiaphylline and epiaphyllidine. L. hintonii and L. aschenbornii are characterized by QAs with long retention times.

Novel analytical strategies for GM fodder targeting in cattle’s biological fluids

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Abstract
The use of GM fodder in cattle feed is substantially invisible to the consumer, who is in a clear lack of information in relation to a matter which is, however, of great importance for its choices of purchasing. In fact, it was demonstrated that in animals the isotopic composition of biological fluids is affected from diet (Knobbe et al, 2006; Tudisco et al, 2010). We were involved in the development of a novel, dedicated method for dairy food characterization, and possibly certification. From bulk studies on cattle’s biological fluids, different matrixes (urine, milk) were considered and some representative target analytes selected to be extracted as appropriate diagnostic markers of genetically modified products soy and corn feeding (Gonzalez-Ronquillo et al, 2003; Godin et al, 2011). A new method based on sample pretreatment and liquid chromatographic separation was developed for targeting, isolation, identification and purification of the selected markers. A sample preparation procedure developed starting from the whole matrix was aimed to obtain sufficient separation on monolithic support without further purification, representing was tuned as the first step of a GC-IRMS analysis (Urban et al, 2008).
Phenolic content of blueberry fruit and ready to eat frozen purées: Comparison of two extraction media

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Abstract

Blueberries (Vaccinium corymbosum L.) are one of the best source of phenolic antioxidant compounds and their processing into high quality ready to eat products may support healthy diet habits. In blueberries, phenolics are confined into the cell vacuoles of few epidermal layers underneath cuticle waxes, and hence a proper tissue rupturing pretreatment is required to release phenolics from fruit matrix, along with an extraction step which ensures a good yield and stability of recovered compounds. Mechanical and thermal processing impact on the istochemical environment of the plant source and the phenolic release operated by the extraction media on processed products may be altered. The aim of this research was to study the effect of acetone addition to the extraction medium on the phenolics recovery from different blueberry processed matrices. Two extraction media, i.e. formic acid-water-acetone (5:35:60 v/v/v) and formic acid-water (5:95 v/v), were tested on unblanched (NB) and blanched (BL) blueberry fruit and their respective frozen purées, which were prepared according to Brambilla et al. (2011), after a 24 h thawing at 4°C. Phenolics (2 replicates/sample) were extracted in duplicate with both the extraction media and were quantified as total phenolic compounds (TPC, Folin-Ciocalteau method), total monomeric anthocyanins (MAP, pH-differential method) and polymeric color (PC%, subtractive method) (Brambilla et al., 2008). Adding acetone to the extraction medium resulted in: a significant TPC increase in the extract for NB products (berries: +36.4%; purée: +27.8%), no MAP increase in the extracts from NB products, and a MAP decrease in BL berries (–10.8%). PC% was higher in all acetone extracts whatever the product, with higher PC% values in NB samples compared to BL ones. These results confirm the ability of acetone to liberate phenolic compounds from blueberry cellular structures and indicate that this enhancing effect is reduced in blanched fruit matrices. The yield in MAP from the products on the contrary was not improved by the acetone extraction which was, indeed, detrimental on thermally treated matrices.

Effects of environment on crop, chemical composition and anticancer activity of Mediterranean sage

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Abstract

Salvia officinalis L. (sage), an aromatic plant from Lamiaceae family, can be found worldwide and its leaves are commonly used as ingredient in food and cosmetic industry. Sage essential oil is applied in the treatment of a range of diseases and has been shown to possess antimicrobial, viricidal, cytotoxic, antimutagenic and antifungal activities. The objectives of our research were the effects of environment on crop, chemical composition and anticancer activity on S. officinalis essential oil. Sage was cultivated at eighteen experimental sites in south-central Italy (Molise) in different growing environments. The essential oils (S1-S18), extracted by hydrodistillation according to the European Pharmacopoeia, were analysed by GC and GC/MS: peak identification was accomplished by comparison of their mass spectra with NIST 02 and Wiley 275 libraries, as well as by comparison of their retention indices with literature values. Results show that the major components of all the oils were α-thujone, β-thujone, caryophyllene, γ-elemene and γ-muurolene for all the samples, but the percentages of these compounds varied depending on environmental factors such as altitude, water availability and pedo-climatic conditions. The anti-growth and proapoptotic effects of the eighteen S. officinalis essential oils were evaluated in two human melanoma cell lines, M14 and A375.
Characterization of volatile fraction of wines obtained with starter in different formulation

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Abstract

Some of the most important components of the wine aroma are already found in grapes, although most of these molecules is formed during the fermentation process. Several studies have been performed in this direction, in order to highlight how the production of these molecules depend on the strain of yeast used [1, 2]. Studies carried out on wines produced with different strains of Saccharomyces cerevisiae have demonstrated variations in the content of alcohols and esters responsible for specific olfactory notes [3]. Among the compounds significant for the aroma, the presence of terpenes (as linalool, citronellol, geraniol) and norisoprenoids (b-ionone and b-damascenone) is characterizing. These aromatic molecules are released from the glycosylated form (non-aromatic) by the action of B-glucosidase enzymes produced by yeasts [4]. The aim of this study was to determine the qualitative and quantitative pattern of terpenes present in the volatile fraction of experimental wines produced with strains of S. cerevisiae, different for geographical origin and formulation (dried, fresh and immobilized yeasts). Four strains of S. cerevisiae were used: three wild yeast, selected according to different technological characteristics and cell viability while the fourth strain was chosen between the commercial strains. All the yeasts, before use, have been subjected to drying by Mini Spray-dryer. The strains, in the fresh or dried form, were immobilized by the technique of entrapment in calcium alginate. Then they were tested both to determine the effectiveness of the treatment of immobilization and to evaluate the performance of the fermentative biocatalysts. The immobilized yeasts were tested in controlled fermentation trials and compared with control consisting of fresh and dried cells yeast, in the free form. After fermentation the wine samples were cooled to facilitate the clarification, then aliquots of the experimental wines obtained were stored at -20°C for the gas-chromatographic analyses. The analysis of volatile fraction was performed using SPME-GC-MS technique. The gas chromatographic analysis has allowed the identification both many aromatic compounds (alcohols, esters, ketones, aldehydes) and the interesting pattern of terpenic compounds. Among them the profile of some molecules, considered most representative for flavour, was performed, such b-damascenone, geraniol, citronellol, terpineol and b-linalool. All samples obtained by fermentation with fresh strains demonstrated a terpenic and damascenone content lower than samples dried using Mini Spray-Dryer. From the comparison between the samples obtained with the strains immobilized it can be observed that this technique does not show any increase, but produces a variable decrease. Wines produced with the wild strains showed a terpenic and norisoprenoids content greater than the wine produced using the commercial strain. The results obtained showed that the drying technique using Mini Spray-dryer strains of S. cerevisiae allows the formulation of starter able to produce a volatile fraction most abundant in the aromatic components such as terpenic compounds.
Recipe composition data: Calculation procedure can be considered a valid alternative to chemical analysis for all nutrients?

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Abstract
To produce data for food composition tables and databases, because of expensive and time consuming analyses, data on nutrient content of cooked composite dishes are often calculated from ingredients. Furthermore the abundance and the variability of food composing the Italian diet, increase the difficulty to analyze most of the Italian recipes. The commonly used calculation method is a procedure that applies a yield factor (WYFs) at the recipe level and the retention factors (NRFs) at the ingredient level; this procedure has been investigated and validated under European Food Information Resource (EuroFIR) (Reinivuo et al., 2009). The aim of the study was to validate the calculated composition data through a comparison with analytical data in 12 frequently consumed Italian traditional dishes and, the same time, to verify if the calculation method leads to a reliable estimation of macro and micro nutrients values. The selected Italian dishes were: Tomato sauce, Pesto sauce, Bolognese sauce, Pasta and beans, Parmesan risotto, Pizza Napoletana Margherita, Italian omelet, Turkey escalope, Braised beef with Barolo wine, Vicentina cod, Castagnaccio, Cannoli Siciliani. Every recipe was prepared according to traditional cooking techniques in a test kitchen following standardized protocols (recipe and preparation) and nutrients analyses (proximate, minerals, vitamins) were carried out by the official methods for each composite dish; procedural steps for calculating nutrient contents of recipes were performed by a computerized program using WYFs and NRFs primarily on Italian food composition data. The results of the computed nutrients show a satisfactory degree of agreement for protein contents, less for lipids.

Validation of isotopic and elemental analyses of hard cheeses for origin traceability: international collaborative study

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Abstract
In compliance with the European law (EC N. 510/2006), geographical indications and designations of origin for agricultural products and foodstuffs must be protected against mislabelling. This is particularly important for Protection Denomination of Origin (PDO) hard cheeses, as Grana Padano and Parmigiano Reggiano, that can cost up to the double of the no-PDO competitors. A recent paper showed as Random Forests statistical models, based on isotopic and elemental composition, can trace the origin of 9 European and 2 extra-European cheeses in grated and shredded forms, for which it is not possible to check the logo fire-marked on the rind (Camin et al., 2012). The most significant variables for cheese traceability common in both models are δ¹³C, δ²H, δ¹⁵N, δ³⁴S and Sr, Cu, Mo, Re, Na, U, Bi, Ni, Fe, Mn, Ga, Se, and Li. This work presents the results of an international collaborative study on the validation of isotopic and elemental analytical procedures organized in order to support their recognition as official methods, for establishing the authenticity of PDO cheeses. The collaborative study has been performed according to the IUPAC protocol and the ISO Standards 5725/2004 e 13528/2005.
Rapid and comprehensive profiling of carotenoids and fat-soluble vitamins in milk from different animal species by LC–DAD–MS/MS

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Abstract
Simultaneous analysis of fat soluble micronutrients is a challenging task, due to the different sensitivity of these substances towards light, oxygen, heat and pH (Rizzolo, 1998). For the same reason, there are still few works that deal with multi-vitamin analysis, especially when it concerns complex matrices such as foods (Gentili, 2011; Heudi, 2004). In this paper, a novel and efficient analytical method was developed and used to define the fat-soluble vitamin and carotenoid profile of milk from different animal species. Overnight cold saponification was optimised as simultaneous extraction procedure and the whole analytical approach was devised to perform both quantitative and qualitative analyses within the same chromatographic run. Analytes were separated by non-aqueous reversed-phase chromatography and detected by diode-array-detector and positive atmospheric pressure chemical ionisation mass spectrometry. Twelve compounds (all-trans-lutein, all-trans-zeaxanthin, all-trans-β-criptoxanthin, all-trans-β-carotene, retinol, α- , γ-, δ-tocophers, ergocalciferol, cholecalciferol, phyloquinone and menaquinone-4) were quantified in selected reaction monitoring scan mode. Owing to the unavailability of authentic standards, other carotenoids were provisionally identified on the basis of the expected retention times, the absorbance spectra and mass spectrometric data. The feasibility of the whole strategy was then verified analysing samples of cow, buffalo, sheep, goat milk. Retinol and α-tocopherol were the most abundant fat-soluble micronutrients, especially in small ruminant milk. Bovine milk contained the least amount of retinol but it was rich in β-carotene; moreover, it showed a large variety of carotenoids which were completely absent in milk from other species with the only exception of all-trans-lutein. All the milks, in particular those from small ruminants, were also a good source of vitamin K vitamers.

Anthocyanins, procyanidins and antioxidant capacity in botanical supplements and in commercial anthocyanin-rich juices

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Abstract
Berries contain a large variety of different phenolic compounds such as anthocyanins, flavonols and phenolic acids. Berries constitute one of the most important sources of anthocyanins and proanthocyanidins, they are especially abundant in the wild blueberry species (as Vaccinium myrtillus) but also in blackcurrant (Ribes nigrum), cranberry (Vaccinium macrocarpon) and cowberry (Vaccinium vitis-ideae). Along with fresh berry, a variety of berry products such as juice, wine, jam and food supplements contribute to the intake of these substances. There is considerable current interest in the possible health effects of anthocyanins in humans owing to their potential antioxidant effects and their reported positive effects on blood vessel walls and on adhesion of bacteria to the cell surface (a risk factor for urinary tract infections). The aim of this study was to investigate the anthocyanin and proanthocyanidins composition and the antioxidant activities in various anthocyanins rich juices and in different categories of botanical supplements: supplements for urinary infections, for helping vision, for cardiovascular health and for reduction of oxidative stress. A suitable analytical methods to separate, by HPLC (reverse phase column and UV-FL detection), the different components of the pool of anthocyanins and proanthocyanidins, were developed to: quantify the content of total anthocyanins and verify the declared value on the label; identify a characteristic fingerprint that allows to verify the source of anthocyanins; express a qualitative judgment of the anthocyanin profile and evaluate the claims of the product; and compare the products having the same claims. The antioxidant capacity was measured by TEAC method and expressed as Trolox equivalent. The samples often do not coincide with the values stated on the labels, and a large variability was found in quality profile of anthocyanins and in the values of the antioxidant capacity.
In vitro and in vivo antithrombotic activities of cherries

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Abstract
Cherry intake has been reported to reduce inflammatory responses. Given the documented interplay between inflammation and thrombosis, we investigated the effect of cherries on cell-mediated fibrin formation and fibrinolysis. An ethanol-HCl extract of cherry cultivar Ferrovia (FCE) was used in the in vitro study. The influence on fibrin formation was investigated by assessing the ability of FCE to down-regulate the synthesis of tissue factor (TF, the physiological trigger of coagulation) in endotoxin-stimulated blood. The effect on fibrinolysis was studied by assessing the rate of lysis of blood clots exposed to a fixed concentration of a fibrinolytic agent (tissue plasminogen activator, 100 ng/ml). The synthesis of TF was inhibited by FCE in a concentration-dependent manner with more than 50% reduction of TF activity at 10 ug/ml catechin-equivalents (P=0.02). The identity of TF was confirmed by quenching experiments with specific monoclonal antibodies. Blood clots formed in the presence of FCE lysed significantly faster than control clots. The effect was concentration-dependent and clinically relevant, the rate of lysis being almost twice as fast in the presence of 10 ug/ml FCE (P=0.008). The profibrinolytic activity of FCE was maintained when clots were prepared from platelet-rich plasma but not cell-free plasma, suggesting a platelet-mediated mechanism. Moreover, the effect disappeared when platelets were treated with an antibody against GP IIb/IIIa. An FCE extract prepared under conditions simulating gastro-intestinal digestion displayed similar antithrombotic activities as the ethanol extract. A randomized-sequence, 2-period crossover pilot study was conducted in 10 volunteers. Blood samples were taken before and 2 h after a single meal of fresh cherries (300 g) or a glucose-equivalent amount of white bread. Cherry intake led to a 20% reduction of TF synthesis in whole blood (P=0.04) and to a 31% increase in the rate of clot lysis (P=0.02). On the contrary, bread intake was associated with a slight increase in TF synthesis and a reduction in fibrinolysis rate. These findings suggest that cherries may exert a clinically relevant antithrombotic activity by reducing the main trigger of thrombus formation (i.e. TF) and by rendering the thrombi more susceptible to endogenous fibrinolysis.

Chemical composition and functional characterization of commercial pumpkin seed oil

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Abstract
The commercial oil of the seeds of pumpkin (Cucurbita pepo L.) has been investigated. The oil is a common product in Slovenia, Hungary and Austria and is considered for its health promoting properties. Therefore, the pumpkin seed oil is considered a preventing agent for different pathologies, particularly for prostate diseases [1]. These properties are related to the carotenoids and liposoluble vitamins high content. The carotenoids (lutein and zeaxantine), vitamin E (α-tocopherol) and fatty acids content has been investigated. Furthermore, the composition of the volatile fraction due to roasting process has been investigated. The preliminary extraction of volatile compounds was obtained by dynamic headspace, while the analyses were performed by gas chromatography-mass spectrometry. The results of this study show that the aromatic profile obtained from the commercial analyzed samples is directly related to the intensity of the roasting process of the crushed pumpkin seeds: the temperature of the roasting process plays a crucial role on the concentration of volatiles substances, originated from Strecker degradation, lipid peroxidation and Maillard reaction. Therefore, high temperature roasting processes lead to the production of an oil with intense aromatic characteristics, while mild conditions, generally employed to obtain an oil with professed therapeutic characteristics, would lead to a product with minor characteristics pumpkin seed oil aroma. The nutraceutic properties of the product are confirmed by the high content in α-tocoferol and carotenoids: in particular, the content in lutein and zeaxanthin is one order of magnitude higher to the virgin olive oil.
Preliminary studies on the phenolic compounds evolution with the storage time of olive fruits via HPLC/MS

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Abstract
Recent studies have shown that the harvest time is a key parameter for the olive oil quality. In particular, the optimal harvesting time corresponds to the reaching of a minimum and constant value in sugar, with a consequent reduction of fermentative activity risk, coinciding with a maximum and constant value in oil, with consequent optimization of extraction yields. These are the optimal conditions of the olive fruit for the production of extra virgin olive oil (EVOO) without sensory defects (Cherubini et al 2009). In order to obtain EVOO with an high nutritional content, it is important to evaluate the phenolic content in the olive fruit and particularly the content of some molecules with proved health and sensory properties (Bendini et al 2007). To obtain EVOO that are as rich as possible in these molecules is necessary to work olives with a maximum content of these phenolic compound. For this purpose maturation curves have been built. (Giusti, 2011). The aim of this work was to understand the quantitative variation of the phenolic compounds in just picked and stored olives. Preliminary tests of storage of olive fruits from three different Tuscan cultivars (Frantoio, Moraiolo, Leccino) at different ripening stage were conducted. The phenolic compounds were extracted immediately after harvest (time 0), after 24h and after 48h. The data show an increase of the total phenolic content during the storage of the olives for all the cultivars in every ripening period, while every single phenolic compound shows a behavior which depends both from cultivar and from ripening stage. Moreover, it was possible to better understand the trend of the phenolic compounds of the different parts of the olive fruit.

Fingerprint of “special” edible oils using a widespread analytical protocol

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Abstract
Some flavoured olive oils (basil, lemon, chilli, rosemary, garlic, white and black truffle flavoured olive oils), and some oils with particular nutritional properties (e.g. linseed, rice, sunflower and hazelnut oils), were analyzed using an analytical protocol which involves conventional analyses (free acidity, peroxide value, UV spectrometry and fatty acid composition) and 1H and 13C NMR analyses at two different magnetic fields (600 and 400 MHz). In this way a widespread analytical protocol has been developed to characterize these oils in terms of quality and chemical composition. Result obtained from the different techniques allowed the metabolite profile, the quality of products on the market, the presence of possible treatments to stabilize the products, and specific markers for each type of oil to be determined. The analytical profile of metabolites gave also indications on similarities and / or differences in the oils of the same type but obtained from different brands and / or producers. It was possible to verify the production system and to study the variation of the metabolic profile during the storage time. The proposed analytical protocol (Mannina et al., 2010) can be particularly useful for the food industry to control reproducibility and quality of products by means of compositional chemical profiling (Comm. Reg. N° 1989/2003), that plays an essential role in ensuring their chemical consistency.
Act locally, think globally: Food specifications as a promotional genre for quality products and as a learning tool in English classes

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Abstract
Quality products, as acknowledged by in force EU legislation, and identified by labels such as PDO and PGI, are those food items linked to a geographical area and to peculiar production methods that preserve the manufacturing traditions and the culinary heritage of a country. This geographical specificity is typified by Product Specifications (FSs) that can be used as the identity card of such items. FS bear a promotional imprint and can serve to raise consumers’ interest and recognition of quality products. To this aim, a simplified FS model has been developed and applied to Sicilian red oranges. It has been targeted to a lay public of middle to high class status in terms of education and affluence interested in safe food issues. The template describes the geographical site where oranges are produced; the relationship with the environment; the main features (e.g. shape, pulp, size, colour); some nutritional characteristics; relevant information that can raise consumers’ interest, such as culinary traditions and literary evidence from British and American travellers from the 1880’s to 1990’s to facilitate product’s identification with the place of origin. In a globalised and competitive market, where many citrus fruits are currently available, the added value of this template is manifold. It makes product’s identification easer by raising interest in products from a specific area, thus facilitating informed choices among the different items available on shelf and by paying national efforts on certified growing practices back. It can be used as a promotional means in gourmet tourism by raising consumers’ curiosity, since it is written in international English and since it appeals an informed and well educated audience by means of the literary and geographical hints. It can be employed in English as a foreign language classes syllabi at university levels to develop language proficiency and communicative skills by the use of a technical vocabulary, thus fostering interdisciplinary education (e.g. foreign language, Food Science and literary studies) as required by the European Community.

Characterization of Sicilian ecotypes of eggplant (Solanum melongena L.)

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Abstract
The eggplant (Solanum melongena L.) fruits are very well known and consumed in various parts of the world and show the highest content of antioxidant molecules. The quantity and quality of these compounds is significantly influenced by cultivar, environment, type of soil and growing conditions.¹,² New cultivars of eggplant provide increasingly high production without taking into account the quality of the product. The local populations, although are not comparable to the F1 hybrids under the aspect of production, can be used as niche products in areas suited to horticulture, especially for their adaptability to the characteristics of low energy inputs. Hence the interest in development of ancient local populations that, if they are not adequately safeguarded and promoted, is likely to undergo a process of genetic erosion. In order to valorize the local populations, in the present research, we have characterized four ecotypes of Sicilian eggplant (Bianca, Marsala, Sciacca e Sicilia), ungrafted and grafted plants (on Solanum torvum). The agronomic results were correlated with the HPLC-MS analysis. From the agronomic point of view, grafting has a positive effect for the dry matter, marketable production, number of fruits. This aspect may be related to the increase of the content of phenylamide, known as promoters of the growth and development.³ Instead, the content in caffeoyl conjugates which represent almost all of the metabolites oxidizable, in grafted ecotypes decrease. Moreover, in all grafted ecotypes (except in Bianca) there is a significant increase of the content in flavonoids. We also have characterized the fruits of Solanum melongena L. both histologically and histochemically.
Aromatic profile of balsamic vinegar of Modena I.G.P.: Comparison between HS-SPME/GC-MS and sensorial analysis

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Abstract
Balsamic Vinegar of Modena (BVM) is a very appreciated typical Italian products and has recently obtained the “Protected Geographical Indication” (P.G.I.) denomination (Gazzetta Ufficiale Unione Europea 2009). It is produced starting from concentrated and cooked grape must submitted to alcoholic and acetic fermentation and added with wine vinegar (10% v/v minimum) and caramel (2% v/v maximum) (Gazzetta Ufficiale Unione Europea 2007). Vinegar is then submitted to a maturation period in wooden barrels that can vary depending on the desired final density: minimum 60 days for a “matured” vinegar and at least 3 years for an “aged” vinegar. Quality of a balsamic vinegar is based on several parameters such as colour, density and flavour. The aromatic fraction of a BVM is influenced by many factors such as raw materials, fermentation process, temperature of must cooking and type of wood utilized for ageing period (Pizzarro et al. 2008). Volatiles those mainly concur to BVM volatile fraction are ethyl acetate, 3-methyl-1-butanol and 3-methyl-1-butanol acetate, derived from fermentation process, furfural and 5-methylfurfural, formed during must cooking, and phenyl ethyl alcohol that derives from grape. It is possible to distinguish BVM of different ageing on the basis of different volatile profiles (Cirlini et al. 2011). This work was aimed to the determination of volatile profile of BVM samples of different ageing coming from a typical traditional Italian vinegar factory placed in Reggio Emilia province by HS-SPME/GC-MS technique. Moreover, the characteristic aromatic profile obtained by gas-chromatographic method was compared with sensorial analysis of the different vinegars, in order to better understand BVM flavour and to characterize products of a single vinegar factory.

Novel peptide based e-nose for the characterisation of food volatiles: Evaluation of olive oils samples

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Abstract
E-noses have been recognized in the recent literature as valuable tools for the quality assessment of olive oils (Cosio et al. 2006, Cimato et al. 2006, Cosio et al 2007). In this work a Quartz Crystal Microbalance based array of gas sensors (20 MHz) was realized by modification of the sensor surfaces with gold nanoparticles (GNPs) bearing short peptide moieties. The array consisted of 7 sensors modified with GNPs, GNP-cys, GNP-glutathione, GNP-γ-glul-cys, GNP-cys-gly, GNP-thioglycolic acid and GNP-Cys-Glu-His-Gly-Gly-Pro-Ser. The synthesized GNPs have been characterized using TEM and VIS spectroscopy. The peptides have been selected in order to characterize the structure/response relationship of the sensors. Preliminary data on model solutions demonstrated the ability of this array to discriminate among different solvents and different aromas in aqueous solutions. The e-nose was then challenged with 40 samples of olive oils coming from Marche and Abruzzo region. A Partial Least Squares calibration model was built in order to compare the sensory evaluation run according to EU N. 61/2011 regulation, e-nose data and gas-chromatographic analysis of the entire set of samples. A good correlation among the different methods was obtained demonstrating that the e-nose is able to qualitatively discriminate the samples. Considering the ease of synthesis and the wide range of peptides with different binding ability, this approach appears very promising for the development of a new generation of e-noses based on different sets of peptides.
Chemical indices of hazelnut roasting: quality and technological descriptors

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Abstract

The quality of food products is undoubtedly a challenging and complex issue; it refers, in a first instance, to the compliance with legal standards on health risks, environment, animal welfare and ethical requirements, but it is also related with the sensory impact due to the flavour, smell and appearance. Due to specific quality properties, some products achieve further added value thanks to their origin and manufacturing and/or to processing practices. EU encourages these productions and recognizes, through a series of quality labels, product peculiarity and specificity (i.e., Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Specialty Guaranteed (TSG)). Origin Labeled Products, as EU Regulation explicitly refers, should be different from standard products for their qualities or characteristics evoking the concept of “specificity”. The product is said to be differentiated if it has characteristics that are specific and measurable in the sense of substantial or intrinsic and, as a consequence, consumers perceive it as such (Bariolle 2002). Italian Corylus avellana L. cultivars received the EU quality labels: “Nocciola Romana” also named “Tonda Gentile Romana” from Viterbo - Lazio is PDO labeled (IT/PDO/51705/0573) while the “Nocciola del Piemonte” from Piedmont (IT/PGI/0217/0305) and “Nocciola di Giffoni” from Avellino province (IT/PGI/0117/1538) are both PGI products. These cultivars are recognized to be specifically suited for roasting, peeling and gauging, qualities that are of a great importance in the confectionary industry for the production of hazelnut paste (manteca), granulate, confectionary specialties or for a direct consumption of the whole nut. Moreover, the Turkey is the leading hazelnut producer, according with the last updated FAO report on world crop production (FAOStat), with the 65% (500,000 tons) of the world production while Italy, the second country in the ranking, covers the 14% (104,900 tons) of the production followed by US and Spain with the 6 and 4% respectively. In this context it is of great importance the possibility to distinguish raw or roasted hazelnuts of different variety/cultivar and geographical origin as well as to provide detailed information on chemical composition and sensory quality of food end and semi-finished products. Analytical methods for product quality assessment should give reliable and robust results and procedures should be developed and validated, in terms of performance, to be effective and informative when adopted for quality/origin qualification as for process monitoring. The present study reports the experimental results obtained by applying a validated HS-SPME-GC-MS method to monitor raw and roasted hazelnut volatiles. The method enables the qualitative and quantitative determination of key-analytes (markers) and allows to reveal technological and quality indicators to classify and qualify hazelnut samples of different geographical origin. In particular in this study an Italian cultivar from Piedmont, named Tonda Gentile Trilobata known also as “Nocciola del Piemonte” (EU Quality registration code IT/PGI/0217/0305) and a Turkish high quality blend from “Ordu” region consisting of three major cultivars: Tombul, Palaz and Kalinkara have been investigated. Roasting have been followed different time/temperature protocols in a lab-scale ventilated oven and in two industrial plants: a hot air dry roasting and an infrared ray roasting. Robust indicators of technological treatments and quality markers for aroma qualification and origin authentication are presented.
Resveratrol and pterostilbene: Protective effects toward induced DNA damage in HEPG2 cells

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Abstract
Polyhydroxylated stilbenes are naturally occurring phytochemicals found in grape and wine. Resveratrol has been the most studied because its presence in wine has been connected to a low incidence of coronary heart disease among people consuming moderately wine. Moreover resveratrol exhibits several beneficial health effects by its antioxidant, antiinflammatory, antiaging and chemopreventive properties. However this molecule has a very low systemic bioavailability, while pterostilbene, a natural dimethylated analog of resveratrol, is more lipophilic and so more permeable across biological membranes. It has been reported that also pterostilbene has many healthy properties. To test the ability of resveratrol and pterostilbene to protect HEPG2 cells from genotoxic effects, the phytochemicals were challenged with two different mutagens (i.e. 4-nitroquinoline-N-oxide and acrylamide). To gain insight into the mechanisms of action of resveratrol and pterostilbene, HEPG2 cultures were treated with the tested compounds during (co-exposure), before (pre-exposure), and after (post-exposure) treatment with the model mutagens. Capabilities of reducing the extent of induced DNA strand-breakage were evaluated by comet assay. Cell cultures were also evaluated for the induction of apoptosis. Up to the higher tested concentration (i.e. 250 µM) both resveratrol and pterostilbene were nongenotoxic. Preliminary data (i.e. co-exposure protocol) showed that these phytochemicals were able to reduce the extent of genotoxic effects induced by both model mutagens. In particular for resveratrol, we observed an antigenotoxic effect showing a typical U-shaped hormetic biphasic dose response relationships, with the intermediate dose (50 µM) shaving the higher effect.

Presence of Fusarium mycotoxins in organic cereal and organic cereal products by liquid chromatography coupled to tandem mass spectrometry

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Abstract
Organic agriculture has benefits with respect to food safety, however another risk for human health is due to Fusarium infection. Fusarium affect conventional as well organic cereal because of its possibility of producing several mycotoxin as trichotheccenes, beauvericin (BEA) and enniatins (EN). Trichothecene mycotoxins have been linked to human and animal diseases through the consumption of contaminated grains (e.g., wheat, oats, barley, maize and rice) whereas BEA and EN mediated cytotoxicity towards various mammalian and cancer cell lines has been demonstrated. The most common trichothecenes found in agricultural products include: nivalenol (NIV), deoxynivalenol (DON), fusarenon-X (FUS-X), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), neosolaniol (NEO), HT-2 and T-2 toxins. While the most studied ENs are ENs A, A1, B, B1 and B4. Currently in the EU the Scientific Committee on Food (SCF), tolerable daily intakes (TDIs) for DON, NIV and the sum of T-2 and HT-2 have been established at 1, 0.7 and 0.06 µg/kg body weight, respectively. TDIs for NIV and T-2/HT-2 were temporary established, because of gaps in the data available. Whereas, EC regulation has established maximum level only for DON, fixing it in the range of 750-1750 ng/g for cereal and cereal products food. The objective of this study was to evaluated the occurrence of the eight above mentioned trichotheccenes, five ENs and BEA in organic cereal food purchased in Campania region (Italy). So that, 93 organic cereal samples (wheat, barley, rye and oat) obtained from supermarkets of have been analyzed using liquid chromatography coupled to triple quadrupole mass spectrometry that was carried out with a method optimized in our laboratory. Limits of quantification (LQs) ranged from 5-50 µg/kg. 53% of analyzed samples contained trichotheccenes and the occurrence of DON, 3-AcDON, NIV and 15-AcDON were 28, 13, 2, 41, 1, 27 and 2 %, respectively. The higher levels obtained of each found mycotoxin were 100, 60, 18, 102, 4, 99 and 29 µg/kg, respectively, non exceeded the maximum limit established. The
average values were 14.39, 3.05, 0.27, 22.31, 0.05, 15.03 and 0.44 µg/kg for DON, HT-2, T-2, FUS-X, 3-AcDON, NIV and 15-AcDON, respectively. Whereas BEA, ENNA, ENNA1, ENNB, ENNB1 and ENNB4 in analyzed samples were below 52%. ENA1 was the most mycotoxin found and levels ranged from < LQ to 301.5 µg/kg.

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Evaluation of beauvericin and five enniatins in Italian cereal products and multicereal food by liquid chromatography coupled to triple quadrupole mass spectrometry

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Abstract
Beauvericin (BEA) and enniatins (ENs) are produced by different Fusarium species such as F. avenaceum, F. poae and F. tricinctum which can grow on many agricultural commodities (wheat) and processed food (pasta or baby food). BEA and ENs mediated cytotoxicity towards various mammalian and cancer cell lines has been demonstrated. Therefore, these mycotoxins may accumulate in the environment and enter the food chain, thus exerting possibly important effects on the health of humans and domestic animals. The most common ENs found in agricultural products include: ENs A, A1, B, and B1 and enniatin analogues (enniatins B2, B3, B4, D, E, F, and G) which have been identified and characterized. In this study, 47 multicereal foods including 25 pasta and 23 multicereal baby foods from supermarkets of Campania region (Italy) were analyzed for evaluating the presence of ENs A, A1, B, B1 and B4, and BEA. It was done using a method developed in our laboratory by liquid chromatography–mass spectrometry with multiple reaction monitoring. A liquid phase dispersion procedure was optimized for the simultaneous extraction of ENs and BEA. The main parameters affecting extraction yield and selectivity, such as extraction solvent were evaluated. The method was validated by analysis of multicereal food samples fortified at different concentration levels (0.5-20 µg·kg\(^{-1}\)). Average recoveries (n = 5) ranged from 67 to 90% with relative standard deviation lower than 14%. Spiked blank samples were used as standards to counteract the matrix effect observed in the chromatographic determination. Limits of quantification (LQs) ranged 1-10 µg·kg\(^{-1}\). Analytical results showed that the occurrence of BEA, ENA, ENA1, ENB, ENB1 and ENB4 in analyzed pasta and multicereal baby food samples were below 52 and 73 %, respectively. ENB was the most mycotoxin found and levels in pasta and baby food ranged from < LQ to 106 and < LQ to 1100 µg/kg, respectively. It was observed a high incidence and high contamination levels of these mycotoxins in multicereal baby food which it is a risk for this sensitive population.

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P-58

Interactive effects of vineyard covering sheet and leaf removal on anthocyanins content of table grape (SUMMER ROYAL, *Vitis vinifera*)

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Abstract
Anthocyanins are water-soluble pigments responsible for blue, purple, and red color of many fruits (Maxcheix et al. 1990). Because their high nutritional importance, within the last decades, many studies have focused on the potential biological activities or health effects of anthocyanins in humans (Bohm et al. 1998; Kong et al. 2003). Grapes, that are economically the most important fruit species in the world, constitute one of the major sources of anthocyan compounds among different fruits. In this research anthocyanins of Summer Royal (*Vitis vinifera*) table grape grown in Apulia region have been determined, from veraison to harvesting, by HPLC-DAD-MS analyses. Two different parameters have been exploited, a different degree of leaf removal and shading of vines with four covering sheets in LDPE (low density polyethylene), characterized by different light absorbing abilities. The principal component analysis (PCA) allowed differentiating the samples affected by the different leaf removal/shading effects; furthermore according to ANOVA analysis, grapes covered by yellow color sheet generally showed the highest level of anthocyanins. The acquired information are quite promising to propose the contemporary application to vineyard of leaf removal and covering sheet as useful tools to increase the levels of beneficial compounds in these grapes.

P-59

Effect of chromosome doubling on secondary metabolite profile of *Solanum bulbocastanum* genotypes

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Abstract
Chromosome doubling of diploid (2n=2x) plant species induces structural and/or functional genome changes that may result in either new or improved phenotypic traits. (Anouche et al.,2009). Among potato species, diploid *Solanum bulbocastanum* possesses several noteworthy traits (e.g., resistance to biotic stresses) that make it a precious genetic resource for potato breeding. In the frame of a breeding work aimed at exploiting this species, tetraploid (2n=4x) genotypes were obtained through oryzaline treatment and evaluated for several traits. Here we compare their secondary metabolite leaf content, evaluated through HPLC and LC-MS, with that of the diploid progenitor (Soltis et al., 2009). Alfa-chaconine was quantified and results showed that the diploid parent had a chaconine content, much higher than its tetraploid derivatives. Ten additional metabolites were identified (Shakya et al., 2006). The effect of chromosome doubling did not affect their content in a qualitative manner but in the amount of metabolites. Although these data should be confirmed by larger tetraploid/diploid combinations, they support the idea that there are not traits systematically associated to poliploidy and that genome multiplication per se may be an evolutionary advantage (Caruso et al., 2011).
Validation of an electrochemical method based on screen printed electrodes for Cd and Pb analysis in Cephalopoda

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Abstract
The presence of potentially toxic trace metals into the marine environment still remains a global problem. Heavy metals are considered to be the most dangerous, since continuous exposure of marine organisms to their low concentrations may result in bioaccumulation, and subsequent transfer to man through the food chain. The toxic risk of heavy metals in fish products has brought about a directive from the European Union 1881/2006 defining the maximum residues limits (MRL) of these contaminants in fish, mollusks and cephalopoda. In order to reduce the time of analysis and reduce instrumental costs it is mandatory to develop alternative analytical methods which require an easier sample treatment and a more convenient instrumentation. For these reasons an electrochemical method based on graphite screen printed electrode was developed and validated as a qualitative screening method according to 2002/657/CE decision. Schematically the measurement protocol consisted in an electrochemical conditioning of the electrode surface followed by the deposition, potential driven, of a mercury film on the electrode surface. Then the modified electrode was used to measure Cd and Pb according to an anodic stripping SWV protocol. A dedicated Pb and Cd extraction protocol was developed based on the modification of that published by Meucci et al. It consisted in sample homogenization, followed by HCl and H₂O₂ digestion, filtration and dilution. The obtained solution was used for stripping analysis. Calibration curves were obtained using blank samples fortified in the concentration range 0.01-0.25 ppm, recovery was also evaluated at 0.5, 1.0 and 1.5 mg/Kg. The CC₁₅, selectivity, specificity, analytes stability and robustness were evaluated both for Cd and Pb.

Validation of a one day analytical diagnostic real-time PCR for detection of Salmonella in meat

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Abstract
Food-borne diseases caused by Salmonella represent a worldwide public health problem. In Europe salmonellosis is still the second most commonly recorded zoonosis. Although various control measures have been adopted throughout the food chain, the microbiological testing of meat products, during production and processing, still plays a significant role in preventing food-borne infections. The standard cultural method for detecting Salmonella (ISO 6579:2002) requires up to 5 days to produce results and the need to develop rapid methods represents an important issue for the producers. Several methods, based on real-time PCR have been developed, but most of these assays are not applicable as a diagnostic tool because an internal amplification control is not included. In this context, the aim of this study was the development and in-house validation of an open-formula diagnostic real-time PCR for detection of Salmonella in meat products. The assay employed specifically primers and a probe target within the trtRSBCA locus, that allows the tetrathionate respiration in Salmonella. A competitive internal amplification control was also included in the assay to avoid false-negative results. The selectivity of the method was tested using 110 salmonella strains and 60 non-salmonella strains. After that 170 strains (110 target strains and 60 non-target strains), 180 various edible meat samples (chicken, bovine, pork) experimentally and not experimentally contaminated, were tested using both real-time PCR and standard cultural method. From the results obtained it was possible to evaluate the analytical performances of the real-time PCR : 100% for selectivity, specificity, sensitivity and accuracy. The overall analysis time of the PCR method was approximately 22 h (about 20 h of pre-enrichment and 2 h of real-time-PCR) in contrast to 5 days for the standard cultural method. In addition to reduce the total analysis time, two different pre-enrichment media are under investigation.
Validation of an optimized real-time PCR for the detection of pathogenic *Yersinia enterocolitica* in fresh and ready to eat vegetables

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**Abstract**

In Italy, the consumption of ready to eat (RTE) vegetables has shown a significant increase from 14,736 tonnes (€138,677 million) in 2002 to 50,723 tonnes (€397,599 million) in 2009. These products received some degree of minimal technological processing before commercial distribution. The presence of pathogenic micro-organisms in these products, mainly in leafy green vegetables, constitutes a potential problem for consumer health and is a public health issue. The presence of *Yersinia enterocolitica* is recently correlated with a suspected foodborne outbreak in Norway, caused by radicchio produced in Italy, and packaged in the United Kingdom. The reference method (ISO 10273: 2003) for the detection of pathogenic *Yersinia enterocolitica* in food is time consuming and inefficient. Therefore, we have in-house validated a new analytical method based on TaqMan probe real-time PCR for the detection of this pathogen in vegetables. Overall, the method consists of the following steps: an enrichment phase, a DNA extraction and a real-time PCR amplification. Firstly, the method was optimized using primers and probe capable of amplifying a specific sequence for the *ail* locus (adhesion invasion locus), highly conserved in all pathogenic bio-serotypes of *Yersinia enterocolitica*. In addition to avoid false negative results, an Internal Amplification Control was included. After that, the method was in-house validated following the guidelines of EN/ISO 16140. For this purpose, 122 strains (59 target strains and 63 non-target strains), 132 experimentally contaminated samples and 40 not experimentally contaminated samples, were tested using both real-time PCR and reference method. The analytical performances of the real-time PCR resulted to be 100% for selectivity, specificity, sensitivity and accuracy. The proposed method could play a key role for the control of *Yersinia enterocolitica* in vegetables, because it is able to provide the results within the shelf life of the products.

Co-pigmentation effects and its importance in understanding the origin of the color

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**Abstract**

Co-pigmentation is an important phenomenon that can affect the color in many foods. This phenomenon occurs when pigments and other colorless organic compounds or metallic ions form supramolecular complexes that adsorb in a different visible region. In wine chemistry, and more in general in food chemistry, these interactions are very important because color is one of the most significant factors in a product acceptance. In this communication we present our recent experimental (UV-Vis spectroscopy) and theoretical (Density Functional and Time-dependent Density functional theories) results on the geometrical and spectroscopic properties of a series of supramolecular complexes obtained by weak interactions between a series of anthocyanins and phenolic compounds. In particular, the binding energies, supramolecular geometries, UV-Vis spectra and their electronic origins will be reported and discussed.
Anthocyanin profile in red radicchio from Treviso (*Cichorium intybus*, L.) by LC-DAD-MS/MS

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Abstract
Anthocyanins are phenolic pigments responsible for the blue-red colour of vegetables and are regarded to possess beneficial effects on human health for their antioxidant, free radical scavenging, antimicrobial and anticarcinogenic properties. Red radicchio is characterized by the highest phenolic content among leafy vegetables, commonly consumed fresh. The higher antioxidant activity exerted by red-leaved varieties of chicory compared to the green-leaved is mainly due to the presence of anthocyanins (Innocenti et al., 2005). The present study aims at a qualitative and quantitative characterization of anthocyanins in the red radicchio from Treviso (*early* and *late* harvested) by liquid chromatography tandem mass spectrometry. Pigments extraction was carried out on freeze-dried samples by two solvent extraction mixtures in order to extract more and different anthocyanins: methanol acidified with chloridric acid 0.1%; methanol acidified with formic acid 5% (Castañeda-Ovando et al, 2009). The chromatographic separation was carry out on a C18 column (2.1x150 mm, 5µm) by gradient elution with a mobile phase composed of 2.5% formic acid in water (A) and 2.5% formic acid in acetonitrile (B) at a flow rate of 0.2 mL min⁻¹. The spectrophotometric detection was performed with a DAD detector and the UV/Vis spectra were collected between 190 and 650 nm, monitoring the absorption at 510 nm. The LC-DAD apparatus was connected online to a QQQ instrument interfaced with ESI source operating in positive mode. The extracts were analyzed by LC-DAD-MS in scan, product and precursor ion acquisition modes in order to identify the molecular structures. At least ten different anthocyanins were identified. Cyanidin-3-O-malonylglucosyde and delphinidin-3-O-malonylglucosyde were found as the most abundant compounds in formic acid extract, whereas cyanidin-3-O-succinylglucosyde was the predominant anthocyanin in chloridric acid extract. Cyanidin-3-O-glucosyde was found in both extracts and all the identified anthocyanins were quantified as cyanidin-3-O-glucosyde equivalents.

Statistical analysis of mineral content in *Cerastoderma edule glaucum* and *Venerupis aurea laeta* from Ganzirri lake

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Abstract
Our study was carried out on two species of clams “*Venerupis aurea laeta*” and “*Cerastoderma edule glaucum*” from Ganzirri Lake considered as “environmental biomarkers” for their changes in the physiology, morphology or distribution under the influence of substances in the environment. The aim of the present study was to conduct a statistical analysis in *Venerupis* and *Cerastoderma* to investigate the difference between the two autochthonous clams according to the presence of mineral contents and to link mineral concentrations to the reproductive cycle of clam during 2009-2010. Metal analysis was carried out by ICP-MS. The multivariate analysis was made using the SPSS 13.0 software package for Windows (SPSS Inc., Chicago, IL). In this study the concentrations of some metals found in clams tissue showed seasonal cycles with higher values in summer than in winter (Mubiana et al., 2005; Saha et al., 2006). The significances of mineral concentrations differences between *Venerupis* and *Cerastoderma* samples were estimated by Mann-Whitney U test. The concentrations of Ag, As, Cd, Mn, Se and Zn show a significant p-level that suggests a difference between the two group samples. Statistical analysis showed that the link of metal concentrations to the reproductive cycles of *Venerupis* and *Cerastoderma* were not evident.
Study on Private label UHT creams

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Abstract
Private label products are widespread in the Italian market because of their cost lower than the of regional, national or international brands. One of the aims of a Italian project "BIOVITA" financially supported by the Italian Ministry of Agricultural, Food and Forestry Policies (MAFFP), is the study of the quality of Private label dairy products compared with other products differently labelled. In this study UHT creams (with fat $\geq 20\%$) were analysed: in particular 7 different Private label of UHT cream ("PL") and 7 different brands of UHT cream ("B"). Proximate composition (AOAC, 1995), vitamins A, E and cholesterol (Panfili et al., 1994), the contents of the main minerals (AOAC, 2002) (calcium, phosphorus, sodium, potassium) were determined in all the UHT cream samples. Moreover the DRI index (Degree of Retinol Isomerization) an index of process and storage severity (Panfili et al., 1998) and the DAP index (Degree of Antioxidant Protection), a parameter used to estimate the potential oxidative stability of fat in foods (Pizzoferatto et al., 2007), were calculated. PL cream samples and B cream samples were not statistically different in the contents of fat, ash, cholesterol, vitamin E and DAP index. There were some differences in vitamin A: trans retinol was different (P<0.05) in these two categories (316.8µg/100g and 262.9µg/100g respectively in PL and B samples). The DRI index was different between the two groups (P<0.05). This index, often used to highlight the effect of heat treatment, is greater in the brand UHT cream samples than in the Private label UHT cream samples. UHT treatment is not a "mild" technology and for this reason the possibility of development of cis isomers is very likely to take place. Moreover, there are no rigid rules describing time and temperature of the process applied to UHT cream, and consequently the samples showed a wide range of variability of this index.

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Different feeding strategies for fattening phase in Nero Siciliano pigs: fat quality and carcass traits

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Abstract
The Nero Siciliano pig is a native breed from Sicily, an island in the south of Italy, traditionally fattened with acorns and grass in an outdoor free-range production system [1]. Meat and meat products from Nero Siciliano pigs fed under extensive conditions reach the highest prices in the market compared to pigs fed in confinement with a concentrate diet. In 2011 Sicilian producers applied for Protected Designation Origin (PDO) certification of Nero Siciliano ham including in the production protocol as pigs reared under extensive conditions as pigs reared in confinement. The main objective of this study was to assess the effect of feeding system during the Nero Siciliano pig finishing period on production results, carcass quality and fatty acid composition of subcutaneous backfat (outer and inner layers). Forty Nero Siciliano pigs, 20 males and 20 females, at an average age of 180 days and with an average initial weight of 29.6 ± 3.75 kg were employed. During the growing period, pigs were kept under confinement and fed the same concentrate diet. At the beginning of the fattening period pigs were divided into two groups with a different feeding system: a group was fed under outdoor free-range conditions with acorns and grass fully available for 60 days (CDO: Concentrate Diet Outdoor group), the other group was fed in confinement with a concentrate diet for the same period (CDI: Concentrate Diet Indoor group). The CDI group had a higher carcass weight and yield than the other group of pigs, while the CDO group had lower backfat thickness and higher percentages of lean cuts than CDI. The fatty acid composition of subcutaneous backfat outer and inner layer from CDO pigs, showed a lower concentration of polyunsaturated fatty acids (PUFA) and a higher content of monounsaturated fatty acids (MUFA), C16:0 and C18:0. These characteristics would produce dry products with higher sensorial characteristics from the meat of pigs fed acorn and grass in comparison with those fed a concentrate diet.
γ-Irradiation as a methods for decontaminating food: reduction/elimination of mycotoxins in raw unpeeled almond kernels (*Prunus dulcis*)

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**Abstract**

Annually over 4,000,000 ton of dried fruits and nuts are commercially produced throughout the world and the main cause of post-harvest losses is the proliferation of larvae and insects, fungal growth and toxic metabolites formation in stored products. *Aspergillus* is the predominant species responsible for fungal contamination and subsequent production of mycotoxins in the field, at harvest, during post-harvest operations, and storage of nuts. Due to relatively high contamination risk of nuts, decontamination methods are of great interest from economic, public health, and environmental aspects. The effect of gamma irradiation process (a method of food preservation physical and non thermal) on the sensitivity of fungi and mycotoxins has been well established for various foodstuffs. The γ-irradiation of foodstuffs up to an overall dose of 10 kGy is permitted in numerous countries for commercial food processing. According to the literature the lowest dose needed to control insects is about 0.5 kGy. As for the control of mold growth it has been shown that gamma irradiation at dose levels above 5 kGy is effective in reducing the mold population on the surface of dried fruits and nuts. The aim of this study was to evaluate the applicability of the ionizing radiation treatment and its effects on mycotoxins content raw unpeeled almond kernels as a function of irradiation dose in order to determine dose levels that causing sensible reduction. The reduction/elimination of aflatoxins B₁, B₂, G₁, G₂ and ochratoxin A was evaluated on artificially contaminated almond samples up to an overall dose of 15 kGy. The results obtained showed that aflatoxin G₁, B₁ and ochratoxin A were more sensitive to ionizing radiation than the G₂ and B₂ and that the degradation is greater with increasing radiation dose. In particular, ionizing radiation treatment at the maximum dose of 15 kGy resulted in a significant reduction of the content of aflatoxins G₁, B₁ and ochratoxin A in almond samples.

Alimentary and nutraceutical profile of Italian onion (*Allium cepa l.*) organic-grown genotypes

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**Abstract**

This study, within the project “Valorbio”, deals with the evaluation of four onion genotypes named: i) “Suasa” and ii) “Precoce di Romagna”, landraces typical from Marche and Emilia Romagna region, respectively; iii)”Tropea”, a commercial cv.; iv)”Density”, a commercial hybrid. All genotypes have been cultivated in 2011 season on 11 years organic-converted soils, in the experimental field of CRA-ORA. All samples, harvested at commercial ripening, have been subjected to some quality aspects as follows. Alimentary quality: dry matter, soluble sugars, unsaturated and saturated sulphur volatile compounds. Nutraceutical quality: unsaturated sulphur volatile compounds (USVC), ascorbic acid, quercetins, anthocyanins, polyphenols and antiradical capacity. The attention was particularly focused on USVC (mainly E together with saturated ones, by enzymatic cleavage of precursors, because of their anti-carcinogenic effects (Galeone et al., 2006). Moreover, quercetins are noted for their antioxidant capabilities among many polyphenols compounds (Rice-Evans et al., 1996). The saturated sulphides content ranged from 1.28-1.35 mg/100g dm for all genotypes, with the exception of Suasa (0.94 mg/100g dm). The USVC content ranged from 3.18-3.71 mg/100g dm, repeating a lower amount for Suasa (1.92 mg/100g dm). Quercetins, highly correlated with antiradical activity (rxy=0.92), revealed high content in Density and Tropea (43.3 and 30.6 mg/100g fw), and low amount in Precoce di Romagna and Suasa (13.1 and 11.4 mg/100g fw). The potential anti-tumor activity, given by USVC resulted better in all genotypes than Suasa, with a contemporary high antioxidant potential in Density and Tropea.
**Abstract**

Varietal and developmental differences in three Montepulciano rootstocks, grown in the same water regimen, were investigated by analysis of protein composition of grape berries. Two dimensional gel electrophoresis, image analysis and mass spectrometry analysis as proteomic approach to protein characterization of grape berries of Montepulciano rootstocks. The results demonstrated that different protein expression can occur as cultivars and viticulture conditions change. Montepulciano red wine grapes were examined to study the variation in protein expression of berries with different rootstock (Kober, 1103 and 140). The grape vine berries were harvested at time of fully ripe (September 2009). Proteins from grapevine ripe berries were extracted with trichloroacetic acid-acetone and fractionated by two dimensional gel electrophoresis. Images of two dimensional maps were acquired and processed to evaluate differences in spot number and their intensities as percentage of volume. The spot after trypic digestion are identified with LC-ESI-Q-TOF (MS/MS analysis). The rootstock is grown under same condition, without water deficit stress and in well watered conditions. The two dimensional maps of berries from rootstock 1103 showed the most number of spots, 130, while the less number of spots were from berries of rootstock 140 were 98; rootstock Kober showed intermediate values (120). In particular, the major expression for rootstock 140 and 1103 was detected at medium molecular weight, from 37 to 20 kDa, and the lowest was detected at the lower molecular weight. It is note of worthy that the percentage volume is higher in rootstock Kober, we can infer that the rootstock strongly influence protein expression. These results were better represented by the density spot (d3: spot number/total volume of spots). In fact, d3 of Kober was 2.87x10^{3}, 4.82x10^{3} for 1103 and 4.53x10^{3} for 140. In conclusion, these results provided data that demonstrated strong influence of rootstock on protein expression. As matter of fact 1103 expressed a major number of proteins but quantitatively lower than Kober that expressed protein at the highest quantitative content. The rootstock 104 expressed a minor number of proteins in a lower percentage volume resulting similar to rootstock in terms of density. Finally, some proteins were identified. **Word key:** Montepulciano, proteins, different rootstock, proteomic

**P-71**

**Resveratrol inhibits TLR2 activation on corneal epithelial cells: A confocal laser scanning microscopic study**

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**Abstract**

TLRs (Toll-like receptors), widely expressed by the ocular surface, are able to recognize microbial pathogens and to trigger the earliest immune response leading to inflammation (1). This ex vivo study was designed to confirm the effectiveness of resveratrol to inactivate the TLR2 induced by *Staphylococcus aureus* on corneal epithelial cells by confocal laser scanning microscopy (CLSM). Rabbit corneas were placed in air-interface organ culture (2). The epithelial wound was created with a sterile cutter in the centre of the corneas. The wounded corneas were divided in two groups exposed to different concentrations of UV-inactivated *S. aureus* ATCC 6538P (5x10^3 CFU/ml and 5x10^8 CFU/ml) and one control group (wounded corneas). Organ cultures were incubated at 37°C in a humidified 6% CO2 incubator for 48 h. The groups treated with inactivated bacteria were treated with 100 μM of trans-resveratrol and incubated for another 2 h. The tissues, fixed and dehydrated, were embedded in paraffin. Primary antibody employed was anti-rabbit TLR2 followed by Alexa Fluor 594 donkey anti-rabbit IgG. The TLR2 distribution was determined by CLSM (3-4). Previously we had shown the effectiveness of resveratrol in inhibiting the activation of the TLR2, induced by *S. aureus*, on the surface of epithelial cells of cornea by immunoperoxidase techniques (5). CLSM has confirmed the presence of the activated receptor by *S. aureus* on epithelial cells and its inhibition after treatment with the resveratrol. The two techniques applied are very effective and complementary in evaluating the influence of resveratrol in inhibiting TLR2 on epithelial cells involved in corneal inflammation induced by *S. aureus*. These results confirm that resveratrol could potentially have anti-inflammatory properties against *S. aureus* corneal infection by repressing TLR2-mediated recognition.
Antioxidant properties of experimental pastas made with different wholegrain cereals

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Abstract
Pasta is a cereal food consumed worldwide and in some countries, like Italy, it is a staple food. Traditional pasta is made with durum wheat semolina only. However, pasta manufactured with different cereals has become available on the market and its consumption is rapidly increasing. In this work, new mixed cereal grains pastas produced in a pilot plant with a high percentage (up to 60%) of whole grain flours of different cereals (wheat, oat, rye, barley and rice) were studied. Pastas were analyzed both in the raw and in the cooked state, ready for consumption. For each product, aqueous-organic extracts and their residues were studied. Their antioxidant properties were evaluated by FRAP (Ferric Reducing Antioxidant Power) assay and total phenols (PFT) were determined by the Folin Ciocalteau method. For cooked experimental mixed cereal grains pastas, FRAP values ranged from 3.26±0.08 µmol/g d.w. to 19.52±1.28 µmol/g d.w. in aqueous-organic extracts and from 19.70±3.11 µmol/g d.w. to 96.61±5.57 µmol/g d.w. in residues. In both raw and cooked products the lowest FRAP values were found for semolina/60% whole rice pasta. The barley pasta represents a good source of total phenols and this result was matched by the FRAP one.

Validation of a method for detection and quantification of polycyclic aromatic hydrocarbons (PAHS) in food

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Abstract
The polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds with two or more aromatic rings, usually found in the form of complex mixtures. These persistent contaminants, formed by the incomplete combustion of many substances, including coal, gas, tobacco and garbage, generally show mutagenic and carcinogenic effects. Human and industrial activities such as the processing of coal and crude oil, the combustion of natural gas, and vehicle traffic, disperse PAHs into the environment, thus, according to their chemical and physical properties, they may migrate through the food chain so, among the many routes of human exposure, food intake is the major contributor. The European Food Safety Authority (EFSA) in 2008 adopted an opinion on PAHs in Food where benzo[a]pyrene is not considered a suitable marker for the occurrence of PAHs in food and a system of four specific substances should be chosen as the most suitable markers in food. As a consequence, Regulation EU 835/2011 amended Regulation EC 1881/2006 in order to set maximum levels for the sum of four PAHs (benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene). For this reason, the Regulation EC 333/2007 laying down analytical performance criteria only for benzo[a]pyrene has been amended by Regulation EU 836/2011 that lays down analytical performance criteria for the other three PAHs. Therefore, in order to ensure food safety, it is necessary to develop a method for determination of PAHs in food, that is the aim of this work. The method, using HPLC with fluorescence detection with a specialized PAH column, was optimized and validated in agreement with Regulation EU 836/2011. The analytes were separated on-column with a gradient of acetonitrile in water and detected using appropriate excitation and emission wavelengths. In the validation of the method the limit of quantification was fixed at 0.5 µg/kg. For all substances good linearity was observed. Mean recoveries for the six PAHs were in the range 60-120% according to the EU Regulation and precision, as repeatability, showed relative standard deviations lower than 20%. Measurement uncertainty was calculated by identifying and quantifying the uncertainty components of the analytical process; the relative expanded uncertainties were calculated for each PAH. The method developed can be considered for the quantitative confirmation, using, as a further confirmatory test, the co-cromatography approach according to Decision EC/2002/657.
Determination of ochratoxin A in chili pepper (*Capsicum spp.*): A suitable method for an official laboratory

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Abstract

The chili pepper (*Capsicum spp.*) is one of the most consumed spices in the world, used not only as a food and in food ingredient but also for its nutraceutical activities (topical analgesic, tonic, antiseptic, carminative). However, the *Capsicum* products are among the foodstuffs for which the EU has issued a series of laws aimed at the consumer safety. Among the risks linked to consumption of red chili pepper, the contamination by mycotoxins, ochratoxin A (OTA) in particular, is one of the most important. Ochratoxin is a by-product of metabolism in *Penicillium* and *Aspergillus spp.*, showing nephrotoxic, genotoxic, teratogenic and immunosuppressive effects and it was classified from the IARC as a possible carcinogenic to humans (Group 2B). On the basis of the opinion relating to OTA in food (EFSA, 2006) and for the protection of public health the European Commission in the Regulation EU 152/2010 fixed maximum levels for some spices (*Capsicum* spp., *Piper* spp., *Myristica fragrans*, *Zingiber officinale*, *Curcuma longa*) that can show very high levels. Due to the maximum limits as well as for the real risk of high intake it was therefore necessary to carry out an extensive monitoring of *Capsicum* both in raw materials and in transformed products, fit for human consumption (dried fruits, whole or ground, chili, cayenne and paprika).

The risk of contamination by OTA also stems from the fact that, once collected, the *Capsicum* is subjected to a procedure for its subsequent drying and grinding. The techniques of processing and storage can lead to the development of fungal strains producing mycotoxins and therefore contamination of the product with great risk to the health of consumers. In the present study, a fast and sensitive method for the quantification of OTA in chili products has been developed. The sample preparation procedure (extraction into acetonitrile/water followed by a simplified single-step clean-up on an immunoaffinity column and analysis by HPLC coupled with a fluorescence detector) has been optimized and tested for several validation parameters (LOD, LOQ, recovery, precision). The procedure has been then applied to samples collected from the market. The ochratoxin A has been occasionally found in some products and some differences were observed between crushed and ground products.

Hydrolytic and lytic activity of hen egg white lysozyme in model wine

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Abstract

Lysozyme, a muramidase from hen egg white, has been widely used in winemaking to prevent microbial spoilage by gram positive bacteria, to control malolactic fermentation (MLF) and to reduce sulphur dioxide addition after MLF. Catalytic activity of an oenological lysozyme preparation (Fordras SA, Svizzera) was investigated in model wine (tartaric buffer, pH 3.2) toward *Oenococcus oeni*, as microbial substrate for lytic activity, and toward glycol chitosan, a peptidoglycan-like amino polysaccharide, as synthetic substrate for hydrolytic activity. Kinetic study was carried out determining the maximum initial reaction velocity (*V*ₘₐₓ) and the kinetic parameter *K*ₘ according to Michaelis-Menten equation by a nonlinear regression procedure (GraphPad Prism 5.0, GraphPad software, Inc.). The saturation curves obtained for microbial substrate showed that, increasing the enzyme concentration (from 300 mg l⁻¹ to 3000 mg l⁻¹), *V*ₘₐₓ and *K*ₘ values decreased (from 15.30 to 0.80 I.U. mg⁻¹ lysozyme and from 70.48 to 9.53 mg *O. oeni*, respectively), this as expression of excess-substrate inhibition. The same inhibition mechanism was revealed for muramidase hydrolytic activity, with *V*ₘₐₓ and *K*ₘ decrease (from 1.29 to 0.92 I.U. mg⁻¹ lysozyme and from 0.034 to 0.004 mgglycol chitosan, respectively), raising lysozyme concentration (from 300 mg l⁻¹ to 3000 mg l⁻¹). Finally, in view of a more effective and sustainable application of lysozyme in winemaking it is worth noting that, the lytic activity toward *O. oeni* retained the 50% of the maximum value at the average minimum pH of wine (3.2) and it retained about the 20% at 20°C (standard wine storage condition) with respect to the optimal temperature (70°C).
Development and validation of method for determination of (E)-resveratrol and related phenolic compounds in wine

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Abstract
Resveratrol (3,4',5-trihydroxystilbene) has been identified as one of the major active compound of the stilbene phytoalexins. At first isolated from the roots of white hellebore and later from Polygonum cuspidatum. Up to now resveratrol was found to be present in grapes, wine, peanuts and peanut products. Resveratrol has been associated with several beneficial health effects such as antioxidative, anti-inflammatory and estrogenic effects as well as anticancer and chemopreventive activities. Epidemiological studies showed an inverse correlation between the consumption of red wine and the incidence of cardiovascular disease increasing the interest in monitoring the presence of resveratrol in wine. The aim of this study was the development and validation of an analytical method based on Molecularly imprinted solid phase extraction (MISPE) and high performance liquid chromatography coupled to UV and MS detector for determination of (E)-Resveratrol and related phenolic compounds in wine samples. For this purpose (E)-Resveratrol was used as the template molecule for the preparation of molecularly imprinted polymer (MIP) stationary phase in a self-assembly (non covalent) approach with the functional monomer 4-vinylpyridine (4-VP) in a 1:3 molar ratio. Polymerization in the presence of a cross-linker resulted in rigid block copolymers with selective capacities towards (E)-Resveratrol when compared to the non-imprinted polymer (NIP). In order to evaluate the efficiency of the MIP as a sorbent, recovery experiments of (E)-Resveratrol and related compounds were conducted from water and spiked wine samples. Aqueous samples were passed through a SPE cartridge packed with 30 mg of either MIP or NIP phases. After sample passage, cartridges were washed with acetonitrile:ethanol (5:1) to remove unbound sample components and finally target analyte were eluted with methanol. Washing and elution fractions were analyzed by HPLC and UV detector set to the absorbance wavelengths of 306 nm. In such conditions quantitative recovery (101.5 % ± 6) were obtained using MISPE cartridges (less than 1% using NIP).

Comparative analyses of oil and biactive compounds from seeds of wild fruits of Prunus and Sambucus species from Southern Italy

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Abstract
Small berries are rich sources of bioactive compounds such as flavonoids, phenolic acids and vitamin C, which display potential health-promoting effects (Tabart, 2006). Blackberry is an edible fruit produced by several species in the Rubus genus of the Rosaceae family. Elderberry is a widespread specie of the Sambucus genus of the Caprifoliaceae family, growing on sunlight-exposed locations in several parts of Europe, Asia, North Africa and the USA. The berry seed materials, removed as waste during the processing of berries, may contain health-beneficial compounds. In this study, we have compared the composition of berry seed oils blackberries (Rubus ulmifolius Schott) and elderberries (Sambucus Nigra L.) growing wild in Calabria (Southern Italy) and characterized some of their potentially health-beneficial compounds. The fruits were picked randomly from different parts of unmanaged wild bushes on mountain slopes at an altitude of 1000 m above sea level (C.da Pallone, Cosenza). The freeze-dried fruits were crushed in a mortar and then sieved using a 60 mesh screen to achieve the separation of seeds from the pulp. Seeds were ground and dried in an oven at T = 110 °C until constant weight (t = 2 h). Total lipids were extracted from ground seeds (5 g) with hexane at 90 °C for 2 h. Elderberry seeds had a higher oil content (22% yield w/w) than blackberry seeds (8% yield w/w) on a dry weight basis. The fatty acid composition was determined by GLC after a direct transesterification procedure carried out in methanol-benzene with acetyl chloride; in both berry seed oils the most represented fatty acids were linoleic acid (51.5% for elderberry and 70.4% for blackberry) and linolenic acid (40.0% for elderberry and 19.2% for blackberry). The radical scavenging activity, determined using the DPPH test, of the methanolic extract of defatted seed flour was higher for blackberries (97.0%) than elderberries (82.4%). The total phenolic content of the methanolic extracts was found to be in correlation with the radical scavenging activity of same extracts. Ellagitannins and free ellagic acid were the main phenolics detected in blackberry seed extract.
Tandem mass spectrometry characterization of high molecular weight glutenin subunits in wheat cultivar: Potential application to wheat breeding

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Abstract
Gluten proteins are responsible for the functional characteristics of wheat flour. They are conventionally grouped into monomeric gliadins and polymeric glutenins. Glutenins are composed of low molecular weight glutenin subunits (LMW-GS) (20–45 kDa) and high molecular weight glutenin subunits (HMW-GS) (70–90 kDa), linked through inter-chain disulfide bonds. A series of investigations showed that HMW-GS are closely associated with bread-making quality of wheat flour, especially dough viscosity-elasticity. Furthermore, allelic variations of HMW-GS contributed to different processing properties. For example, the subunit pair 1Dx2+1Dy12 were confirmed to have negative effects on bread-making quality whereas 1Dx5+1Dy10 were considered to be superior quality subunits. Consequently, the considerable interest in understanding the influence of these subunits on flour quality has provided a stimulus for determining structure–function relationships of wheat glutenins. The analysis of HMW-GS is hindered by several difficulties because of the complexity resulting from the presence of sequence repeating motifs. This last event produces a number of protein isoforms differing in length only for a few amino acid residues. Mass spectrometry techniques have in part contributed to the assessment of HMW-GS heterogeneity. In particular, MALDI-MS of purified HMW-GS yielded molecular mass determinations consistent with their cDNA derived amino acid sequences. The limit of this approach is that the experimental value falls within the experimental error of those expected from the glutenin gene sequence, leading to imprecise identification of the protein classes. In the present study we report the analysis of glutenin extracted from several Italian durum wheat cultivars. RP-HPLC separation of each sample reduced the protein profile complexity. Purified subunits were then digested with trypsin and the resulting peptides analyzed by LC-ESI-Q/TOF MS/MS. Data processing with Protein Prospector search engine allowed coverage of up to 98% of sequence, leading to an unambiguous identification of each HMW-GS. This strategy can be used for rapid identification and screening of desirable HMW-GS associated with superior quality in wheat breeding programs.

Yeast strains and production of odorous thiols in Sauvignon blanc wine

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Abstract
Odorous volatile thiols have been identified as impacting compounds responsible for the “tropical fruity” Sauvignon Blanc wines character. Volatile thiols are generated during alcoholic fermentation by carbon–sulphur β-lyase enzymes acting on the cysteinylated and glutathionylated precursors present in grape. There also seems to be no direct correlation between precursor concentration in grape and the amount of free thiols found in the resulting wines regardless of the Vitis vinifera variety. Since different yeasts have significant variation in the ability to convert precursors and release volatile thiols, the main objective of this study was to investigate ten commercial wine yeast strains modulation effects on the quantitative levels of fruity volatile thiols and of their precursors. The production of 3-mercapto-hexanol (3MH), 3-mercapto-hexylacetate (3MHA) and 4-mercapto-4-methylpentan-2-ol (4MMPOH) has been evaluated in Sauvignon Blanc wines at the end of fermentation and after fining in bottle. Headspace solid-phase micro-extraction (SPE) coupled with gas-chromatography mass spectrometry (HS-SPME-GC-MS) allowed quantification of 3MH, 3MHA and 4MMPOH volatile aroma compounds. The cysteinylated and glutathionylated precursors were extracted by SPE and analyzed by LC/ESI-MS/MS. The results suggest that the yeast strains affect significantly the amount of volatile thiols released in wine and some of them enhanced the thiols volatile production. The quantitative levels of 3MH, 3MHA and 4MMPOH released compared to the respective odor theresholds, allowed the identification of strains with an high performance on desirable thiol aroma compound sensory perception in wines.
The role of peach volatile compounds in relation to the impact of the Mediterranean fruit fly (medfly) *Ceratitis capitata*

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Abstract

The Mediterranean fruit fly (medfly) *Ceratitis capitata* is one of the most destructive agricultural pests nowadays causing enormous economic losses in fruit crops of many regions worldwide. Understanding the interaction mechanism fruit-insect is crucial for developing control strategies for such biological pests (Pitts, R. J. and Zwiebel, L. J., 2001). In this study, the relation between the fruits' tolerance level of different peach cultivars toward the medfly and the volatile composition of ripe fruits of each cultivar has been investigated. Volatile compounds were analyzed by SPME-GC-MS in three cultivars highly attacked by the medfly (Fair time, Flaminia, Sicilia piatta), and in two cultivars little attacked (Percoca romagnola and Doctor Davis). From the factor and multivariate analysis on the main classes of compounds, a different volatile profile has been found for the different cultivars, with the most tolerant cultivar (Percoca romagnola) being clearly different from impacted cultivars. The main differences concerned the composition and relative abundance of esters. The most tolerant cultivars, above all Percoca romagnola, contained higher amounts of hexenyl, hexyl, isoamyl, butyl and isobutyl esters and a lower relative content of methyl esters, some of which are known to act as specific pheromones of the medfly (Warthen, J.D. et al., 1997; Wicker-Toms, C., 2007). Doctor Davis contained lower levels of aliphatic hydrocarbons. These results let hypothesize a role of some volatile compounds in the interaction fruit-insect and in the selection mechanism of the host.

Characterisation of pig fat by means of FT-NIR spectroscopy and VIS/NIR imaging

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Abstract

An increase in the degree of unsaturation of lipids, although being appropriate by the nutritional point of view, may create serious problems caused by oxidative phenomena in products like cured ham, which requires a maturing period of at least 12 months. During this period, thanks to the lipolysis and favourable oxidizing phenomena that occur in ham lipids, a number of volatile compounds are formed, which play an important role in the development of the sensory characteristics of the product. Due to greater susceptibility to oxidative degeneration, an excessive amount of unsaturated fatty acids, in particular of linoleic acid, can lead to rancidity which, in turn, is reflected into flavour-, colour- and texture-related defects in the final product, (Lo Fiego et al., 2005). Aiming at the prediction of unsaturation and peroxidation values of pig fat by means of fast and non-destructive methods, such as FT-NIR spectroscopy and VIS/NIR hyperspectral imaging, we present here a preliminary investigation, done in order to check the suitability of these techniques for the characterisation of swine adipose tissue. Two-hundred and five fat samples were cut to separate the external layer (close to the rind) from the internal one, since the fatty acids composition is different between the two layers. Then, the samples were repeatedly measured both by integrating sphere and fiber optic probe using a Bruker Optic MPA FT-NIR spectrophotometer. The spectra acquired on external and internal layers were discriminated by using Partial Least Squares - Discriminant Analysis (PLS-DA) and considering a wide number of different options for signal pretreatment. Classification results showed the possibility to recognize fatty samples belonging to the two different classes with a Classification Efficiency equal to about 90%, which demonstrates that NIR spectroscopy is suitable to characterise the lipid fraction of swine meat. To gain a better understanding of NIR results, some of the samples were analysed by VIS/NIR hyperspectral imaging: in this way, it is possible to have a visual representation of the variation of the chemical composition of fat tissue directly on the sample surface.
Detection of red skin defect on raw hams by classification and reconstruction of RGB images

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Abstract
The assessment of qualitative characteristics of fresh pig thighs to be used for seasoning is becoming a key aspect in the production of PDO ham. The quality of fresh pig thighs is preliminarily evaluated by expert assessors on the basis of the presence/absence and of the extent of defects. Notwithstanding the experience of the assessors, the human visual evaluation is often affected by subjectivity, (i.e., the evaluation is operator dependent and not easily transferable) and by other drawbacks, like e.g. the possible inconsistency of the human eye or the need for skilled personnel. In this context, the development of automated systems able to collect and process data related to visual aspect of the analysed samples is an effective solution to gain objective, reproducible and transferable information about product appearance. In this work, in order to quantitatively estimate the degree of objectivity of human visual evaluation for the detection of the so-called red skin defect, 198 digital images were acquired on a series of raw ham samples and then were subjected to the classification in three quality categories by a panel of expert assessors (Lo Fiego et al., 2009). The results obtained by assessors evaluation allowed to identify 95 samples whose class assignation is reasonably indubitable. The RGB images of these samples were converted into one-dimensional signals, namely colourgrams (Antonelli et al., 2004), which bear all the colour-related information of the original images, and were then used to build and validate multivariate classification models. PLS-DA and the feature selection/classification algorithm WPTER (Cocchi et al., 2003) were used as classification methods. Finally, the selected features were used to reconstruct the RGB images highlighting the regions of ham surface where the red skin defect is mainly located.

Nitrate in the Italian total diet: Levels in fresh vegetables

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Abstract
Nitrate is naturally present in plant foods as part of the biological cycle of nitrogen. Leafy vegetables are, among plant foods, those accumulating larger quantities of nitrate and therefore, these crops greatly contribute to the total intake of nitrate from the daily diet. The accumulation of nitrate in vegetables is determined by several factors, among these genetically based plant metabolism, harvesting time and nitrogen fertilization are predominant. The dietary nitrate could be a health risk because some of its reaction products (e.g. nitrite, N-nitroso compounds, nitric oxide) may adversely affect human health (methaemoglobinemia, carcinogenesis). For these reasons, the European authorities (EFSA, 2008) set maximum acceptable levels for nitrate in some leafy vegetables among the richest in nitrate (spinach, lettuce). This study represents the first stage of a larger project planned to provide up-to-date data on nitrate levels in the most commonly consumed vegetables in Italy and to assess their contribution to the dietary nitrate exposure. The following fresh vegetables and some of their cultivar were analysed: two cultivar of chicory, Red Radicchio of Treviso (Early, Late) and Radicchio of Castelfranco (both PGI), three species of lettuce, Romaine lettuce, Foglia di Quercia and Canasta, two cultivars of tomato, Nerina and CXD271BIO. Both lettuce and tomato crops were grown by conventional, organic and biodynamic cultivation systems, this because the choice of farming techniques, with particular reference to nitrogen nutrition, can be crucial in the accumulation of nitrate in leaves. Lettuce cultivar were collected and analyzed both in summer and winter in order to highlight possible differences due to changes in plant metabolism along seasons. Furthermore, samples of purple asparagus of Albenga and four cultivar Garlic (Piacentino, Sulmona, Castelliri, Proveno), grown in different geographical areas (Viterbo and Alvito) were also studied. Nitrate content was determined on freeze-dried samples by HPLC-DAD according to the method of Ferreira et al. (2007). Nitrate levels in both lettuce and radicchio cultivar varied greatly suggesting that both genetic factors and cultivation systems strongly influence nitrate accumulation capacity. Furthermore, the nitrate concentration found in garlic varied significantly both among cultivar and between the two growing geographycal areas.
Evolution of phenolics and astringency during aging of red wine: effect of oxygen exposure before and after bottling

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Abstract

During aging in bottle of red wines changes in phenolic composition occurs and the chemical transformations involved depends on the oxygen intake of wines. These changes are responsible for colour stabilization and astringency decrease of red wine. Astringency is a tactile sensation that derives from the interaction between salivary proteins and wine tannins, resulting in their complexation, and the subsequent precipitation in the oral cavity. Although several studies aimed to determine the effect of oxygen intake during winemaking on phenolic composition of wines were reported, few studies evaluated the changes occurring during aging in bottle and no study deals on the effect on reactivity of wine toward salivary proteins. This study was aimed at evaluating the effect of oxygen exposure of red wine, before (micro-oxygenation) and after (nano-oxygenation) bottling, on phenolic composition and astringency of wine. Two experiments were performed. In the first two level of micro-oxygenation were applied to two red wines after malolactic fermentation. Wines were bottled and analysed 3 and 42 months after the end of the micro-oxygenation treatments. In the second experiment red wines were sealed with different closures. Three oxygen transfer rate (OTR) conditions, ensured by using synthetic closures with controlled oxygen permeability, were compared. Colour indexes, anthocyanins, tannins and total phenolics were analyzed by spectrophotometric methods and HPLC. The astringency was evaluated by sensory analysis and by a method based on the SDS-PAGE electrophoresis of salivary proteins after reaction of saliva with wine (SPI saliva precipitation index). Micro-oxygenation caused a decrease of wine astringency and a stabilization of colour. A dramatic enhancement of these effects was observed 42 months after micro-oxygenation treatments. Concerning the effect of nano-oxygenation, there was a positive correlation between the OTR of synthetic closures and the decrease of astringency and SPI.

Production of experimental wines without sulfites from sicilian grape varieties Catarratto and Nero d'Avola

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Abstract

In order to assess the possibility of producing wines without or with low-sulfite content, during the 2011 vintage we conducted fermentations without SO2 of Sicilian grapes "Catarratto Bianco lucido" and "Nero d'Avola" and compared the results with those obtained in the presence of SO2. We carried out microbiological and molecular analyses to evaluate yeast species and strains involved in fermentation; finally we performed oeno-chemical and sensory analysis to evaluate the wines quality. As far as concern the fermentation of the white wines, after the cold static clarification we detected differences in the microorganisms concentration in the must. In fact, the sample with SO2 showed only 2% of non-Saccharomyces yeasts detectable at the moment of the crushing, whereas in the absence of SO2 the quantity of these yeasts was doubled compared to the initial amount. This led to significant difference during the first phase of the fermentations; in the first case, the low concentration of indigenous yeasts allowed to maintain the ratio between the inoculated Saccharomyces strain and non-Saccharomyces always higher than 100. On the other hand in the absence of SO2, even if the selected Saccharomyces strain was able to drive and complete the fermentation, during the first three days of fermentation the ratio between Saccharomyces and non-Saccharomyces was lower than 20 and it is not possible to exclude a role for indigenous yeasts in the fermentation process. As far as concern the fermentation of red musts, in which no clarification process is used, we didn't observed any significant microbiological difference between the fermentation with or without SO2. Furthermore, the ratio between Saccharomyces and non-Saccharomyces was optimal since the beginning of the process and both fermentations were carried out always by the inoculated yeasts.
Biogenic amine levels in the traditional Sardinian cheese Casu Marzu

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Abstract
Casu marzu is a traditional Sardinian (Italy) sheep milk cheese known as maggot or rotten cheese, notable for containing live insect larvae. This cheese is derived from pecorino by the digestive action of the larvae of the cheese fly Piophila casei. In this work we evaluated levels of eight biogenic amines (putrescine, cadaverine, histamine, tyramine, spermidine, spermine, 2-phenylethylamine, and tryptamine) in traditional Sardinian pecorino and in the Casu Marzu cheese. Biogenic amine analysis was performed on the starting material pecorino cheese and after three months on casu marzu using an HPLC-MS/MS equipped with a Synergi Hydro column. Average content of individual biogenic amine ranged from 1.5 ± 0.2 mg/kg for tryptamine to 155.2 ± 5.8 mg/kg for 2-phenylethylamine in pecorino cheese whilst for Casu Marzu cheese ranged from 11.4 ± 0.7 mg/kg for histamine to 343.9 ± 14.9 mg/kg for cadaverine. Proteolysis induced by the fly Phiophila casei gave rise an increase of the concentration levels of putrescine, cadaverine, histamine, and tyramine. This is the first study that describe levels of biogenic amines in the traditional Casu Marzu.

Development of strategies to combat food poisoning from Clostridium perfringens

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Abstract
The problem of foodborne pathogens associated with meat consumption has a significant impact both on health and on the economy. Clostridium perfringens, one of the most pathogenic species in the Clostridium genus, is causing increasing concern because it is responsible for severe infection both in human and animals, especially poultry. It is considered the third leading cause of food poisoning death in the UK and USA and causes necrotic enteritis (NE) in poultry. Banning of the prophylactic administration of antibiotics for the control of NE in poultry has led to an increase in the incidence of this disease. To improve poultry health and reduce the number of these pathogenic bacteria in food chain, novel strategies can be applied to control the infection in the animal. Previous studies have demonstrated that lactobacilli can be considered as competitive exclusion agents to reduce the number of pathogenic bacteria colonizing the crop and cecal mucosa of poultry. Particularly Lactobacillus johnsonii FI9785 (a poultry-derived isolate) was shown to reduce colonization and shedding of Clostridium perfringens in chickens after a single oral dose. We are investigating the interaction between Lactobacillus johnsonii FI9785 and Clostridium perfringens in vitro, using a batch culture simulating the gut environment, to understand the mechanism of competitive exclusion. We are also assessing the efficacy of novel antimicrobials for the control of Clostridium perfringens in this environment.
Irradiated meat: Analysis of dose-correlated volatile compounds

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Abstract
The ionizing radiation treatment can be used to inactive microorganisms and help maintain food safety. Linear hydrocarbons and 2-alkylcyclobutanones derived from fatty acids are known as irradiation markers in meat. In order to overcome the complexity of official methods adopted for their determination, innovative procedures have been proposed, among which head-space solid-phase micro-extraction (HS-SPME) (Li et al, 2010; Barba et al., 2012; Soncin et al. 2012). In this work volatile compounds produced by irradiation in ground beef patties, packaged in vacuum bags, were extracted by HS-SPME and quantified by gas-chromatography coupled to mass-spectrometry (GC-MS). The study has been carried out at 5 different levels of irradiation ranging from 0 to 8 kGy. Operative conditions were optimized in order to detect the highest number of significant compounds, both low and high boiling squares regression (PLS regression), a linear combination of the so far individuated stable dose markers has been obtained. This linear combination can be used as an indicator of dose more robust than single compounds taken alone. In view of future potential applications of ionizing radiation to improve meat products safety, the present method of dose determination appears more rapid, simpler and efficient than current literature procedures. Acknowledgments: Research Project (Politecnico di Milano and the Italian Ministry of Education, University and Research MIUR) within the National Research Program of Relevant Interest (PRIN 2008) (Research Project no. 20087R5ER5).
NMR methodologies in the food characterization: The *Solanum tuberosum*

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**Abstract**

Texture is a very important quality attribute in most starch-based food and is closely related to water distribution in them and water-starch interaction. In potato tubers, the texture quality is also influenced by biological variation and is of paramount importance in industrial processing and final product evaluation by consumers. For this reason, the possibility of predicting the textural characteristics and the ability to select high- and uniform-quality products are among the major challenges for the food industry. Due to its rapid non-invasive nature, the Nuclear Magnetic Resonance Spectroscopy (NMR) is one of the most attractive methods for the development of on-/at-line protocols and increasingly relevance in the food quality determination. In particular, the low-resolution $^1$H-NMR relaxometry is suitable for studying the water distribution in heterogeneous systems, and therefore have great potential to predict food quality attributes [Brosio and Gianferri, 2009].

The poster presented a protocol to potato tubers characterization by low- and high-resolution NMR. Low-resolution NMR was involved in water relaxation times in different structural components study, while high-resolution NMR was used in molecular fingerprint elucidation. The study is preliminary to the development of an on-/at-line method for a grading and sorting of potatoes in relation to their quality before marketing, storage or manufacturing processes. Particular attention was given to experimental relaxation data production and their analysis. In fact, heterogeneous systems characterized by such a high structural complexity, generally, give rise multi-exponential relaxation curve [Belton and Colquhoun, 1989; Hills et al., 1990; Belton et al., 1993] and experimental data interpretation is not obvious. For this reason, in the present work, an accurate experimental strategy was followed to optimize the measurements and, therefore, the analysis of the relaxation curve. Strategy can be summarized as follows:

1. Appropriate choice of measurement parameters of sequences NMR, IR and CPMG, to sample the best of the experimental curves;
2. Best procedure choice for experimental data processing to obtain a good relaxation curve deconvolution.

The experimental protocol developed for this purpose has allowed the distinction between different relaxation time values, attributable with certainty to the different chemical and physical components of the system.

**Determination nitrofuran in food by liquid chromatography tandem mass spectrometry**

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**Abstract**

Nitrofurans are a broad spectrum antibiotic drugs; due to concerns about their toxicity the use of these antibiotics on food sources and edible products is banned by EU Authority. Detection of nitrofurans is made difficult by their rapid metabolism to protein-bound metabolites such as AMOZ, AOZ, SEM and AHD. A specific, sensitive and robust liquid chromatography tandem mass spectrometry (LC-MS/MS) method for detection of nitrofuran metabolite in food samples is presented. The method is based on hydrolysis, derivatization and extraction with organic solvent (ethyl acetate). The present method was validated for muscle, eggs and honey according to Commission Decision 2002/657/EC and ISO 17025 requirements. Recovery studies were performed by spiking the different matrices samples in the range 0.5 - 2.0 µg kg$^{-1}$ and resulted ranged between 70 to 86% for muscle, 67 to 83 % for honey and 67 to 88 % for egg. The precision (CV) ranged between 12.2 and 16.1% for muscle, 11.2 and 15.6% for honey and from 10.3 to 16.3% for egg. Linearity for the investigate nitrofurans were calculated from 0.2 to 2.0 µg kg$^{-1}$. Under the Official Control Plan activity, several samples were found positive for nitrofuran residues, such as SEM and AMOZ, including those from non-European regions. The method is used for confirmatory purposes in the research of nitrofuran residues in food, therefore it is suitable for laboratories involved in official controls.
Beneficial effects of *Candida zemplinina* in wine fermentation: Lower alcohol level and higher glycerol content

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Abstract

Much of recent oenological research has focused on the isolation and utilization of non-*Saccharomyces* yeast species which are naturally found on grapes and/or musts. We have demonstrated the presence of *C. zemplinina* yeast species in Sicilian grapes and musts and we have successfully selected one strain with promising features (Cz3). The results obtained using Cz3 together with *S. cerevisiae* commercial strains in micro-vinification experiments performed in the laboratory, demonstrated the possibility of obtaining wines with lower alcoholic levels and an higher glycerol content than those obtained with just the *S. cerevisiae* strains (Di Maio *et al.*, in press). Here we report on 100-liter vinification experiments conducted in a winery, using the *C. zemplinina* Cz3 and *S. cerevisiae* commercial yeast strains. Molecular analyses showed that although the first part of the fermentation was dominated by Cz3, the second part of the process was completed by *Saccharomyces* yeasts which were indigenous of the winery, even when the commercial strains were inoculated early in the fermentation. The features of the wines that were produced clearly showed the positive impact of the Cz3 strain: alcohol levels were lower (of about half a degree) and glycerol content was higher (50% more on average) compared to wines fermented with only *S. cerevisiae* strains. These features are of great interest given that wines with lower alcohol levels are a preferred choice because of consumers’ health concerns, while a higher glycerol content increases wine’s pleasantness.

Evaluation of total lipase activity in bovine milk

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Abstract

Among milk enzymes, lipases are of particular interest because lipolysis may present different implications in terms of dairy technology. Among the unfavourable consequences, there are some deleterious effects of lipolysis on the sensory properties of milk and cheese, while for some cheeses the lipolytic action is a desirable event (Humbert *et al.*, 1997; Chen *et al.*, 2003). The indigenous milk lipoprotein lipase is a dimer of glycoprotein chains; it is a relatively unstable enzyme, and can be inactivated completely by heating skim milk for 20 min at 65°C, or exposing to ultraviolet light (Chen *et al.*, 2003). Many microorganisms can produce more than one type of extracellular lipase that hydrolyse different chain length fatty acids, especially short-chain fatty acids (Macrae, 1983; Chen *et al.*, 2003). A protocol was developed for the evaluation of the total lipase activity of milk on the basis of the method proposed by Humbert *et al.* (1997) and modified according to Caroli *et al.* (2011). The linearity of the method was satisfactory. The method was simple and repeatable, and could be used routinely in the evaluation of the quality of cow's milk. The method was applied preliminarily to bovine milk samples analysed to evaluate the shelf life of UHT milk. It was noted a significant decrease of the lipase activity in UHT milk than in pasteurized milk; this decrease continues over time. The residual lipase activity, however, may be responsible for undesirable organoleptic characteristics, such as precipitated formation in packaged milk.

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Evaluation of total lipase activity in caprine milk

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Abstract
The evaluation of total lipase activity (TLA) can be of interest also in caprine milk, due to the possible unfavourable or favourable effects on the quality of dairy products, as described in bovine milk (Chen et al., 2003). The method developed for the evaluation of the TLA of bovine milk (Humbert et al., 1997; Caroli et al. 2011) was adapted successfully to goat’s milk. Results on the variation of TLA in individual goat milk samples are presented. A total of 24 milk samples from Camosciata primiparous goats were collected in an experimental flock. The TLA mean was 27.8 μmol/L substrate p-nitrophenil butirrate (standard deviation = 15.6 μmol/L, minimum value = 3.3 μmol/L, maximum value = 75.8 μmol/L), with a positively skewed distribution. Relationships between TLA and different milk traits were evaluated by the CORR and GLM procedure (SAS, 2008). Preliminary results are discussed. In particular, TLA was positively and significantly associated with days in milk and somatic cell counts.

Acknowledgement: This study was supported by Regione Lombardia (BIOLAT contract).

Focus on biopeptides from bovine milk proteins: reasons for monitoring the genetic variation

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Abstract
Many bioactive peptides may originate from the six major milk proteins. It is important that milk and milk products are a balanced source of such peptides, especially considering the different actions of each of them. For example, the effect of opioid agonist peptides that may arise from αs1-casein and β-casein is somehow balanced by antagonist peptides derived from κ-casein, which is also the source of 7 peptides with antithrombotic action. Recent studies have shown that β-lactoglobulin is a source of bioactive peptides that inhibit the degradation of incretin hormones, molecules produced by the intestine that favour the production of insulin in healthy subjects and those with type 2 diabetes. In particular, the tripeptide Ile-Pro-Ala (IPA) has this inhibitory action, the same peptide showed an antihypertensive action. A special mention deserves the A2 variant of β-casein. Australia is currently on the market a milk (a2 Milk) which contains mainly the fraction of β-casein A2, derived from cows selected for the production of this variant (http://www.a2australia.com.au/). Beta-casein A2 differs from the other variants, in particular from beta-casein A1 and beta-casein B, for the presence of proline (Pro) instead of histidine (His) in position 67 of the mature protein. The presence of His67 determines the enzymatic cleavage of the Ile66-His67 peptide bond with a possible release of beta-casomorphin-7 (BCM7) which exhibits opiate properties. This bio-peptide was supposed to be implicated in the etiology of type 1 diabetes, autism and other diseases as immune suppressor. However, the European Food Safety Authority (EFSA) has not seen any formal risk associated with the consumption of milk carrier of His67 as the available scientific literature to date is unable to establish a cause-effect relationship between oral consumption of BCM7 and the etiology and development of various diseases. In all cases, the distribution of β-casein variants should be monitored because a reduction of Pro67 in cattle selected populations could increase excessively the possibility of BCM7 release, with an imbalance in the biological effects of milk protein peptides. Some results of milk protein monitoring are discussed.

Acknowledgement: This study was supported by Regione Lombardia (BIOLAT contract).
Characterization of 12 Capsicum varieties by evaluation of their carotenoid profile and pungency determination

Daniele Giuffrida, Paola Dugo, Germana Torre, Chiara Bignardi, Antonella Cavazza, Claudio Corradini, Giacomo Dugo, and Luigi Mondello

Abstract

In this research 12 different varieties of Capsicum cultivars belonging to three species (C. chinense, C. annuum, C. frutescens) and of various color, shape, and dimension have been characterized by their carotenoids and capsaiacinoids content. The berries were cultivated in the region Emilia-Romagna and provided by the Azienda Agraria Sperimentale Stuard (Parma). Several samples of Habanero of different colors (golden, orange, red, chocolate and white), Naga Morich, Scotch bonnet, Serrano, Japaleno, Sinpezon, Tabasco were analysed. The native carotenoid composition was directly investigated by an HPLC-DAD-APCI-MS methodology, for the first time. In total, 56 carotenoids have been identified and considerable variation in carotenoid composition was observed among the various cultivars investigated. Among the cultivars with red colour, some Habanero, Naga morich and Sinpezon showed an high β-carotene content, whereas Serrano, Tabasco and Jalapeno showed an high capsanthin content and the absence of β-carotene; probably specific carotenoid biosynthetic enzymes are associated with specific carotenoid profile in chilli peppers. Habanero golden and Scotch Bonnet showed a high lutein, α-carotene and β-carotene amounts, and Habanero orange was rich in antheraxanthin, capsanthin and zeaxanthin. cis-cryptcapsin was present in high amount in Habanero Chocolate. The qualitative and quantitative determination of the capsaiacinoids, alkaloids responsible for the pungency level, has also been estimated by a validated chromatographic procedure (HPLC-DAD) after a preliminary drying step and an opportune and optimized extraction procedure. Results have also been expressed in Scoville units. Dry matter and water activity have also been established on the fresh berries. The dried peppers of each variety were then submitted to the evaluation of the total nitrogen content, measured by a Dumas procedure (HPLC-DAD) after a preliminary drying step and an opportune and optimized extraction procedure. The formation of hydroperoxide was followed by 1H NMR and CG MS.

Effect of chlorophyll photosensitization on the change of volatile compounds in virgin olive oil

Angela Alessia Giuliania, Angelo Cichelli, Enzo Perri and Nicola d’Alessandro

Abstract

Fresh and genuine extra virgin olive oil contains high amounts of chlorophyll pigments that both influence the oil color and its stability during light exposure. Light greatly increases the oxidation of virgin olive oil due to the presence of an efficient photosensitizer such as chlorophyll. The mechanism of photogeneration of singlet oxygen can be schematized as follow: the chromophore adsorb a quantum of light giving its excited singlet state (1Chl) which can undergo intersystem crossing (ISC) to produce the triplet state (3Chl). The interaction between 3Chl and molecular oxygen can occur generating the singlet oxygen (1O2) via a type II process. 1O2 is an extremely reactive species which quickly react with electron rich compounds such as unsaturated fatty acids leading to several kind of oxidized derivatives. Cycloaddition reactions, both [2+2] and [4+2], lead to endoperoxide derivatives which decompose in several oxygenated species; alternatively the ene reaction lead to the formation of hydroperoxides which again decompose to give short chain oxygenated species. The result is that the chlorophyll-sensitized photo-oxidation of lipids makes a rapid deterioration of olive oil changing its color, developing undesirable odor and flavor constituents and thus decreasing its nutritional value. We report in this research the ability of chlorophyll pigments (chlorophyll a, chlorophyll b and in the form of crude extracts of several vegetable species) to act as singlet oxygen photosensitizers in ene reactions under visible light irradiation. To standardize the photosensitized reaction, a model study employing both chlorophyll a and b and a standard electron rich derivative like methyl tiglate, was carried out. The formation of hydroperoxide was followed by 1H NMR and CG MS. The same experimental procedure was applied on olive oil samples added with known amount of chlorophylls. The photooxidations were measured monitoring the relative amounts of volatile products formed by head space analyses.
Influence of the temperature of deodorization on the oxidative stability of refined olive oils

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**Abstract**  
Deodorization is an essential and delicate step of the refining process of oils: the high temperatures used cause the increase of oxidation products and the appearance of new-formed compounds (de Greyt et al., 1997). The aim of this research was to assess the effect of different temperatures of deodorization on the oxidative stability of a refined olive oil. A *lampante* virgin olive oil, neutralized and bleached using a laboratory-scale pilot plant, was deodorized at temperatures varying from 180 °C to 240 °C. The refined samples were then subjected to High Performance Size-Exclusion Chromatography (HPSEC) of the Polar Compounds (PC), in order to assess the contents of oxidized triacylglycerols (ox-TAG) and triacylglycerol oligomers (TAGP). The oils, deodorized at different temperatures, showed different amounts of ox-TAG and TAGP. Two of them, showing the greatest differences, were submitted to an accelerated oxidation test (Oven Test at 60 °C for 25 days) and repeatedly sampled and analyzed by HPSEC of PC. The oil deodorized at the highest temperature – having higher amounts of TAGP and lower content of ox-TAG respect to the other oil – showed the best oxidative stability (Figure 1). The differences observed can be attributed to the pro-oxidative effect of ox-TAG, that has been proved to be higher respect to that shown by TAGP (Gomes et al., 2011).

Development of an extraction and purification method for the characterization of the high molecular weight fraction of traditional balsamic vinegar

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**Abstract**  
Traditional balsamic vinegar (TBV) is a seasoning produced in the provinces of Modena and Reggio Emilia (Italy) and known and appreciated worldwide. During his years-long seasoning many chemical reactions takes place, leading to the formation of antioxidants and bioactive compounds, including melanoidins [1]. They are a class of polymeric substances originating from sugars and amino acids, as a consequence of Maillard’s reaction. In literature only a relatively low number of studies on melanoidins contained in foods are present, mainly because of objective difficulties in their isolation. For this reason we decided to develop a method suitable for the extraction and the purification of high molecular weight melanoidins from a complex matrix, such as vinegars. Extraction with solvents as reported by Xu et.al [2], dialysis and ultrafiltration, were tested on TBV and on a good quality industrial balsamic vinegar (IBV). On the obtained fractions dry matter, antioxidant power and total phenolic content were determined. To evaluate the effect of purification procedures, $^1$H-NMR spectra of the different fractions were also recorded and compared to raw vinegars spectra. In the ultrafiltered sample low molecular weight compounds were still presents, so it is possible to affirm that dialysis is crucial to obtain an effective purification of the melanoidins. Nevertheless it is noticeable that polyphenols concentration is higher in ultrafiltered samples: although this step is not suitable, alone, to achieve a satisfactory purification, it is useful to concentrate the high molecular weight fraction isolated by dialysis in a smaller volume. IBV showed a behavior very similar to TBV, but its high molecular weight fraction generally presents a lower phenols concentration and antioxidant activity. Moreover, in general, does not appear to exist any kind of correlation between the fractions molecular weight and their antioxidant capacity. In conclusion, the combination between dialysis and ultrafiltration appears to be a valid procedure to extract and purify the high molecular weight fraction of TBV in a small volume, but results obtained from dialysis alone are more consistent and reproducible.
Development of an extraction and purification method for the characterization of the high molecular weight fraction of traditional balsamic vinegar

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Abstract
Traditional balsamic vinegar (TBV) is a seasoning produced in the provinces of Modena and Reggio Emilia (Italy) and known and appreciated worldwide. During his years-long seasoning many chemical reactions takes place, leading to the formation of antioxidants and bioactive compounds, including melanoidins [1]. They are a class of polymeric substances originating from sugars and amino acids, as a consequence of Maillard’s reaction. In literature only a relatively low number of studies on melanoidins contained in foods are present, mainly because of objective difficulties in their isolation. For this reason we decided to develop a method suitable for the extraction and the purification of high molecular weight melanoidins from a complex matrix, such as vinegars. Extraction with solvents as reported by Xu et.al [2], dialysis and ultrafiltration, were tested on TBV and on a good quality industrial balsamic vinegar (IBV). On the obtained fractions dry matter, antioxidant power and total phenolic content were determined. To evaluate the effect of purification procedures, 1H-NMR spectra of the different fractions were also recorded and compared to raw vinegars spectra. In the ultrafiltered sample low molecular weight compounds were still presents, so it is possible to affirm that dialysis is crucial to obtain an effective purification of the melanoidins. Nevertheless it is noticeable that polyphenols concentration is higher in ultrafiltered samples: although this step is not suitable, alone, to achieve a satisfactory purification, it is useful to concentrate the high molecular weight fraction isolated by dialysis in a smaller volume. IBV showed a behavior very similar to TBV, but its high molecular weight fraction generally presents a lower phenols concentration and antioxidant activity. Moreover, in general, does not appear to exist any kind of correlation between the fractions molecular weight and their antioxidant capacity. In conclusion, the combination between dialysis and ultrafiltration appears to be a valid procedure to extract and purify the high molecular weight fraction of TBV in a small volume, but results obtained from dialysis alone are more consistent and reproducible.

Endogenous levels of nitrites and nitrates in wide consumption foodstuffs: results of five years of official controls and monitoring

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Abstract
In the past 50 years the massive introduction of nitrogen fertilizers has brought to triple the global food production, but, at the same time, to increase notably the residual amounts of nitrites and nitrates in the products. Notoriously, if these compounds are accumulated in humans, they may exercise some toxic effects (1) (2). The Chemistry Department of the Institute Zooprofilattico Sperimentale della Puglia e della Basilicata effects official controls for the determination of nitrites and nitrates in food products using accredited analytical methods. Moreover, from 2007 to 2011, it has developed several monitoring on wide consumption foodstuffs in order to appraise the endogenous levels of nitrites and nitrates. The investigations have been performed on 200 samples of fresh meats (cow, pork, horse and chicken), 180 samples of dairy products (“mozzarella” cheeses, short and lengthy maturation cheeses), 120 samples of shellfishes (mussels and clams) and 90 samples of leafy vegetables (fresh and frozen spinaches and lettuces). By analysing the obtained results it is possible to underline a high presence of nitrates in foodstuffs, also in some matrices for which the actual Normative (3) does not foresee a presence and, consequently, a maximum admissible level (fresh meats and dairy products). Some “not-compliant” samples of frozen spinaches have been registered, with concentrations higher than the limit established by the actual Normative (2000 mg · kg−1) (4); in addition, some shellfishes samples (mussels) with high concentrations of nitrates (up to 400 mg · kg−1) must be highlighted. During leafy vegetables samples analyses (spinaches and lettuces), the presence of nitrates has been registered, both at high concentrations (up to 197.5 mg · kg−1) and at low quantifiable concentrations. The results obtained in the last five years of official controls and monitoring have confirmed the necessity to develop most controls. Moreover they suggest the introduction of new legal limits, not established by the actual Normative, related to some combinations contaminant/matrix. Such new limits may fill some legislative gaps that may cause wrong interpretations of the results obtained during official controls.
Implication of meptyldinocap treatment for grapes, fermentation and wine

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Abstract

Meptyldinocap [2,4-dinitro-6-(1-methylheptyl)phenyl crotonate] is a new dinitrophenol fungicide and is one of six isomers found in the older fungicide dinocap, recently introduced to control powdery mildew (Uncinula necator). In this paper the fate of Meptyldinocap residues from vine to wine was studied, to monitor residue levels. In addition, the effect of fungicide on the fermentation of Saccharomyces cerevisiae has been evaluated. This study was carried out in comparison with Quinoxyfen, which residual profile and the effects on fermentation are well known (1, 2, 3). After the last of four applications at the recommended rate, a 0.42 mg/kg residue was found in the grapes. Vinification was carried out with maceration. Meptyldinocap was separated by ECD-Gas Chromatography and identified by comparison with commercial standard. During the vinification no detectable residues passed from the grapes to the musts. At the end of fermentation with maceration no Meptyldinocap as well as Quinoxyfen residues were determinable in the wine. Moreover, also the new fungicide showed to have no effect on alcoholic or malolactic fermentation. Finally, the wines resulting from both treated grapes had comparably sensorial qualities.

On-Line HS-SPME-QMS quality and process monitoring of roasted foods

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Abstract

The flavour quality of a roasted food depends on the roasting process to which it has been submitted. Correct conditions for industrial roasting process are in general obtained from a suitable scaling up of the results of experiments carried out in pilot or laboratory plants monitored by on-line easy and fast control methods. This study deals with a non separative HS-SPME-MS approach in view of its application to on-line monitoring of a roasting process (Pérez Pavón, Marsili). HS-SPME is a high concentration capacity technique characterized by high and reliable recovery, it is easy to automate and to be directly on-line combined with mass spectrometry (MS-nose or mass sensor). The resulting system can give quick representative and diagnostic fingerprints of the volatile fraction of samples in a set and in combination with a suitable chemometric analysis (Armanino, Rodriguez) can successfully be applied to characterize, differentiate or discriminate them. TIC fingerprint or diagnostic ion abundance(s) can therefore be successfully used both as marker or as Analytical Decision Maker (ADM) (Sandra) to monitor roasting processes on-line and in the definition of quality of roasted food. The experimental HS-SPME-qMS results in the monitoring of coffee and hazelnut roasting process are here reported and critically discussed.
Kinetic study in model wine of two commercial acid urease preparations tested in the free and immobilised form

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Abstract

Ethyl carbamate, a probable human carcinogen (Group 2A-International Agency for Research on Cancer), is spontaneously produced by the reaction between urea and ethanol in all fermented foods and beverages. In European Union, the use of acid urease, from Lactobacillus fermentum for wine urea degradation is authorised by the Commission Regulations (EC)1493/1999 and 1332/2008. Two acid urease preparations (from Nagase Enzyme and Enzyme Development Corporation with a protein content of 6.5 % and 7.6 %, respectively), were tested in model wine to determine the kinetic parameters of free and immobilized (on chitosan beads from shrimp shells-SIGMA) forms. The substrate saturation curves, of both enzymes in solution, showed a double hyperbolic behavior, with an intermediate plateau at urea concentration of about 1 g/L. This result is indicative that both preparations contain an urease with sites of different substrate affinity. The kinetic parameters (Vmax, K_M, K_a), of each hyperbolic sections were determined according to Michaelis-Menten equation using a non linear regression procedure (GraphPad Prism 5.0, GraphPad software, Inc.). The Scatchard plot allowed to obtain information about the enzyme different subunits and about the two sites with an higher and lower substrate affinity. The former characterized by a K_M 6 times lower and double K_a value with respect to the latter (active at substrate amount more than 1 g/L). Using different glutaraldehyde and urease concentrations, the optimal conditions assessed for carrier and biocatalyst preparation, were 3% (v/v) glutaraldehyde and 1 mg protein/g carrier of immobilizing solution, respectively. The immobilization procedure, despite of high immobilization yield (80%), revealed low biocatalyst activity. Nevertheless the bounded enzyme maintained its native quaternary structure, showing a double saturation hyperbolic curve, with the intermediate plateau at about 1 g/l of urea, similar to the free form. In view of both urease immobilization for oenological purposes, substrate degradation activity should be mainly ascribed to the high-affinity site of enzyme which hydrolyzes urea at typical average concentration of wine (2-20 ppm).

Determination of ochratoxin A in wine by means of immunoaffinity and aminopropyl solid-phase column clean-up and spectrofluorometric detection

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Abstract

An analytical method for the determination of ochratoxin A (OTA) in Primitivo red wine has been developed by using immunoaffinity and aminopropyl solid-phase column clean-up, and spectrofluorometric measurement (λ_ex 330 nm, λ_em 470 nm) after spectral deconvolution. Average recoveries from wine samples spiked with OTA at levels ranging from 0.5 to 3.0 ng/mL were 85% with relative standard deviation (RSD) less than 20%. The limit of detection (LOD) of the method was 0.3 ng/mL. The spectrofluorimetric method was tested on 20 red wine samples (naturally contaminated and spiked with OTA at levels ranging from 0.9 to 3.3 ng/mL) and compared with the AOAC Official Method 2001.01, based on immunoaffinity column clean-up and HPLC with fluorometric detector. A good correlation (r=0.9778) was observed between OTA levels obtained with the two methods highlighting the reliability of the proposed method, whose main advantage is the simple OTA determination by spectrofluorometer, instead of HPLC, with evident reduction of costs and time of analysis.
Influence of climatic conditions on alimentary and nutraceutical profiles of organic-grown sweet red-peppers (*Capsicum annuum* L.)

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Abstract

This research, within the project “Valorbio”, showed the evaluation of two red-pepper genotypes named “Alce”, a commercial hybrid, and “RS08”, a selected landrace typical from Marche region. The evaluation has been made over two seasons, on 2010 and 2011 in 11 years organic-converted soils, located in the experimental field of CRA-ORA. All samples, harvested at commercial ripening, have been subjected to some quality aspects as follows. Alimentary quality: dry matter, soluble sugars, pyrazine volatile compounds (PVCs). Nutraceutical quality: ascorbic acid, polyphenols, carotenoids and antiradical capacity. The attention was particularly focused on PVCs (mainly 2-methoxy-3-isopropyl pyrazine and 2-methoxy-3-isobutyl pyrazine), for their interesting odorous properties (Buchbauer et al., 2000). Moreover, the antioxidant profile (mainly ascorbic acid and carotenoids) was examined for its role in the nitrosamine inhibition, that is responsible of some tumors (Ramirez-Victoria et al., 2001). The main finding was a clear difference in both genotypes in the behavior of phytochemicals: pyrazine increased of 30% in 2011 with respect to 2010, while sugars and the nutraceutical profile clearly depleted (30% sugars, 8% ascorbic acid, 57% polyphenols and 34% carotenoids). The antiradical capacity, measured by DPPH (2-dipheny-l-1-picrylhydrazyl), on hydro and lyophilic extracts was well correlated with phytochemicals amounts, also showing the same decreasing trend in 2011. This behavior can be correlated with the different climate conditions in the two years: in the period August-September of 2011 the rainfall was significantly lower and the average daily temperature was increased by about 5°C, with respect to 2010. The plant stress was evidenced in 2011 also by a significantly decreased productivity.

Nitrate and nitrite in the italian total diet: levels in traditional cured meats

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Abstract

Nitrate and nitrite can be contained in water, vegetables and meat products. They play a crucial role in the curing of meat process, which results in microbial growth prevention and typical sensory properties development (colour, flavour, texture and sensory characteristics). High residual nitrite levels in foods could have severe health consequences due to the formation of carcinogenic N-nitroso compounds. Therefore, nitrite and nitrate addition in meat manufacturing is strictly controlled by legal regulations, aimed to technological and safety requirement. Accordingly, the EU 2006/52/CE directive established a maximum ingoing quantity of nitrite and nitrate, in place of the residual content in the final product, set in the previous directive (EU 95/2). Italy is characterized by an ancient tradition in the production of a large number of cured meats, typical of the various Italian territories. The possible health concern associated to an excessive consumption of these products has led to a systematic monitoring of nitrate and nitrite in a variety of Italian cured meats. Therefore this study represents the first stage of a larger project designed to update the current knowledge on nitrate and nitrite content in the most popular cured meats in Italy. An extensive sampling of cured meats (PDO, PGI, typical) was carried out, involving the main national manufacturers. The most traditional Italian cured meats were analyzed, each provided by five companies: raw hams (*San Daniele, Parma, Modena, Nazionale)*; cooked hams (three quality brands); salami (*Cacciatorre, Milano, Napoli, Ungherese*); bresaola Valtellina; mortadella Bologna; speck Alto Adige; coppa; rolled bacon; ciccioli; zampone; cotechino; wurstel. Nitrate and nitrite levels were compared, when possible, to those found fifteen years ago on the same cured meats. Nitrate content in cured meats from this study ranged from 4 ppm in zampone to 30 ppm in bresaola samples. These values were different from those reported in a previous INRAN study. In fact nitrate content found in this study tended to be lower than those reported in the 1993 study (the era before the directive 2006/52/CE). The nitrites were almost absent in all the samples analysed.
Micronutrients in Italian ham: A survey of traditional products

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Abstract
Ham belongs to the oldest Italian tradition of cured meats, the first description about its preparation dates back to Romans time. A large variety of products with a consolidated preparation technique has been originated along the time. Among the traditional Italian hams, 7 have got the PDO or PGI recognitions according to EU rules; in addition, there are many other local hams characterized by a specific geographical identity and manufactured by a small number of farms. Ham is among the most consumed traditional Italian cured meat and can be an important source of micronutrients in the Italian total diet. This study belongs to a wide survey addressed to chemical and nutritional characterization of Italian traditional hams. The present study was planned to estimate B vitamins (B1, B2, PP, B6, B12) vitamin E and trace elements (Fe, Zn, Se, Cu, Mn) level in the Italian most consumed hams. The prominent types of ham (raw, cooked, smoked) were studied selecting products among the most representative in Italy. Therefore an extensive sampling of Italian hams, produced in specific geographical areas by different processing methods, was carried out. The main types of traditional Italian ham were analysed: 4 raw hams (San Daniele, Parma, Modena, National), 3 types of cooked ham and 1 smoked ham (Speck of Alto Adige). The selected samples were provided by five manufacturers, each sample was analyzed individually. B vitamins and vitamin E were determined by HPLC (Ndaw et al., 2000). Vitamin B12 content was assessed by the performance of a fully automated chemiluminescence analyser (Saccani, 2011). Trace elements were quantified by Inductively Coupled Plasma Optical Emission Spectroscopy. The contribution of ham portions to vitamins and trace elements daily average requirement (Italian RDA) was also estimated.

The yogurt amino acid profile’s variation during the shelf-life

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Abstract
Yogurt is obtained by lactic fermentation of two microorganisms, Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus. During the fermentation process, the greatest changes are obtained on glucose and proteins that undergo a partial hydrolysis with consequent release of peptides and free amino acids. The objective of this study is to analyze the amino acid profile of the yogurt from the placing on the market until the end of shelf-life. The evaluation of the proteolytic activity of the bacterial strains concerned allow to deduce their vitality during the period of shelf-life of 45 days. We analyzed three types of yogurt – all natural white, sweet white and whole banana- in three times: t0 (first day of shelf-life), t1 (mid shelf-life) and t2 (end of shelf-life). Proteins were determined by the Kjeldahl method and the amino acid profile by HPLC. In natural white yogurt we observed an increase in the amount of free amino acids quite constant throughout the period of shelf-life (36% between t0-t1 and 40% between t1-t2). As for the sweet white and whole banana yogurt we observed a greater increase in the first half of the shelf-life (56% and 82% respectively) to decrease thereafter between t1 and t2 (10% and 45% respectively). Based on data, the proteolytic bacterial activity appears to persist throughout the duration of the shelf-life and this can be considered an index of the survival of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. Thermophilus in yogurt for the entire period of commercialization.
Healthy and nutrictional characterization of dressing obtained by co-crushing and malaxation of olive (cv Moraiolo) and tomato

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Abstract
Virgin oils obtained from olive fruit can be identified as the main condiment of Mediterranean diet and it is recognized an important role, also functional in this diet. This function is given, in particular by compounds with antioxidant activity, belonging to different families of phenolic compounds (simple phenols, phenolic acids, secoiridoids, lignans and flavonoids).

In this study was characterized the phenolic profile of oils extracted from specific cultivars (moraiolo), carefully selecting the variables of the extraction process (temperatures, times, concentrations of oxygen, etc.). Was also performed co-crushing of olives with tomatoes rich in lycopene and the condiment thus obtained was characterized in terms of healthy and nutrition property (fatty acids profile, phytosterols profile, phenolic compounds profiles, lycopene, vitamin E, antioxidant capacity [ORAC]). In particular the samples of seasoning obtained by co-crushing with tomato were in a range of phenolic compounds of 500 mg/kg and lycopene greater than 130 mg/kg and ORAC values greater than 2000. These dressings were submitted to the in vivo studies to evaluate their antioxidant activity compared to oxidative stress induced by an high fat meal, which can certainly be considered among the risk factors involved in the development of cardiovascular disease (Valko, 2007). This risk is particularly high in western society, where most of the population consumes three or more meals for day and then spending most of the day in the postprandial phase (Lopez-Miranda, 2007). In vivo studies showed that with regard to the Th2 cytokines, there has been a significant reduction of IL-10 following ingestion of these condiment rich in lycopene.

Effects of isoflavons and phenolic compounds extracts from food industry waste on antinflammatory and antioxidant pathways

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Abstract
The waste from the processing of plant products in the food industry, are often a special waste and thus have an environmental problem as well as a cost to the company that produces them. The extraction with water of biologically active components in addition to reducing the environmental impact of this waste can be an important source of phytochemicals that can be used pharmaceutical, cosmetics or food supplements. This process then allows to transform a problem into an asset. In this study we looked in particular to evaluate the nutritional genomics effects. Nutritional genomics reflects gene/nutrient interactions, utilising high-throughput genomic tools in nutrition research. Nutrigenomic approaches, especially transcriptomics, enable simultaneous study of various signalling pathways and networks. We test the activity of extract from by-products from breweries and mills very rich in isoflavones (Lapcik O, et al. 1998), or polyphenols, on hepatocyte cell line (HepG2) to evaluate their potential activity on two important metabolic pathway: inflammation and antioxidant mechanisms. We define the maximum concentration not cytotoxic for any extract and any cell line and then exposed the cells for 24 h to the treatment and then we extract the RNA and perform the real-time PCR analysis. We used two Applied Biosystem Gene expression array every one with 96 different assay: TaqMan® Array Human Inflammation and TaqMan® Array Human Antioxidant Mechanisms. Our results demonstrate that every extract show a significative influence on the expression of different genes belonging to the two pathways.
Optimization of the agronomical and wine-making process of Grechetto and Vermentino grape to improve the sensory and healthy characteristics

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Abstract

In recent years the focus on natural antioxidants has greatly increased, phenolic compounds, in particular, are a class of compounds ubiquitous in the plant world and widely present in food daily. The wine contains a large number of phenolic substances the concentration of which depends both on the type of grape that the wine-making technique used. This study assessed the effect of the optimization of agronomic procedure on the content of phenolic substances in grape and the effect of the optimization of winemaking with the goal of preserving the content of phenolic substances and obtain wines that possess the highest healthy activity. Moreover, since these same substances also mainly responsible for the color, aroma, flavor and astringency, preserve it increase the potential sensory characteristic. The study was made on white grapes of the variety Grechetto and Vermentino from Umbrian winery. The determination of the polyphenolic profile through advanced analytical methods such as HPLC and head space mass spectrometry techniques, side by side in sensory tests, have allowed us to identify, for the two types of grapes, the best conditions of fermentation in order to optimize the content of functional substances. The results showed that different agronomic conduction (biodynamic, organic, conventional) induce significant differences in antioxidant content, while optimization of the wine making process (cryo-maceration and pressing in a modified atmosphere) increase the content of antioxidants (from 20 to 40%) moreover the increasing of amount and variety of aromatics compounds greatly improving the sensory profile of the finished product as evidenced by the results of panel and consumer test.

Plant extracts in swine nutrition: Effects on some hematochemical parameters and sensory characteristics

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Abstract

There is an increasing public interest in the use of plant extracts in livestock feed. The objective of this study was to investigate the effects of inclusion of oregano (Origanum vulgare L) and chestnut wood (Castanea sativa) extract in pig diets, on some blood parameters, and chemical and sensory characteristics of the meat pork. Ninety Suffolk hybrids pigs were randomly divided into 6 groups (3 indoor and 3 outdoor; 15 animals/group) and, after the adaptation period, both indoor and outdoor pigs were assigned to one of the following iso-nitrogenous and iso-energetic diets: a) control, a commercial pellet diet (16.0% CP, 4.3% CF, 1.0% Lysine) groups (3 indoor and 3 outdoor; 15 animals/group) and, after the adaptation period, both indoor and outdoor pigs were assigned to

P-113
Predictive models of wine tartaric stability using fourier transform infrared spectroscopy

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Abstract
The feasibility of using Fourier Transform Infrared Spectroscopy (FT-IR) for the prediction of tartaric stability of wines was investigated (Guerrero et al., 2010; Kupina & Shrikhande, 2003; Palma & Barroso, 2002; Patz et al., 2004; Romera-Fernández et al., 2012; Soriano et al., 2007; Versari et al., 2011). The calibration set was made up of 252 white, 150 red and 38 rosé wines, representing the most diffused Italian varieties; the validation set was made up of 81 white, 33 red and 3 rosé wines. Two of the experimental approaches most commonly adopted in wine testing laboratories, the Microcontact Conductometric Test and the Cooling Test (–4°C for 5 days), were used as reference methods to evaluate the tartaric stability of the samples collected. Discriminant Analysis (DA), Artificial Neural Networks (ANN) and Partial Least Square regression (PLS) were considered for proposing new predictive models for tartaric stability, separately for white and red or rosé wines. The results demonstrated the possibility of developing new predictive models for wines, so long as tartaric stabilisation products have not been added, starting from FT-IR analysis. The best results were obtained by the models based on the Cooling Test as reference method. In particular, for white wines DA allowed to correctly classify into the right category (“stable”, “unstable” or “suspect”) the 84% of the calibration samples and the 73% of the validation samples, whereas ANN for red and rosé wines permitted to correctly classify the 83% of samples in both datasets. Nevertheless, also PLS for white wines and DA for red and rosè wines gave a right classification for more than 70% of samples.

Polyphenolic profile in berry skin and wine of Vitis vinifera l. cv. Aglianico (Basilicata, Italy)

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Abstract
Anthocyanins are a very large group of red-blue polyphenol plant pigments, located within vacuoles of the skin cells of red grapes and characterized by a positive charge on the molecule which enables them to absorb light. These naturally occurring pigments from grapes are glycosides having a sugar bonded at the 3 position. The presence of this sugar helps the anthocyanins maintain solubility in water and allows pigments to diffuse into the must and wine during maceration, contributing to the color and antioxidant properties of red wines. The antioxidant properties, named as total antioxidant capacity (TAC) are well known and are correlated with electron transfer processes promoted by glycosilated and methoxy derivates anthocyanins as malvidin-3-glucoside arising from the free anthocyanin fraction of the red wines. Based on the widely accepted knowledge that antioxidants are potent scavengers of free radicals and serve as inhibitors at both initiation and promotion-propagation stages of tumor promotion-carcinogenesis and protect cells against oxidative damage, it seemed of interest to investigate anthocyanin presence and composition within the grape berries of Aglianico (Basilicata-Italy) [1], one of the most ancient vineyards introduced from Greece in the southern Italy in pre-Roman times.
**P-116**

**Tocotrienol supplementation: A novel approach to the complementary treatment of Friedreich’s Ataxia**

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**Abstract**

Oxidative stress is always associated with Friedreich’s Ataxia (FRDA) also accompanied by impaired mitochondrial functions. Patients are currently treated with idebenone, a CoQ10 analogue, believed effective in view of its ability to counteract free radical damages. Taking into account that efficacy of Vit. E and phenols on oxidative stress-related pathologies is well documented, and considering our experience in the field of natural phenols, we started a preliminary investigation with natural extracts containing phenols. The radical scavenging activity was measured by instrumental methods, and antioxidant potential was screened in fibroblasts. From this preliminary investigation, tocotrienol based extracts emerged as the most prominent candidates and thus its ability to counteract in vivo oxidative stress was evaluated in FRDA patients. In particular, five young FRDA patients were treated and several biochemical parameters were monitored in blood samples. Patients assumed tocotrienol (5 mg/kg/day), for two months. The following features were studied: white blood cell gene expression of SOD-1, SOD-2, catalase, GPX-1, GSR and GSTM-1; plasma content of GSH and GSSG; plasma Oxygen Radical Absorbance Capacity; amount of plasma carbonylated proteins; urinary levels of Hexanoyl-Lysine adduct; lipid composition of erythrocyte membranes. Such wide array of different markers consistently pointed to the presence of oxidative stress in FRDA patients, despite the fact that the idebenone therapy had not been discontinued. However, even a two month low-dose tocotrienol supplementation led to the decrease of oxidative stress indexes and to parameter values that approached those of healthy controls. Moreover, there are evidences that a longer tocotrienol treatment may be more effective in reducing oxidative stress.

**P-117**

**Zespri, CI.GI. and hayward kiwifruits: Metabolic profiling and water status by nuclear magnetic resonance**

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**Abstract**

The metabolic profiling of the aqueous extract as well as the water status of the entire kiwifruits of CI.GI. (*Actinidia deliciosa*, a selection of Hayward cultivar) and Zespri (*Actinidia Chinensis*) kiwifruits were monitored from June to December and compared with the corresponding ones of the Hayward kiwifruits. A more complete assignment of the kiwifruit 1H spectrum with respect to that reported in the previous paper (Capitani et.al.) is reported: histidine, phenylalanine and a quercetine derivative were additionally identified in the kiwifruit 1H spectrum. Zespri kiwifruits were characterized by the presence of a significant amount of aminoacids such as phenylalanine, triptophane and histidine, whereas quercetine derivative is completely absent. The water status of kiwifruits was measured non destructively on a single kiwifruit both attached to the plant and detached from the plant measuring the T2 spin-spin relaxation time of water by means of a portable unilateral NMR instrument. Zespri kiwifruits showed a net T2 increase between October and November whereas in the case of CI.GI. the variation of T2 was more gradual.
Authentication of trappist beers by $^1$H NMR-chemometric approach

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Abstract

An NMR and chemometric analytical approach was developed within the European TRACE project (FP6-2003-FOOD-2-A, contract number: 006094) to characterize and authenticate Rochefort 8 beers, very famous Trappist beers with a yellowish brown colour and a pronounced fruity aroma. Different types of beers (233 samples) namely Rochefort 8 samples, other Trappist beers (Achel, Chimay, La Trappe, Orval, Westimalle…) and other special non-Trappist beers (Afligem, Binchoise, Bon Secourse, Brigand, Bruges, Charles Quint, Leffe, Grinbergen, Gueuze, Jupiler…) were analyzed by $^1$H NMR spectroscopy (Duarte et al.). PLS-DA modelling analysis applied to selected $^1$H resonances was used to build up a statistical model to discriminate between Rochefort 8 beers and all the other beers. Alanine, pyruvic acid, lactic acid and acetic acid turned out to be responsible for beer discrimination.

Metabolic profiling of percoca and flaminia peaches by nuclear magnetic resonance

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Abstract

NMR is recognized as one of the main analytical methodologies for metabolite profiling giving a complete view of the foodstuffs metabolites and, together with a suitable statistical analysis, providing relevant results in terms of foodstuffs quality, geographical origin, processing, raw material, safety and so on (Capitani et al., Mannina et al., Sobolev et al.). The metabolite profiling of the aqueous extracts of two peach varieties (Percoca and Flaminia) were investigated by means of high field NMR spectroscopy. Water soluble metabolites belonging to different classes such as organic acids (malic acid, citric acid, quinic acid and shikimic acid), sugars (glucose, sucrose, myo-inositol, fructose, xylose), amino acids (alanine, threonine, asparagine, valine, isoleucine) and other metabolites have been identified. The metabolite profiling together with a suitable statistical analysis were used to characterize and discriminate Percoca and Flaminia varieties.
A selection of french and British cheeses commercialised in Italy

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Abstract
The Italian consumers can choose from a wide selection of national and European PDO (Protected Designation of Origin) cheeses. One of the aims of an Italian project "BIOVITA" supported by the Italian Ministry of Agricultural, Food and Forestry Policies (MAFFP), is to collect information and evaluate the nutritional quality of some PDO European cheeses commercialized in Italy. In particular five French cheese (Chaource, Époisses, Langresse, Fourme d'Ambert made from cow's milk and Crottin de Chavignol made from goat's milk) and one British cheese (Blue Stilton made from cow's milk) were purchased from different commercial sources and studied. The cheese samples were analysed for their proximate composition (AOAC, 1995), cholesterol, ergosterol, vitamin A, vitamin E (Panfili et al 1994), fatty acid (Prandini et al., 2007) and the contents of the main minerals (calcium, phosphorus, sodium, potassium) (AOAC, 2002). Two scientific tracing parameters the “Degree of Antioxidant Protection” (DAP), related to farm systems (Pizzoferrato et al., 2007) and the “Degree of Retinol Isomerization” (DRI) related to heat-treatments and other environmental stresses (Panfili et al., 1998) were applied to the cheeses studied. A large variability in the composition of these cheeses was found and it can be probably due to milk from different animal species, processing and ripening time. In particular high levels of some short chain fatty acids (C6:0, C8:0 and C10:0) in Crottin de Chavignol made from goat's milk were detected. From a nutritional point of view a serving of 50g of the cheeses studied guarantee good level of calcium, and vitamin A (33% for man and 39% for woman) required for an adult man of 75 kg body weight, while the percent of average requirement for vitamin E is low (8%).

Acknowledgments: This research was financially supported by the Italian Ministry of Agricultural, Food and Forestry Policies (MAFFP), “BIOVITA” project (DM 3684/7303/08).

Contemporary analysis of some vitamins of B-group in vegetable matrices

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Abstract
The B-group vitamins play an important role in human metabolism, because of their cellular activity as coenzymes. This group includes many vitamins (Thiamine, Riboflavin, Biotin, Pantothenic acid, Folic acid, B6, Cobalamine, Niacin) all water soluble, an important feature that could origin the risk of vitamin B deficiency due to an unbalanced diet. Methods commonly used for the analysis of B vitamins in food include separate determination for thiamine and riboflavin [2], for the three isoforms of vitamin B6 [3],[4] and for niacin [5] or even a simultaneously liquid chromatography separation using mass spectrometry detection[6]. The aim of this study was the application of a HPLC method for simultaneous determination of B vitamins. The method was carried out using a C8 column (250x4.6mm) and a mobile phase gradient using phosphate buffer (pH 2.5, 50mM) and methanol. Riboflavin, piridoxamine, pyridoxal, piridoxol and thiamine with niacin were revealed using respectively fluorimetric and UV detector. The method has been applied to five selected vegetables: red radish, green olives, garlic, tomatoes and pistachio; all samples were extracted by acid hydrolysis followed by enzymatic step [7] after being freeze-dried or defatted to obtain homogeneous samples. The method application allowed simultaneous determination of piridoxamine, pyridoxal, piridoxol, riboflavin, and niacin; because of the thiamine low quantities in plants, samples required potassium ironcyanide derivatization to be visible in fluorimetry as its oxidized form.
MIR and NIR milk casein spectra: Dependence on pH, temperature and calcium ion concentration

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Abstract
Casein is a complex milk protein consisting of subunits of different nature, \(\alpha_s\)-, \(\beta\)- and \(\kappa\)-casein-linked by calcium and phosphate ions. It’s commonly recognized that variations in temperature, pH and ionic strength affect the delicate balance of forces and can change the status of micelles association. This work aimed to evaluate modifications in Mid and Near InfraRed (MIR and NIR) spectrum of milk casein in function of pH, temperature and calcium ion concentration. Commercial preparation of casein fractions \(\{(\alpha_s,\beta\) and \(\kappa\)-casein (Sigma-Aldrich Srl, USA)], whole ‘technical grade’ casein (Sigma-Aldrich Srl), and samples of native casein, obtained by centrifugation, were dissolved in phosphate buffer solution (pH 6.8) at the same concentration as in milk. Tests were carried out to check: i) the influence of pH in phosphate solutions from pH 6.8 to 10.0; ii) the effect of temperature using solutions at pH 6.8 at five different temperatures from 20° to 60°C. Calcium ion concentration like in milk was obtained by adding CaCl\(_2\) (Merck, Milan, Italy) to the solutions of commercial casein. MIR spectra (4000-500 cm\(^{-1}\)) were recorded with a FT / IR 400 spectrometer (Jasco Europe, Como, Italy) using multiple reflection Attenuated Total Reflectance system (ATR), with a cell of ZnSe crystal for liquids. Transmission NIR spectra (10000 and 4000 cm\(^{-1}\)) were obtained with a NIRFlex N-500 spectrometer (BUCHI, Italy) with a quartz cuvette (optical path = 0.2 mm). The spectral response was little affected by temperature modifications. MIR detected shifts in peaks maxima of amide I bands of casein fractions, related to modifications of H bonds. These shifts were also related to hydrophobicity properties of caseins. NIR could only detect the water bands modifications. Regarding pH, MIR could only detect modifications of phosphate groups of PBS. Abs from the NIR mean spectrum evidenced some modifications due to the number of negative charged amino acid residues at pH>6.80 in the casein sub-fractions. By adding Ca\(^{2+}\) ions to commercial casein, shifts of signals attributed to phosphate and carboxilate groups in MIR spectra were observed. These variations were explained with the formation of complexes with casein. Similarly, the subtraction of Ca\(^{2+}\) by EDTA provided shifts in the opposite direction.

NIRS ability in the determination of casein genetic variants content in reconstituted casein samples

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Abstract
Milk casein and casein fractions contents have great influence on rennet properties and cheese yield so that the selection of dairy cattle with genetic characteristics suitable for milk transformation is of great interest for dairy farms and firms. The possibility of a rapid and accurate determination of these parameters would be very useful to predict milk aptitude to cheese making. The aim of this work was to determine casein genetic variants content by Near InfraRed Spectroscopy (NIRS) by comparing the performance of different NIR equipments and sample presentation modes. NIRS ability in detecting bonds involved in the micelle complex was also verified. This work was realized inside the RiProSel project funded by MiPAAF. Raw bulk milk samples, collected from different farms in the Asturia region (Spain), were analyzed by Kjeldahl’s method to measure Total Protein (TP%) and Non Caseinic Nitrogen (NCN%) contents. Casein content was calculated as the difference
between NCN and TP contents. Samples were split in two parts: the first one was ultra-centrifuged (native casein); the second one was acidified (pH=4.6) and centrifuged (acid casein). All samples were then reconstituted in phosphate buffer (pH=6.8). Milk and casein spectra were collected at 37°C with two spectrometers, an FT-NIR (Perkin-Elmer, USA) in transflectance mode, and a Foss-NIRSystem 6500 (Foss, Denmark) in both reflectance and transflectance mode. Data were processed by the Unscrambler software v.9.2 (Camo Inc., Norway). Partial Least Square Regression (PLSR) analysis performed on milk transflectance spectra showed very satisfactory results: good prediction ability was highlighted for all casein fractions (min R²=0.70 for β-casein; max R²=0.92 for κ-casein), with the exception of αs2 casein. NIRS ability to determine and quantify casein genetic variants could be used for milk selection for its final purpose. The explorative PCA, performed on casein reconstituted spectra, allowed the separation between native and acid casein samples, suggesting the possibility to investigate casein interactions and structure.

Carbohydrate prebiotic activity of typical Italian garlic cultivar (Allium savitum L.)

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Abstract
Garlic (Allium savitum L.) is a food ingredient widely used in global gastronomy; besides to be used like food, it has been used as medicinal plant since ancient times and it is still being employed in folk medicine all over the world in treating many diseases. The health benefits attributed to garlic consumption should also include the high fructooligo/polysaccharide (FOS) content which is responsible of garlic prebiotic activity together with dietary fiber. These water-soluble fructose polymers represent the short-term carbohydrate storage material in garlic bulbs; they are fermented in animal colon by competitive microflora, generating adverse conditions for microbial colonization and increasing resistance to infection (Choque Delgado et al., 2010). In this study garlic carbohydrates were characterized: FOS, dietary fiber, sucrose, fructose and glucose contents were investigated in four popular garlic cultivars (Rosso di Castelliri, Bianco Piacentino, Rosso di Sulmona, Rosso di Proceno). All the analysis were carried out according to official methods. Total dietary fiber was evaluated by enzimatic–gravimetric method (AOAC, 1995) and sucrose, fructose and glucose contents were determined by anion exchange liquid chromatography using an amperometric pulsed detector (HPLC-PAD) according to reference (Lee, 1996). Dietary fiber and total sugars didn’t show large variability among different cultivars with respectively average value of 4.30 and 0.99 g/100g on wet weight basis. FOS were measured using enzymatic kit purchased from Megazyme (AOAC, 2002); their results ranged from 17.89 to 20.52 g/100g wet weight. Interest in fructooligosaccharides is growing worldwide as they are being recognized as health-promoting food ingredients, for this reason garlic it could be added like functional food to highly consumed products.
**Variation of bioactive compounds and biological activity of Ficus carica L. cv.**

**Dottato aerial parts collected in different months**

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**Abstract**

Figs (Ficus carica L., Moraceae) are perhaps the oldest of all cultivated fruit crops and are grown in many areas of the world with subtropical climates. The barks, leaves and fruits are considered to be very effective in various diseases. The content of phytochemicals in plant material is influenced by eco-physiological conditions, genotype, cultivation techniques and phenological stage which influences the compositional quality of fruits and vegetables, since, during different months, a series of biochemical, physiological and structural modifications occur and these determine the attributes of phytocomplex quality. Leaves, bark and woody part of F. carica cultivar Dottato collected in three different months (June, July and September) were examined to assess their chemical composition, antioxidant and phototoxic activity on human melanoma cells. Components in Ficus samples, collected periodically, were extracted by hydroalcoholic solution (70% ethanol). Linoleic acid peroxidation and DPPH test were used to assess antioxidant activity while MTT assay was used to evaluate antiproliferative activity on C32 human melanoma cells after irradiation of an UVA dose of 1.8 J/cm² (Menichini et al., 2012). The phytochemical investigation revealed different composition in the coumarins, psoralen and bergapten, fatty acids, polyphenol and flavonoid content. The highest total phenolic content was found in the second harvest of leaves (12.9 mg chlorogenic acid eq/g of dried material) while flavonoids were more abundant in the first harvest of leaves (2.1 mg/g). Second harvest of leaves possessed the highest content of bergapten (8.2%) while first harvest of bark showed the major content of psoralen (23.3%). The fatty acids analysis revealed that the highest content of linolenic acid was found in first harvest of woody part (14.1%) while linoleic acid showed the same content in leaves and woody part samples. The harvest II of leaves possessed the highest antiradical activity using DPPH test with an IC₅₀ value of 64 μg/mL while harvest III showed the best inhibition of peroxidation with an IC₅₀ value of 1.48 μg/mL. F. carica leaves samples showed also the best antiproliferative activity in comparison to bark and woody part. Among leaves samples the harvest III showed the lower IC₅₀ (3.92 μg/mL).

**Detection of allergens in food products commercialized in Italy**

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**Abstract**

Food allergies are an important health problem in industrialized countries. Today more than 25% inhabitants suffer from allergic rhinitis, conjunctivitis, asthma, dermatitis or food allergies. Undeclared allergens in the label represent therefore a risk for consumers. European Union (EU) legislation currently requires declaring in the label all the ingredients and derived substances used in food products and also requires that specified allergenic ingredients used in pre-packed foods are clearly declared. The regulations that are the object of this consultation will implement Directive 2007/68/EC into national legislation. In this work a monitoring plan of allergens in food products commercialized in Italy is presented. The aim of study was to detect the residues of sulphite in crustaceans and gluten, egg-proteins and β-lactoglobulin in different food products commercialized in Italy. The analyses were carried out with an ELISA test for the detection of gluten, egg-proteins and β-lactoglobulin. For SO₂ detection the method proposed by Person et al. (1971) was used. In the Codex Alimentarius, the term “gluten free” indicates that the total amount of wheat gluten, barley and rye products is ≥ 20 ppm. Unlike expected, the samples that had external breading (cordon bleu, fish sticks, etc.) showed the lowest values. Content higher than the limit set by the Codex in the sample of yogurt with cereals can be explained by the presence of this ingredient rich in gluten. The presence of SO₂ concentrations exceeding the limits established by Directive 2000/13/CE, particularly in fresh unpacked product sold in bulk, confirms the frequent use of this compound. Some samples contained undeclared presence of β-lactoglobulin and egg protein. All milk-positive products proved to contain casein, although “milk” was not declared in the label. Similarly, the term “egg” was not present on the label of all samples positive for egg-protein test. The obtained data confirm that the method used is an effective tool for the determination of food allergens in processed food products. Finally the results showed a high percentage of allergens positive foods, for this reason it is important to continue a constant monitoring.
Presence and significance of cyclopropyl and \( \omega \)-cyclohexyl fatty acids in cow milk

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Abstract

Presence and contents of alicyclic fatty acids as long chain cyclopropyl and \( \omega \)-cyclohexyl fatty acids, probably of microbial origin from silage (Montanari et al. 2010) and rumen (Oshima et al. 1975) respectively, were determined by GC-MS and confirmed by \(^1\)HR-NMR in more than 200 milk samples from lactating dairy cows fed with forages containing or not silage maize. Cyclopropyl fatty acids (0.1% on the milk fat) were detected in all the milk samples from cows fed with forages containing silage maize. On the contrary, all milk samples from cows fed without silage maize were negative. Because in the Disciplinary of Production of some valuable cheeses as Parmigiano Reggiano (PDO product) the feeding of lactating cows with silage maize is forbidden, the presence/absence of cyclopropyl fatty acids can be considered as quality molecular markers of milk and milk products. In fact, samples of Parmigiano-Reggiano cheese tested on the market for the cyclopropyl acids presence were found all negative, while Grana Padano samples (silage maize allowed) were all positive. Therefore the determination of cyclopropyl fatty acids can be proposed as a parameter for the quality control and authentication of Parmigiano Reggiano cheese, especially for products sold as grated cheeses. Other alicyclic acids detected in milk samples were \( \omega \)-cyclohexyl derivatives (mainly C17 and C19). Their content varied from zero to 0.15% of the total fat and the presence was correlated with cow daily rations very rich of corn starches, suspected of favouring the development of subacute ruminal acidosis (SARA).

Development of protein-bound and free D-amino acids during processing of cocoa beans to chocolate

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Abstract

The presence of D-amino acids in foods can be related to the microbial activity (racemase activity during fermentation or preservation) and to the thermal impact. Both protein-bound and free amino acids are affected by racemization, but with different mechanisms. The racemase activity is a characteristic of fermented / microbial-contaminated foods (Gandolfi et al. 1994). Roasting processes influence the contents of chiral compounds depending on technological procedures, and high degrees of protein-bound D-amino acids were previously correlated to the effect of roasting processes (Caligiani et al. 2007). Fresh cocoa beans undergo both fermentation and thermal treatments during their transformation in chocolate or related products, so the aim of this study was to understand how D-amino acids develop during the transformation steps of beans to chocolate, analyzing five complete cocoa series of different geographical provenience, including cocoa beans fermented in the countries of origin, pre-roasted nibs, roasted nibs, liquor, non-tempered chocolate and chocolate. As regards the results, it was found that the percentage of free D-amino acids (expressed as D/(D+L)%) in fermented cocoa beans strongly depends on the cocoa origin, suggesting a significant effect of microbial racemases specific of the indigenous micro flora. For example, free D-ala percentage ranged from a minimum of 2.0 ± 0.1 for Nigeria beans to a maximum of 5.9 ± 0.2 for Ivory Coast beans. The value of free amino acid racemization detected in chocolate depends on the initial content in cocoa beans. As regards protein bound D-aminoacids, an unexpected significant racemization degree (bound D-ala percentage 1.5-2.2 %) was observed in fermented but not roasted cocoa beans, in a strict contrast with previous results that indicated a relevant racemization of protein-bound D-amino acids only in consequence of roasting treatments. In order to better understand the effect of cocoa bean fermentation on protein bound D-amino acids development, analyses on fresh unfermented cocoa beans, bad fermented cocoa beans, partly fermented cocoa beans and on cocoa beans maintained at room temperature for several months were performed.
GC-MS profiling of minor components in nuts: Determination of simple phenols, indole derivatives and phytoestrogens (lignans)

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Abstract
Consumption of nuts has been linked to a lowered risk of cardiovascular heart disease and to the prevention of cancer, even if the potential mechanisms of action of components in nuts have not been completely understood (Albert et al. 2002). To date, nut research has been mostly focused on the lipid profile and little research has been concentrated on the phytochemical profile of nuts polar compounds. To more fully characterize the antioxidant profile and possible associated health benefits of different nuts, it is worth quantifying these bioactive compounds (Yang et al. 2009). Therefore, the objective of this study has been the characterization of secondary compounds of ten types of common edible nuts (almonds, peanuts, hazelnuts, walnuts, pistachios, pine nuts, cashews, pecans, macadamia nuts and brazil nuts) with regard to their phytochemical profile. Tens of compounds belonging to the chemical classes of benzoic acids, phenylacetic acids, cinnamic acids, simple phenols, indole derivatives and phytoestrogens (lignans) were identified and quantified by GC–MS. Many of these compounds were not previously reported in literature for nuts. Between the large amount of data collected, of particular interest resulted the contents of lignans found in macadamia nuts (770 µg/100 g of whole seed) that is higher than the most commonly used vegetables and fruits, except broccoli, flax seeds and sesame seeds, considered the important source of lignans. Macadamia nuts and peanuts were also found to be an excellent source of tyrosol, even better than extravirgin olive oil. An interesting and unreported abundant presence of indole compounds, mainly 5-hydroxy-indoleacetic acid derivatives was also found in nuts, especially in hazelnuts, suggesting to undertake in the future further analytical and structural studies in order to completely identify their structures and understand the biological activity as they might represent a novel class of nutraceuticals.

A fast and sensitive method to detect powdered milk in fresh cow milk based on MALDI-TOF-MS

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Abstract
Among the foodstuffs, milk and cheese are often subject to adulterations because of their economical and nutritional values. Depending on the local laws of the country, the most common practices concerning these products are the milk dilution by whey or by water, the mixing of different milk species, the addition of milk anhydrous products (caseins and caseinate, milk protein concentrate, whey proteins, powdered milk) to liquid milk. In some cases, these practices can be fraudulent and are often committed to totally or partially substitute a high price raw material with a cheaper one, especially in the production of an high quality and expensive product (e.g.: P.D.O. labelled cheeses). Besides PDO products, the Italian regulation, in contrast with the EU food legislation, does not allow the use of powdered derivatives in any type of cheese (L.11 aprile 1974, n. 138). Unfortunately, milk adulteration by powdered derivative seems very difficult to detect because the adulterant material has exactly the same chemical composition. Milk and milk proteins powder production is mainly based on the spray drying technology, where milk or milk protein concentrate obtained by micro/ultrafiltration is atomized by a hot air stream. As a consequence of the thermal stress on milk constituents, the occurrence of some protein modifications (e.g., glycation, oxidation, dehydration) is possible. Among the analytical methods employed for the milk adulteration investigation, the most used are the HPLC and GC for quantitative determination of furosine produced from the acid hydrolysis of lactosylated
proteins [Siciliano et al.,]. Few studies have been dedicated to the detection of milk adulteration considering the protein modifications occurred after thermal processes in powder milk [Cozzolino et al.,]. In this work MALDI-ToF MS was employed to analyze the tryptic digests relevant to samples of commercial cow milk, raw cow milk and powdered cow milk. The analysis was carried out on liquid milk samples additioned at different percentages by powdered milk. In all the samples, the whey and casein fractions were separated and subjected to tryptic digestion. The samples were also subjected to 2D-gel electrophoresis, digested in-gel and pre-treated with an Affi-gel resin to enrich lactosylated peptides before mass spectrometry analysis. Some diagnostic peptides of powdered milk, attributed to modified proteins, were identified.

Food quality control: Application of near infrared spectroscopy for dried egg-pasta characterization

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Abstract
Food quality control is not an optional extra in food processing; neither is it something done only by large manufacturers. It is an essential component of any food processing business. Quality control need not be time consuming or expensive, and the results of quality control tests should help save money in the long run. In general, quality control procedures should be as simple as possible and only give the required amount of information. Quality control is used to predict the quality of the processed food and then control the process so that the expected quality is achieved for every batch. This means that quality specifications must be written and agreed with suppliers or sellers, and control points must be identified in the process. Today pasta has become a dietary staple all over the world. Dried egg-pasta is important in the market, since the range of about 50 different dried egg pasta shapes reflect traditional regional Italian cuisine. The success is due to the unique characteristic that dried egg-pasta looks and tastes like home made and is available in many unusual shapes and sizes. When considering dried egg-pasta, three are the main parameters which can affect the quality of the final product, drying time and temperature, and the amount of eggs used. Indeed, on one hand, thermal processes, have an influence on the quality of pasta on a macromolecular level due to reciprocal interactions between proteins and starch. In particular, changes in dried and in cooked pasta structure were determined regarding protein solubility, thermal properties and digestibility of starch, microscopic and rheological measurements. On the other hand, the color, taste, flavor, texture and cooking properties of different dry pasta products are determined primarily, besides the quality of ingredients used, by the quantity of eggs added. Based on these considerations, in this study the possibility of using NIR spectroscopy as a rapid and non destructive tool to assess dried egg pasta quality was investigated, by determining the influence of the three main parameters (egg percent amount, drying temperature and drying time) on the spectroscopic fingerprint of the final product. Reference pasta samples were prepared with different egg percent concentration (20%, 22%, 25%, 27%, 30% and 33%), and different drying temperatures and times were tested. The results show that all of the three parameters have a relevant impact on the shape of the spectroscopic signal. Therefore, NIR spectroscopy appears a very promising tool to be applied at-line in pasta industry since it is able to monitor the modifications induced by the change of each considered parameter. A similar approach has never been reported in the literature, where only one study can be found and it is simply voted to the egg percent determination of few commercial samples.
Study on active compounds in cow, buffalo and goat butter

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Abstract
Among the dietary fats, the butter from cow’s milk is the most widely consumed in Italy but recently buffalo and goat butter can be also found in food market. These products very similar in proximate composition show interesting differences in minor components. Butter, made primarily from the fat fraction of milk, differs in fatty acid composition, fat soluble, vitamins and cholesterol contents. The consumer’s choice is based mainly on the different colour, due to the presence (cow) or absence (buffalo and goat) of carotenoid molecules and flavour (C4:0-C10:0 fatty acids). The aim of this research was to evaluate in 14 butters from cow’s milk (Italian origin), 2 butters from cow’s milk (North Europe), 1 goat butter and 1 buffalo butter, purchased in local market, some natural functional compounds and colour parameters. Among the active compounds alpha tocopherol, retinol isomers and beta carotene were determined by HPLC method (Panfili et al., 1994), ethyl ester of beta-apo-8’-carotenic acid (sum of all substances present in an extract of sample, which absorb light at 440 nm) was determined by an official spectrophotometric method (Reg. CE, 2008) and fatty acids were determined by GC-FID according to Prandini et al. (2007). The results showed that Italian cow butter were different (P<0.05) from North European ones in all molecules studied: beta carotene 122.6µg/100g Italian butter vs 328.7µg/100g North European butter; alpha tocopherol 1516.7µg/100g Italian butter vs 1995.6µg/100g North European butter; ethyl ester of beta-apo-8’-carotenic acid 2.8µg/g Italian butter vs 5.1µg/g North European butter. As expected, in goat and buffalo butter beta carotene was absent. All the results of ethyl ester of beta-apo-8’-carotenic acid, were compared with b*(D65), a colour parameter, the correlation was quite high (R²=0.86). The C4:0, functional short fatty acid that exerts numerous anti-inflammatory effects, was higher in buffalo and cow than in goat butter (3.5; 3.1 and 1.6 g/100g fatty acid respectively); the amount of C10:0 was higher in goat than in cow and buffalo butter (7.5; 2.9 and 1.9 g/100g fatty acid respectively), while C18:2 conjugated linoleic acid isomers were higher in buffalo than in goat and cow butter (1.0; 0.7 and 0.7 g/100g fatty acid).

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Characterization of some wild edible plants gathered in Umbria

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Abstract
Up to a relatively recent past, the use of wild edible plants in human nutrition has been an important sustenance for especially rural people who had inherited from past generations the art of their gathering and use. Still considered "the reference diet", by the entire International Scientific Community, the Mediterranean Diet claims the prevailing consumption of plant food, like the “country herbs”, for their nutritional and health benefits. This work is grounded in a renewed consumer interest in respect of agricultural products and foods of the rural tradition, such as wild edible species, which could provide a valuable contribution to rebalance the dietary irrationalities generated in recent years by changes in eating habits. Therefore, we proceeded characterizing the chemical composition (1), the main components with antioxidant activity and the total antioxidant capacity (2) on some "country herbs" among the best known locally and about whose little or nothing is found in nutritional scientific literature.
Comparative study on carotenoid composition of milling fractions and bread samples of three Italian durum wheat cultivars
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Abstract
In this study, carotenoid composition in milling fractions of three Italian durum wheat cultivars was assessed, in order to evaluate the effect of milling process on the distribution of these bioactive molecules. Moreover, durum wheat semolina was processed into breads, to study carotenoid stability during bread making. Total yellow pigment content was also investigated, according to ICC Standard Method No. 152, to evaluate the extent to which carotenoids contribute to the characteristic yellow colour of wheat products. The qualitative and quantitative characterization of considerable carotenoids i.e., lutein and zeaxanthin, was carried out by an efficient RP-HPLC method. The analytical determination, following saponification and extraction processes, enabled to separate these bioactive molecules and to identify them by comparing retention time and UV/VIS spectra of standard and sample chromatograms. Regression equations were used to quantify carotenoids in samples, and statistical analysis was performed on HPLC data to determine the significance of different results for raw-materials and end-products. Data showed that carotenoids are unevenly distributed in milling fractions, and this finding may be of nutritional relevance due to their healthy properties. All-trans lutein was the main carotenoid in all fractions and the highest content was found in coarse semolina, whereas, as regards zeaxanthin, coarse bran was the richest milling fraction. A moderate decrease of carotenoid concentration was determined in bread samples. The mismatch between yellow pigment and carotenoid content strengthened the hypothesis that not yet identified compounds strongly contribute to the yellowish colour of durum wheat products.

Estrogenic activity of isoflavonoid-rich red Cuban propolis
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Abstract
Propolis is a resinous hive product collected by honeybees from various plant sources and is used to seal holes in their honeycombs, smooth out the internal walls, and protect the entrance against intruders. Propolis is widely used in traditional medicine and is reported to have a broad spectrum of pharmacological properties. Besides its uses in traditional medicine, it has recently gained popularity as a health food supplement and is used extensively in foods and beverages in various parts of the world, where it is claimed to improve health and prevent diseases such as inflammation, heart disease, diabetes, and even cancer. Propolis contains sticky plant substances mixed with beeswax and other bee secretions, and its chemical composition is qualitatively and quantitatively variable, depending on the vegetation in the area from which it was collected. Chemical investigation by means of spectroscopic analysis techniques of a red Cuban propolis sample, allowed the elucidation of isoflavonoids (isoflavones, isoflavans, and pterocarpans), together with isoliquiritigenin, and (-)-liquiritigenin. This group of natural compounds has a very restricted distribution in the plant kingdom and occurs almost exclusively in legumes (Leguminosae family), with soybeans, chickpeas, and lentils representing the major dietary sources. Dietary consumption of foods and food additives containing isoflavone phytoestrogens has been associated with a variety of health benefits, including relief from symptoms of menopause and reduced risk of hormonal cancer, osteoporosis, and coronary heart diseases. The potential estrogenic activity of red Cuban propolis was investigated in vivo using the immature rat uterotrophic effect. Effects on uterine wet weight gain were examined using female rats treated subcutaneously with propolis (50, 250 y 500 mg/kg of body weights). The positive control agent was 17β estradiol. Animals treated with propolis (I, II, III group) exhibited decrease in uterine wet weight, in comparison with positive control (IV group) and negative control (V group). The results suggest that red propolis rich in isoflavonoid produces estrogenic effects on estrogen receptors.
**Exploitation of synthetic DNA to monitor real-time PCR assay quality in food analysis**

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**Abstract**

Real-time PCR is the method of choice for DNA quantification also for GMO or pathogen quantification in various food matrices. Analytical parameters of the assay are the basic prerequisite for its use as a reliable analytical procedure. Several factors could affect these parameters, among them the reaction efficiency. Reaction efficiency plays the main role for precise DNA quantification. If the reaction is optimised well, reaction efficiency reflects almost just a purity of DNA used in the reaction. Resulting DNA quantification could be thus affected depend on the DNA source, DNA extraction method or DNA purification method and from that follows occurrence of PCR inhibitors or enhancers. Namely food matrices are complex and may contain chemicals affecting final results of analysis. Usually, occurrence of the real-time PCR inhibition is evaluated by 10-times dilution of analysed sample, when the signal decrease should be around 3.3 Ct. However, that approach is not sophisticated and is not applicable in cases of low amount of analysed DNA in the reaction, when another dilution of sample is impossible. We introduced a new approach for detection of real-time PCR inhibitors/enhancers based on the artificial control DNA sequence, inserted into a plasmid vector. Artificial DNA sequence was designed in a way excluding any homology with known either microorganism or animal and plant sequences. Primers and probe for real-time PCR quantification of artificial insert were designed and reaction was optimized and analytical parameters were determined (PCR efficiency, LOD, LOQ). We showed that the plasmid is suited to be used to check: 1) linearity range of real-time PCR assay in any reaction mixture; 2) detection or quantification limit of any PCR assay in any reaction mixture; 3) detection of inhibitors or enhancers of the reaction. As the DNA sequence inserted in the plasmid does not occur according our knowledge in the nature, such plasmid could be used easily for quality monitoring in control and research laboratories.

**Cultivar based selection of strawberry fruits with high levels of health promoting compounds**

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**Abstract**

The strawberry is the most popular and consumed type of berry fruit for its sweet flavor different in flavor, size and texture. In previous study strawberry secondary metabolites showed protective antioxidant, antiinflammatory and antiproliferative capacities (Zhang et al. 2008). Anthocyanins are the most important type of polyphenols in strawberry, and it was demonstrated as the genetic background may play a pivotal role in the secondary metabolites biosynthesis and accumulation, hence the important to investigate the plant genetic background (Lopes-da-Silva et al., 2007). The aim of this work was to study 20 different strawberry genotypes from genetic and metabolomic point of view. We measured 12 different secondary metabolite, total phenolic contents, and extract radical scavenging activity thanks to the DPPH test. Genetic relationships among strawberry genotypes were also underlined using RAPD markers (Kapteyn et al., 2002; Milella et al. 2006). Strawberry genotypes showed high differences in their total antioxidant activity and metabolite content. For the genetic analysis a total of 32 decamer primers were used and total of 124 bands were detected. In order to study the influence of the genetic basis of each cultivar on the chemical composition of fruits, data were processed for Analysis of Variance (ANOVA), the least significant difference (LSD) test and Principal Component Analysis (PCA). Dendrograms were also built to compare chemical and genetic results. This aspect provides a valid opportunity to choose varieties rich in nutraceutical moieties. The results of the present study highlighted that health-promoting compounds and molecular biology technique provided a good prospect for discriminating strawberry fruits by cultivar and genetic analysis and that it can be suitable to discriminate among cultivar with different health promoting compounds.
Characterization of confectionary products containing chocolate and hazelnuts

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Abstract
Chocolate is one of the most relevant confectionary product in Piemonte (Italy) where it is traditionally processed together with hazelnuts for the making of numerous famous products like the chocolate candy called “Gianduiotto”, known also at international level. The development of GC/MS analytical techniques has allowed to detect, both in cocoa and hazelnuts, hundreds of substances and in particular those defining the aromatic profiles; the combination of these flavor molecules can influence consumer preference. The two raw materials are subjected to roasting which is fundamental for the definition of the concentration of good or bad aromatic molecules as regard products taste and consumer safety. Moreover chocolate is subjected to significant further modification during the conching process. The analysis of numerous conched chocolate samples and roasted hazelnuts has allowed to point out the fundamental aromatic constituents and to undertake a chemical interpretation of the final product taste. Indeed some chocolate components have a typical aroma of dried fruit or roasted which can combine analogous compounds found in roasted hazelnuts. It has been experienced that the concentrations of these products, also those unpleasant, in the raw materials can be controlled by means of the thermal operations conditions. The main aim of the present study is to define the operational conditions for the achievement of the best aromatic profiles in relation with the desired final product; it is based on an extensive analytical activity. In particular, the preparation of the sample, submitted to GC/MS analysis, is the original and qualifying key point also because in the scientific literature there is limited published information. The technical application of the analytical procedure is extensively described and discussed in the full work.

Prediction of geographical and entomological origin of pot honey by NMR

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Abstract
Honey is a very complex food, produced by bees from the nectar of blossoming plants or exudates secreted by other insects such as aphid or plant sap-sucking insects. Consequently, its properties and composition depend on bee species, species of the nectar-providing plants, geographic areas, weather conditions, mode of storage and even harvesting technology and conditions. Consumer choice is linked to unique organoleptic and aromatic properties of honey that depend principally on the botanical and geographical origins of the product. For this reason, origin assessment is important in quality control of honey. In this study ¹H-Nuclear Magnetic Resonance (NMR) spectroscopy was used to analyze pot honey samples of different geographical and entomological origin. An NMR-based metabolomic approach, applied to 67 samples, tested and confirmed the validity of the multivariate statistical analysis in the discrimination. We developed an efficient tool to differentiate the honeys by their geographical origin; additionally, we showed that within limited geographical areas it is possible to distinguish honey samples in terms of the bee species that produced it. Furthermore, the structural identification of a geographical marker compound (in the Brazilian honeys) was achieved, and a specific region in the NMR spectrum was found to be responsible for the entomological separation.
Abstract
The use of flavour enhancers in the food industry could be beneficial for several reasons: they ensure homogeneity of the final products, reduce costs for condiments and favor consumer's acceptance. On the other hand, the consumer's attention for convenient, minimally-processed, nutritious, healthy, yet tasty food prompts the food industry to an accurate choice of the ingredients. In this scenario, naturally occurring kokumi substances could play an important role. Kokumi is a japanese term that refers to mouthfulness, thickness and long-lasting savory sensations. Kokumi substances are represented mainly by γ-glutamyl derivatives of amino acids. They are nearly tasteless for themselves, but they elicit a strong taste sensation, especially in conjunction with protein-rich food [Dunkel 2007]. In vegetables of the genus Allium, kokumi substances were identified in γ-glutamyl derivatives of S-alkyl and S-alkenyl cysteines and their S-oxides [Ueda 1990]. There is a number of difficulties connected with the supplying of these materials. Isolation from natural sources is laborious, and their content in vegetables varies with cultivation and storage. In addition, upon crushing the plant, they are enzymatically degraded. The chemical synthesis is not economical, due to the need of protection/deprotection steps. We exploited recently the enzymatic synthesis at the laboratory scale of the γ-glutamyl derivatives of S-allyl cysteine, S-methyl cysteine and methionine, catalyzed by a commercially available mammalian GGT [Speranza 2012]. In this communication we report that such flavour enhancers can be obtained as well by using a purified, home-made bacterial GGT from Bacillus subtilis, a GRAS (Generally Recognized As Safe) organism, suited to food processing.

Production of high added-value peptides from hempseed proteins
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Abstract
Hydrolysates Vegetable Proteins (HVPs) are raising a tremendous interest due to their content of bioactive peptides having important nutraceutical properties. HVPs are produced by chemical and/or enzymatic hydrolysis of vegetable raw materials rich in proteins, such as wheat, soybean, sunflower. We have produced and investigated peptides from hempseed (Cannabis sativa L.) proteins because of their potential in different segments of food industry. Protein content of hempseed is comparable to soy’s one and it is reported to be far more digestible and nutritional in all essential amino acids. Hempseed have been ground and defatted by repeated extraction with n-hexane. Resulting defatted hempseed cake was then hydrolysed using two commercial food grade protease cocktails, Flavourzyme and Umanizyme. Hempseed protein hydrolysates were subjected to ultrafiltration and the antioxidant activity of the fractions <10 kDa was measured by the DPPH radical-scavenging assay and the ferrozine test. A few samples displayed a remarkable Fe2+ chelating ability and were found to exert radical scavenging properties comparable to those of glutathione. Preliminary results suggest that enzymatic hydrolysis of hempseed proteins can be efficiently used to produce high added-value products. Not less important, this approach allows exploiting a usually discarded industrial waste as a valuable source of proteins for human nutrition. Current studies are being focused on identification of peptides mainly responsible for biological activity as well as on assessment of novel nutraceutical and food properties.
The chemical controls, chemical-physical and microbiological in the production of mineral waters, from the fount to the consumer

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Abstract

Are examined in this work the quality control in the production of mineral waters, from natural sources, that the capture, through the production facilities, are packaged for consumers. The definition of "mineral water" comes from far more ancient thermal waters, which were used for treatment outside of the body, only for bathing. Only since about 1300, by the "first doctors", among which he distinguished Ugolino da Montecatini with the book "De Balneis", there are traces of the use of these thermal waters for drinking cures. This extension of the use of mineral water drinking therapy has necessarily determined the discipline relating to the new definition of "mineral water" and monitoring of chemical and microbiological testing, these are food for human consumption. In this note examines the controls responsible for proper maintenance of catchment areas, starting from piezometric surveys, ending with the same chemical and microbiological investigations of water sources. These controls, in accordance with the regulations, providing a "quality management" based on the retention characteristics of the spring, the proper practice for packaging, according to the analytical to the dictates of legislation, are established to protect the consumer. Subsequently, are examined the packaging materials, provided by the EEC regulation 19/2007, suitable for bottling of mineral waters, describing the characteristics of the most used of these materials, polyethylene terephthalate (PET). The extrusion of this polymeric material, determines, through controlled reaction, the formation of acetaldehyde, which may confer mineral water a strong flavor of rowanberries: is described a method of controlling gas chromatography for its determination, that in the mineral water does not exceed a few ppb. The controls are also extended to the seals of the caps, which should not contain esters of phthalic acid. These checks are performed with SPME (solid phase microextraction) also on the water, packed on pallets and places of storage, since the polyester polymer permeable to environmental vapors. Because in the phase of formation of the PET is used as the catalyst, antimony trioxide, is carried out such a control of water, kept at 40°C for ten days, using the AA spectrophotometry or ICP. (They are shown in power point graphs obtained by mass spectrometry GCMS).

Determination of volatiles in italian red pepper (Capsicum annuum L.) powder by HS-SPME/GC-MS technique

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Abstract

Red pepper fruits from genus Capsicum annuum L. are some of the most popular plant foods worldwide cultivated. The adaptation to many different regions and human taste preferences contributed to their diffusion and diversity of uses as these fruits can be consumed as fresh vegetable or used, after appropriate technological treatments, as a spice [Cremer and Eichner, 2011; Rodriguez-Burruezo et al. 2010]. Their powder is one of the most widely utilized food colourants, both for culinary and industrial purposes, because of its particular characteristics such as pungency, colouring and also flavouring capacities [Topuz et al. 2011]. To make paprika, the dehydrated and milled pepper fruit, able to be received and used by food industry, some quality, chemical and physical parameters had to be satisfied like colour, aspect, odour, taste, umidity, ashes, hotness, presence/absence of added colourants (Sudan I, II, III, IV), foreign matter and pesticides. In this work, a further parameter was proposed to characterized quality properties of this product. The aromatic profile of six pepper powders and two blends of these, coming from a totally Italian production, was analysed by HS-SPME/GC-MS technique, showing the presence of volatile compounds from varietal origin and also due to dehydration processes.
Development of a new disposable electrochemical sensor modified with Au and carbon black nanoparticles for the determination of As in drinking water

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Abstract
Arsenic is recognized as one of the most harmful elements to the environment and human health. The toxicity of arsenic depends on its chemical speciation, generally inorganic arsenic is more toxic than the organic one. Moreover, the As (III) is considered more harmful than the AS (V). The analytical techniques most commonly used for the determination of arsenic are the atomic absorption spectrometry with generation of hydrides (HG-AAS) and mass spectrometry coupled plasma source (ICP-MS). The purpose of this work was the development and evaluation of the efficiency of a screen printed sensor (SPE) modified with carbon black and gold nanoparticles for the determination of As (III) in water samples by anodic stripping voltammetry. The large surface area of carbon black was found to be crucial because it allowed an uniform gold nanoparticles film on the electrode surface, which, in the absence of carbon black, could create aggregates therefore decreasing the electrochemically active. The method was carefully optimized, obtaining a detection limit, estimated as three times the standard deviation of the blank, of 1.83 μg/L, far lower the legal limit (10 μg/L) and an operating linearity range up to 300 μg/L. Analyses of drinking water did not evidence the presence of As (III), but recovery studies, carried out adding 10 μg/L of As to drinking water samples ranged between 81-105 %. The advantages of this technique consist in: the simplicity (samples do not require complex pre-treatment, but it is enough to acidify and add ascorbic acid); the low cost (tools for voltammetry cost less than buying and managing the ICP-MS and HG-AAS); the time of analysis (the determination of As (III) in a real sample requires little more than twenty minutes) as well as to the possibility of application in situ. Another feature of this technique is the ability to analyze selectively arsenic in oxidation state +3, considering its greater toxicity compared to the As (V). However, the legal limit for the concentration of arsenic allowed in drinking water refers to total arsenic, so future work will focus on developing a methodology that is able to determine the inorganic arsenic present in oxidation states (III) and (V).

Mass spectrometric elucidation of carotenoids in chili red peppers using ultra high pressure comprehensive two-dimensional liquid chromatography

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Abstract
Some of the important ingredients in paprika extracts are carotenoids formed in the fruit during ripening. These compounds are not only used in the food industry but also in pharmaceutical and cosmetic products. A total of 33 different compounds have been separated and identified in red chilli peppers. A comprehensive normal-phase x reversed-phase (NP-LC×RP-LC) liquid chromatographic system was developed, and applied for analysis of the intact carotenoid composition of chili red peppers, with PDA (photodiode array) and MS (mass spectrometry). The combination of ion trap technology coupled with accurate mass measurements was used for the identification and classification of the different compounds. A micro-bore cyanic column (250x1.0 mm, 5 mm d.p.) was chosen for the first dimension separation, interfaced to a secondary (2D) C18 column (30x4.6 mm, 2.7 mm d.p.) packed with fused-core particles. Subsequently, two columns of the same stationary phase were coupled serially for second dimension separation, and operated under UHPLC (ultra high pressure LC) conditions, within a cycle time of 90 or 60 sec. The advantages of the latter set-up over the single-column one are demonstrated by the higher peak capacity values (ne 836 or 1325 vs. 571); especially for the separation of the more polar chemical classes (mono-esters and free carotenoids), better focusing at the head of the 2D column resulted in improved separation.
Multi-class pesticide analysis in challenging vegetable matrices using fast 5msec MRM with 15 msec polarity switching

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Abstract
Over one hundred pesticides spiked into complex vegetable matrices at 5 ppb were successfully detected in a single analysis. Many regulatory authorities have established multi-class residual pesticides methods for the analysis of vegetables, fruits and other food stuffs. However, there is no global agreement on the provision of a target list of pesticides and this presents a risk with products moving between different regulatory requirements. In order to eliminate this risk, food safety laboratories need to ideally screen as many compounds as possible in a single run which may reach maximum residual limits (MRL); typically 10ppb in food matrices. In this study we report the application of ultra-fast 5msec SRM with 15 msec polarity switching for the analysis of 138 pesticides, while still obtaining excellent LOQs for the majority of compound < 10 ppb. Leek and Paprika (yellow and red) were purchased from a local Japanese grocery store, with the country of origin Japan (Leek) and New Zealand (Paprika). Sample preparation was carried out by the use of a quick, easy, cheap, effective, rugged and safe (QuEChERs) method. A triple quadruple mass spectrometer (8030, Shimadzu, Japan) with modified ion optics was used to monitor 276 SRM transitions (two SRMs per compound). Each transition was monitored ±0.8 minutes of the target analytes retention time. The maximum loop time was 0.58 seconds and the maximum number of overlapped MRM transitions was 98 (Dwell time: 5 msec, Pause time: 1 msec).

Quality control and stability over time of a composed hydroalcoholic tincture based on curcuma radix, rosemary, boldo and maple

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Abstract
The tinctures are liquid formulations, usually obtained by fresh plants after maceration in hydro alcoholic solvents. These products are widely diffuse on the market and, with the exception of the mother tinctures that are included in the homeopathic medicines, all the others obtained by macerating the dried herbal drug in a hydro alcoholic solvent, can be considered foods. Their composition is strongly related to the quality of the raw material, to the extraction processes and to the stability over time of the main components. Aim of this work was to evaluate the content of the bioactive metabolites in composed hydro alcoholic tincture (CHT) constituted by four plant extracts: curcuma radix together with boldo and rosemary leaves and maple buds. Notably, this product, used as food supplement, was designed to facilitate and improve the physiological functions of liver and gall bladder. An HPLC/DAD/MS analytical procedure was developed to control the composition of the (CHT) tincture and the chromatographic profiles of each single plant extract in order to well detect the major metabolites. As expected the composition of this product was complex and characterized by the co-presence of more than 20 different phenolic compounds; the principal molecules were curcumin and demetossi e bisdesmetossi derivatives and also the contribute of the boldo leaves, with the glucorhamnosides of kaempherol, quercetin and isorhamnetin, was highlighted but without the detection of the aporfinic alkaloid, boldine. On the opposite, the absence of rosmarinic and carnosic acids, typical markers of the rosemary leaves, was also confirmed by the EI technique. Mainly due to the limited studies on maple composition, any marker was identified to recognize the presence of this plant in the CHT. Successively, without apply accelerated ageing protocols not suitable for these liquid forms, the stability over time was evaluated for CHT at ambient temperature up to 18 months highlighting an evident reduction of the curcuminoids content while the flavonoids were more stable. This study contribute to reduce the gap that exist in this area mainly due to the scant data available on the composition of the tinctures but almost all to their stability over time.
Antimicrobial activity of phenolic extracts from leaves of *Rosmarinus officinalis* L.

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Abstract

The aim of this study was to investigate the antimicrobial activity of three ethanolic extracts from rosemary leaves, containing phenolic compounds. The antimicrobial activity was tested against *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 82221), *Escherichia coli* (ATCC 10536) and *Pseudomonas aeruginosa* (ATCC 15442).

The antimicrobial activity of these extracts was determined through the agar disc diffusion with serial dilution of 100%, 75% and 50% (100% corresponds to 1mg/mL of rosmarinic acid); it was also determined the antimicrobial activity of the standards carnosic acid and rosmarinic acid, which are the main constituents of these phenolic fraction. The minimum bactericidal concentration (MBC) as the highest dilution at which no growth occurred was estimated by the broth dilution method with concentrations ranged from 90% to 10%. All the samples tested by broth dilution method showed an higher activity compared to agar disc diffusion method. In detail, carnosic acid showed the highest activity with broth dilution method, while the activity of rosmarinic acid was not significant. The distribution of the phenolic components in these extracts was variable because they were obtained from leaves at different stage of development, and the carnosic acid, belonging to non-volatile terpenoids, was poorly present or absent. These extracts showed similar antimicrobial activity against all the microbial strains tested and comparable with the results from carnosic acid. It is interesting that the extract containing the highest concentration of flavonoids, but less concentration of total polyphenols and absence of carnosic acid showed an inhibitory activity comparable to the other two extracts. Our results suggest that the flavonoids from Rosmarinus officinalis leaves can contribute to the antimicrobial activity and probably have a synergetic effect with the other co-present phenolic compounds.

HPTLC synoptic profiling for a rapid characterization of the phenolic fraction of extra virgin olive oils

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Abstract

Extra virgin olive oil is indubitably the main lipid source in the diet of the Mediterranean countries. The hypothesis that minor components such as phenolic compounds could play a major role in its healthy effects has been increasing during these last years. Numerous are the phenolic compounds identified in the olive fruit and most of them are in glycosilated forms. During the milling process these compounds are strongly modified, the non glycosidic forms are dissolved in the oil phase, but only a small percentage (≤1%) are transferred to the virgin olive oil during the production process. The minor polar compounds of the EVOOs are a complex fraction including simple phenols, secoiridoids, lignans, and flavonoids, and the analytical profile of this fraction could be elected as a significant marker for assessing oil characteristics of different cultivars and for controlling oil production processes. The phenolic fraction influences the organoleptic virgin olive oil properties (mainly bitterness and pungency) and acts as primary antioxidants to preserve the product against the auto-oxidation processes. Therefore a rapid method aiming to evaluate the above mentioned features should be highly desirable. In this work we developed an HPTLC method on a C18 wettable reverse phase to obtain a fingerprint of the extra virgin olive oil phenolic fraction and to evaluate the antioxidant contribution of each phenol with a DPPH in situ derivatization. The main secoiridoids, the minor flavonoids and the lignans present in the phenolic fraction of different Tuscan EVOOs were recognized and identified by their UV-Vis and mass spectra measured respectively in situ and after a selective desorption from the stationary phase by a TLC-MS Interface. This approach allowed to highlight the differences among different samples obtaining specific densitometric profiles not only related to the chemical structures of these molecules but also to their antioxidant potency.
Antifungal activity against Candida spp. of phenolic extracts from leaves of Rosmarinus officinalis L.

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Abstract

Actually candida infections are an important problem for public health, because the wide diffusion, the increasing drug resistance and the adverse effect of usual antifungal treatments. The aim of this study was to determine the antifungal activity of a phenolic extract from leaves of Rosmarinus officinalis L. against Candida spp. The fungal strains tested are C. krusei (ATCC 6258), C. albicans (ATCC 90028 and ATCC 10231), and C. dubliniensis and C. albicans both coming from clinical samples. At present there are no data about an antifungal activity of these extracts. The antifungal activity of the extract was determined through the agar disc diffusion with serial dilution of 100%, 75% and 50% (100% corresponds to 1 mg/mL of rosmarinic acid); it was also determined the antifungal activity of the standards carnosic acid and rosmarinic acid, which are the main constituents of the extract. The chemical characterization of the sample showed that for 1 mg of rosmarinic acid there were 1.04 mg of total flavonoids and 7.2 mg of terpenes (carnosic acid and derivatives). The minimum bactericidal concentration (MBC) as the highest dilution at which no growth occurred, was estimated by the broth dilution method with concentrations ranged from 90% to 10%. The results obtained with both the methods (agar disk diffusion and broth dilution) are very different probably because a different sample distribution in the two media. The inhibitory activity was detectable only by the method with broth dilution and carnosic acid showed more effective than rosmarinic acid. All the fungal strains tested showed sensitivity to carnosic acid at concentration of 40% and 50%, while only three fungal strains were inhibited from rosmarinic acid but at a high concentration (90%). The tested phenolic extract showed an inhibitory activity at low concentration ranged from 40% to 20%. These fungal species are often resistant to traditional drugs, but the moderate activity of these natural extracts against Candida spp. suggest to investigate further with the object to show possible synergisms among the components of with the traditional antifungal drugs.

Fast determination of D-amino acids in cheese extracts with a coated chiral ligand-exchange chromatography stationary phase

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Abstract

Due to the recognized difference in the physiological effect of many amino acid enantiomers (Friedman 2010), and also for marking out the food quality and origin as well as the impact of human intervention, the exact knowledge of their presence and relative ratio in foodstuffs, is increasingly considered an hot topic. In this scenery, we became interested in identifying the presence of D-amino acids in a selected set of Spanish cheese samples. With the aim to rely upon a rapid, direct and easy-to-set-up chromatographic procedure, we analyzed six cheese extracts with a chiral ligand-exchange chromatography-based chiral stationary phase (CLEC-CSP). The CLEC analyses were run without any pre- or post-column derivatization of the extracted amino acidic mixture. An appreciable enantioselectivity along with a profitable chemoselectivity was at once achieved with the use of a physically coated CSP (C-CSP) based on the S-trityl-L-cysteine (L-STC) as the chiral selector (Natalini 2008, Natalini 2009). With the established CLEC-CSP procedure, the presence of D-alanine, D-aspartic acid and D-glutamic acid was diagnosed in all the analyzed samples and then confirmed via conventional chiral gas chromatographic (CGC) analysis. A certain degree of peak overlapping was found to be the main drawback of the simplified sample analysis, which is nevertheless balanced by the advantages of the rapid detection. Indeed, although pre-analysis purification steps of the extracted material must be carefully optimized, the adopted method bears high potential in the control of the natural and artificial ageing process as well as the preservative treatments of the analysed products.
The extractor naviglio in food productions

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Abstract
For over a decade the Extractor Naviglio has been a good alternative to solid-liquid extraction techniques such as maceration and percolation. That has been broadly demonstrated since the extractor provides the same quality or even higher of the extracts obtained by the traditional extraction techniques, with a significant reduction in the duration of the extraction process (ten days of maceration extraction correspond to about one hour of extraction by the Extractor Naviglio under the same extractive conditions) and a more efficient extraction. The principle on which it works (Naviglio’s Principle) is studied in graduate courses in Herbal Techniques of several Italian Universities. Currently the Extractor Naviglio is widely used in many fields of research and production (herbals, nutritional supplements, cosmetics, beverages etc.). In the food sector, in particular, the Extractor Naviglio has been shown to be a viable alternative to maceration for: production of lemon liquor (limoncello) and similar liquors; production of bitters and elixir of juniper; rapid aging of wines, brandies and distilled liquors; extraction of lycopene from tomato processing waste. Recently, more unconventional applications of the Extractor Naviglio have been studied, as the rapid rehydration of legumes and their simultaneous aromatization, cleaning of washers for the production of cork stoppers, cleaning of rubber polymers, tanning of leather. Finally, many other applications and uses of the Extractor Naviglio are being studied in our laboratory.

Liquid chromatography tandem mass spectrometry confirmatory method for beta-agonist residues in liver, urine, hair and retina

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Abstract
Beta-agonists are phenyl/ethanolamine-derived adrenergic drugs. These compounds are mainly used in human and veterinary medicine as bronchodilator and tocolytic agents, respectively. Moreover, they are also illegally used as growth promoters in livestock production due to the anabolic effects exerted when higher doses are administered to animals. The European Union banned the use of beta agonists in food-producing animals since 1996, except for tocolysis in mares and cows and bronchodilation in horses. The aim of the present work is the development of a fast and reliable analytical multi-residue method for the confirmation of beta-agonists residues in liver, urine, hair and retina. It is based on hydrolysis, SPE clean up and liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis of different matrices samples. Validation was performed according to Commission Decision 2002/657/EC [1] and ISO 17025 requirements. Independent samples spiked with the investigated drugs at 0.5, 1.0, 2.0 and µg Kg⁻¹ are showing average recoveries, were always higher than 70.5% and intra-day and inter-day precision (RSD) lower than 12.2 % and 16.8 %, respectively for all matrices taken in consideration. Drugs linearity in the range of 0.5 - 5.0 µg Kg⁻¹, resulted with r > 0.998. The decision limits (CCα) for the all investigated beta agonists resulted in the range of 0.6 – 1.5 µg Kg⁻¹. For forensic purposes liver, urine, hair and retina seems to be the biological fluid most suitable for beta agonists detection, therefore this method is suitable for laboratories involved in official controls.
Influence of puréeing and stabilization processes on the antioxidant properties of a new ingredient based on rose petals

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Abstract
In previous studies the aromatic (Bianchi et al., 2007; Nuzzi et al., 2009), antioxidant and technological properties (Nuzzi et al., 2011) of rose petals of some cultivars organically grown in Italy were investigated in order to create a new healthy ingredient. In the present work, petals of genotypes Jeoff Hamilton®, Eglantyne® (English roses), Chianti, Belle Poitevine, Roseraie de l’Hay (Old roses) were processed in duplicate to get a flower purée using a new recipe containing raw petals/lemon juice/sugar/water (15/5/40/40 w/w). Raw petals were homogenized with a sucrose solution, and lemon juice was added to reduce pH to stabilize colour. Then, in order to preserve the original flowers’s properties, the purée was pasteurised at 85°C and at 98°C and not pasteurised purée was used as control. Total phenolic content (TPC), antioxidant capacities by DPPH and Fremy’s salt tests (spectrophotometric methods), soluble carbohydrates and organic acids by HPLC, pH, titratable acidity, soluble solids content, colour (L*a*b* values) were evaluated on raw petals, on control purée and after pasteurisation following specific methods reported in Nuzzi (2010). Old roses differed from English roses for hydrophilic TPC and DPPH test (TPC, 50.24 and DPPH, 30.23 mgGAEg⁻¹ DW) values 2 folds higher than those of English roses, while there was no difference for Fremy’s salt test (13.35 and 15.06 mMAGAEg⁻¹ DW respectively). Petal puréeing and the chemical stabilization with sucrose and citric acid by addition of lemon juice modified not also the nutritional composition, as evidenced by soluble carbohydrates and organic acids HPLC profiles, but the antioxidant capacities as well. The control purée had lower hydrophilic TPC and antioxidant capacity (TPC 103.05 mgGAE 100g⁻¹ DW, DPPH test 61.67 mgGAE 100g⁻¹ DW, Fremy’s salt test 18.53 mMAGAE 100g⁻¹ DW). Nevertheless, with purée pasteurization at both temperatures there was not a significant decrease in the antioxidant pattern, which was comparable with that of the commonly fruit-base preparations of high nutritional value used to enrich yoghurt (Plessi et al., 2007).

Induction of CYP1A in the gills and muscles of Trachurus trachurus living in the Gela coastal area (Italy)

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Abstract
With the development of industry and agriculture, the cases of cancer have been increasing gradually in the last 30 years, caused by persistent organic pollutants (POPs), especially in the aquatic ecosystem. The study area was Gulf of Gela placed along the Sicily’s southern Mediterranean coast in Italy. The environmental damage caused by the activities of the Petrochemical Center of Gela have meant that this area of Sicily has been declared ”area at high risk of environmental crisis”. Cytochrome P4501A monoxygenase has an important function in the biotransformation of many xenobiotics (1), including polynuclear aromatic hydrocarbons, and planar organochlorine compounds. The metabolism can lead to detoxification or activation to reactive intermediates and exposure of fish leads to induction response that can be identified in different organs (2,3). Immunohistochemical and morphological studies have provided qualitative information on cell and tissue distribution of CYP1A in gills and muscles of teleost fish Trachurus trachurus. In gills, the mean length of primary and secondary lamellae was found to be decreased; cellular proliferation developed with the secondary lamellae fusion, and as a result the loss in intervals between the secondary lamellae was observed. Moreover, there were alterations of cartilage cells and increase of perichondrium thickness in primary lamellae and rupture of capillaries and erythrocyte’s release. In muscle tissue were seen an increase in fibres in the interfibrillar area, cellular dissolution, cellular debris in intermyofibrillar area and a decline or loss of striation in muscle fibres. The results demonstrated an induction of CYP1A in gills and muscle of Trachurus trachurus. CYP1A is a marker of toxicity; thus the environmental pollution of Gulf of Gela is related to the petrochemical industry.
Impact of pollutants on fish collected from coastal area of Milazzo (Sicily/Italy): Histopathological study

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Abstract

The coast of Milazzo is characterized by the presence of several production activities such as the Refinery, the steelworks, the electric point station, the commercial-tourist port, the shipyards, the fishery and the agricultural plants which involving the land-sea center. The aim of this research is to make a check on the fish fauna that inhabits both coasts, the west and east, to evaluate health status of fish, creating a biological model of inquiry that can be used both for assessing the toxicity of produced water, which for the degree of environmental contamination of this coastal area Milazzo potential toxicological impact [1]. A morphological analysis on the most common sedentary fish was carried out in particular Boops boops, Lithognatus mormyrus, Diplodus annularis, used as a bioindicator fish [2]. After sampling in different parts of the coast, it is made the removal of organs of interest, then we proceeded to the preparation of durable prepared for light microscopy. Were examined intestine samples that, in fish caught in the eastern, showed a morphological picture altered, with an increase of cells with mucous hypersecretion in the ridges, disorganization of the parenchyma and disintegration of the apical cells [3, 4]. By contrast it has been observed in fish of the western coast, exposed to less impact toxicology, a least altered morphological picture.

Fish quality analysis: Bioaccumulation of pollutants in the tissues

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Abstract

Intensive fishing is practicized along the coastal area of Milazzo (Sicily, Italy) being a source of the economics and culture of this village. Also toxic compounds, mainly heavy metals, are introduced to the sea via industrial discharges that contribute to a toxicological impact to the rich fish biodiversity and biomass. Fish living in polluted waters tend to have structural damage and functional impairment of the various organs. The bioaccumulation of the pollutants occurs in various tissues in a descending order: from liver, kidney, intestine, gill and to muscle [1]. The present study is aimed to describe the effects of the polluted environment on several organs from a variety of fish species sampled along the coast of Milazzo. Tissue specimens are then fixed for routinary histology and preparations are viewed at light microscope. Both muscle and intestine preparations yielded very different results by comparing histological data. While muscle fibres show a compact structure with no losing striatation and no developing fibres in the interfibrillar area [2], intestine epithelia show an alteration of parenchymal cells with a bad damage of the surface epithelial cells, an increase of mucous secretory cells and their mucous products emptying to exterior surface and onto the microvillar ridges [3]. The results presented in this study could be used as a histological tool to give to consumers more information on the safety of fish products.
Relationship between the blend of grapes of Bonarda D.O.C. red wine from Oltrepò Pavese and red color

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Abstract
Several parameters contribute to the definition of food quality and nowadays consumers’ demand regards high food safety, absence of defects, good sensorial properties, attractive appearance, authenticity and product traceability. As regards red wine, the color is one of the key sensorial characteristic. Red wine color is due to anthocyanins that occur naturally in grapes in the form of glycosides. Anthocyanins can be classified into many types based on chemical structure modifications, such as substituent groups on the B ring, type and number of conjugated sugar, and the presence or absence of an acyl group. In grapes and red wine there are all the six main types of anthocyanins: pelargonidins, cyanidins, delphinidins, peonidins, petunidins, and malvidins. The hue of anthocyanins may vary according to different substituent groups present on the B ring, and color saturation increases with increasing number of hydroxyl groups and decreases with the addition of methoxyl groups. Under highly acidic conditions, anthocyanins assume the form of a flavylum ion, exhibit a red color, and are relatively stable. The hue of anthocyanins may vary also according to the occurrence of sulfur dioxide and interaction with metals, such as iron. Of particular interest is the reaction with sulfur dioxide in which the carbons in positions 2 and 4 have a partial positive charge and therefore can attract nucleophilic groups such as the HS0 3-sulphonic acids. The subsequent loss of charge on the flavylum system leads to loss of color. As far as metals are concerned, the role of metals in wine is very important because metals affect the organoleptic characteristics of wine, including color. Because of the reductive conditions in wine, metals are mainly present in their lower oxidation states. They may exist as free ions and complexes with organic acids, amino acids, polysaccharides, peptides, proteins, and polyphenols. Especially condensed tannins and anthocyanins are the most important metal ligands, since these species have numerous coordination sites capable of binding metal cations (e.g., cyanidin-3-glucoside anthocyanin with two -OH groups in the ortho position can complex Cu and Zn and Fe at the ratio 2:1).

In the present paper the color evaluation (performed both spectrophotometrically and with sensorial analysis) of Bonarda DOC red wine, a typical red wine produced in Oltrepò Pavese, was correlated to the chemical composition, and metal content. Furthermore, the relationship between red wine color and blend of grapes is established.

The antinitrosating action of dietary polyphenols

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Abstract
A regular intake of polyphenols widely found in fruits and vegetables is believed to decrease the incidence of certain forms of cancer, due in part to their ability to act as antinitrosating agents capable of lowering the impact of toxic nitrosation processes and carcinogenic nitrosamine formation within the acidic environment of the stomach. As a result, the study of the interactions between reactive nitrogen species derived from nitrite and phenolic antioxidants has emerged as an area of great promise for delineating innovative strategies in cancer chemoprevention and guiding the design of food supplements. This communication provides an account of the chemical mechanisms through which key food components including caffeic acid, chlorogenic acid, epigallocatechingallate, hydroxtyrosol, resveratrol and piceatannol can interact with nitrite-derived reactive nitrogen species (RNS) under conditions that model those occurring in the stomach. Typically, the phenol compounds were reacted with nitrite ions in the μM–mM concentration range in aqueous buffer at pH 2-4 and the products were isolated and structurally characterized. Analysis of product features and distribution revealed competition of different reaction pathways, including nitrosation and Nnitration but also oxidation, via electron transfer to nitrosonium ion or nitrogen dioxide. The effect of conjugation with glutathione on the antinitrosating potency was also evaluated, and the glutathionyl-piceatannol conjugate was found to be one of the most potent inhibitors in the 2,3-diaminonaphthalene assay. This finding provides new clues for the design of novel structural prototype for antinitrosating agent.
High levels of phenols with superior free radical scavenging activity in apple cultivars from Southern Italy

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Abstract
Eight genotypically characterized varieties from old apple trees of Irpinia area (Campania Region, Southern Italy) were selected and subjected to morphometric and physicochemical analysis. The phenolic composition as determined by HPLC-UV-MS analysis and the free radical scavenging activity as determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay were compared with those of standard “Annurca” and “Gold Chief ® Gold Pink*” cultivars. Significant differences were observed in the total phenol content but also in their distribution within the three main classes of polyphenols that is hydroxycinnamates, flavanols, and dihydrochalcones. “Cape ‘e Ciuccio” cultivar was prominent for the high content of all the three classes of phenols, particularly dihydrochalcones, while hydroxycinnamates and flavan-3-ols were comparable to those found in “Sergente” and “Rosa di Serino”, respectively. The reference variety “Gold Chief ®GoldPink*” showed the lowest levels for flavan-3-ols and dihydrochalcones. For all the cultivar tested flavanols represented from 67 to 97% of the total phenol content, hydroxycinnamates corresponded to the second abundant polyphenol class, accounting for 1.7% to 29.3%, dihydrochalcones ranged from 0.8% to 2.3% of total polyphenols, while flavonol glycosides had the lowest concentrations in all the varieties examined. High levels of procyanidin B2 and phloridzin, whose chemopreventive actions have amply been documented, were found in “Cape ‘e ciuccio” and “Rosa di Serino”. The percentage of reduced DPPH showed a good correlation with the total phenol content ($R= 0.79$), with the highest values ($R= 0.77$) associated, within the phenol classes, to flavan-3-ols. Varieties such as “Arancio” and “Cape ‘e Ciuccio” showed a much higher hydrogen donor activity (1.5 to 4 fold) than “Annurca” and “Gold Chief ® Gold Pink*”. This study hints to hitherto unrecognized nutritional value of ancient local varieties which may represent a source of valuable traits for genetic breeding.

Potential antigingivitis activity of shiitake mushroom (Lentinus edodes) extract

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Abstract
Gingivitis is one of the most prevalent infectious diseases of humans, affecting most of the population at some point during their lives, that has long been implicated as a potential precursor to periodontitis. It is caused by the buildup of the plaque biofilm at the gingival margin. Due to poor oral hygiene among the general population, gingivitis is prevalent and results in high treatment costs; so, the option of treating gingivitis using functional foods, which promote oral health, is an attractive one. The funding from the European Union’s Sixth Framework Programme (FP6) under the Contract no. FOOD-CT-2006-036210 (Project NUTRIDENT) let us study a number of food/beverage potentially active against both caries and gingivitis, among which shiitake mushroom that has been studied for its antioxidant, antitumor properties, and antibacterial properties, but it has not as yet been assessed for its oral health benefits. In the present study, a set of assays was used to monitor the
bacterial community structure changes within an in vitro gingivitis model and to assess the effect of shiitake mushroom extract on these communities. The comparison of the different treatments applied in the model system has given a valuable insight into the community dynamics of dental plaque as well as an indication of the efficacy of the treatments. Shiitake mushroom extract was shown to be effective at reducing the numbers of the oral pathogen *F. nucleatum*. The shiitake mushroom fractionation by chromatographic techniques has allowed the isolation of the components most responsible for the registered activities.

**Phenolic compounds in red *Cichorium intybus* vegetables**

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**Abstract**

Polyphenols are biologically active compounds widely distributed in the plant kingdom and so present in plant-derived foods and intrinsic components of human diets. Polyphenols have been shown to possess a range of biological activities, that are consistent with them contributing to the protection afforded by a diet rich in fruit and vegetables against degenerative diseases, inflammatory process, and aging, and modulating the immunity system. *Cichorium intybus* with its different varieties is a genus rich in polyphenols; in fact, it is known to contain a number of hydroxycinnamic acid derivatives and flavonoids, with anthocyanins characterizing the var. *silvestre*, but the qualitative evaluation of polyphenolic fraction of red chicories, commonly used as food, is far to be completed. As the degree of hydroxylation of polyphenols is important in determining the biological activity and the degree and type of glycosylation are important in determining the ability of these compounds to be adsorbed in humans, the aim of this work was to investigate red chicory variety largely consumed in Italian diet, characterizing the different isomeric forms of polyphenols. The investigation was carried out using HPLC-DAD-ESI-MSn. The obtained results indicated the presence of a variety of phenolic acids in their different isomeric forms, flavonols (kaempferol and quercetin derivatives), and anthocyanidins glycosilated with one or more sugar moieties.

**Diacylglycerol isomers in extra virgin olive oil: effect of different storage conditions**

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**Abstract**

Diacylglycerols (DAG) are found in virgin olive oil in low amounts (between 1 and 3%) as intermediate products in the biosynthesis of triacylglycerols (1,2-isomer) or by enzymatic or chemical hydrolysis of triacylglycerols (1,3-isomer) occurring before or during oil extraction process. It is well known that during storage the 1,3-isomers increase, and consequently the 1,2-isomers decrease. Several authors emphasized the importance of DAG fractions to the evaluation of the quality and genuineness of the virgin olive oil. In particular, Pérez-Camino et al. (2001) determined the evolution of the two isomer classes of DAG in oils obtained from olives of different quality and stored at different temperatures, as well as during the refining process; Catalano et al. (1994) and Cossignani et al. (2007), instead, evaluated the influence of temperature and time of storage on the isomerization processes. This study aimed to compare the effect of different storage conditions on the processes of isomerization of DAG. With this aim two oils deriving from two different cultivars (*Coratina* and *Ogliarola*) were stored for two years in the following storage conditions: in bottles at dark; in clear glass bottles at light; in green glass bottles at light; in bottles at dark, with repeated opening and samplings to simulate domestic use. The results (Table 1) showed that the isomerization of DAG, taking place during time, was not affected by the storage conditions. Among the parameters considered, the most suitable as an age parameter, being not affected either by storage conditions or by the cultivar, was the total DAG/1,3-DAG ratio. On the contrary, the 1,3-DAG/1,2-DAG ratio resulted to be affected, only in the initial phase of storage (6 months), by the starting DAG content of the two cultivars.
Influence of drying conditions and of semolina quality on the volatile compounds of pasta

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Abstract
Drying is the most important unit operation during pasta manufacture owing to the high capital investment in the dryers and the operation costs. The so-called high-temperature (HT) drying technology has been widely applied by pasta manufacturers, due to its advantages regarding the increased plant productivity, respect to low temperature (LT). This technology has a positive influence on the mechanical properties of pasta, such as breaking strength and surface state, but may provoke the undesired Maillard browning reaction. Yet, not only thermal treatment, but also the characteristics of semolina affect the evolution of such reactions during pasta-making. Several markers of the Maillard reaction, among which furosine is very common, have been indicated (Anese et al., 1999). Up to now, no studies have been carried out about the characterization of the profile of the volatile compounds of pasta as a function either of processing parameters or of the quality of the semolina used. The aim of this work was to compare the effect of drying temperature on the volatile compounds, determined by SPME-GC/MS, in pasta samples obtained from two different types of semolina by LT and HT drying. This, to characterize the profile of the volatile compounds of dried pasta, as well as to explore the feasibility of identifying specific patterns, better than single compounds, as putative markers of semolina quality and of thermal treatment. Pasta-making trials were performed in duplicate at a local pasta factory. The results showed that the pattern of volatile compounds was significantly affected by both the thermal treatment and the quality of semolina. Strecker aldehydes and furan derivatives, derived from non-enzymatic browning, increased in relation to the intensity of the thermal treatment and the degree of starch degradation. Meaningful information was obtained also from the lipid oxidation volatile compounds: the pattern and abundance of volatiles from linoleic and oleic acids were affected by both semolina quality and thermal treatment. Products of further oxidation of volatile compounds abounded when increasing drying temperature, while the ratio between compounds deriving from linoleic and oleic acids varied on the basis of starch degradation and free/bound lipids content in starting semolina. These results suggest that specific volatile markers could allow to investigate on the effects of both drying conditions and semolina quality and possibly discriminate between them.

Effect of salt reduction on volatile compounds of white bread

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Abstract
Epidemiological studies suggest that dietary salt (sodium chloride) intake is a contributor to the prevalence of hypertension. It has been reported that the dietary intake of salt associated to bread consumption is relevant (Miller and Hoseney, 2008). The aim of the work was to assess the effect of the decrease of salt level in bread-making on product aroma. At a local bakery were prepared bread loaves containing 20 (control), 15, 10 and 5 g salt kg⁻¹ flour. A consumer test involving 95 people was effected to assess the minimum level of salt accepted. Control bread was the most appreciated, but also breads with 15 or 10 g salt kg⁻¹ flour were positively scored. Bread with 5 g salt was rejected by the majority of consumers. Then, volatile compounds were profiled by SPME/GC-MS to compare bread with 10 g salt kg⁻¹ flour (test) and control bread. Quantitative descriptive analysis (QDA) of sensory properties, specific volume, and crust color were also determined to define the overall quality of the final product. The obtained results showed significant differences (p < 0.05) for all the parameters considered. Test bread showed higher specific volume, less colored crust and weaker aroma than control breads. Twenty-three volatile compounds were identified in the aroma profile. In particular, test bread showed higher level of ethanol, due to minor inhibition of yeast activity consequent to the use of smaller quantity of salt. Higher amounts of pyrazines, furans and pyrrols, were observed in control bread, due to more relevant Maillard browning associated to higher salt levels, as confirmed by color determinations. Control bread also showed higher levels of aldehydes imputable to catalytic action of salt on lipid oxidation. QDA evidenced significant differences affecting taste and color descriptors. In conclusion, although being accepted, the reduction of salt content affected aroma profile and overall quality of bread.
Susceptibility to denaturation of caseins in milk samples for improving protein conformational study and their identification

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Abstract
Caseins are phosphoproteins and constitute the major protein component of bovine milk. Caseins occur as micelles in the native form, which are kept together by non-covalent interactions between proteins and by calcium phosphate linkages and appear as a highly stabilized dispersion in milk. In order to optimize the chromatographic resolution for a better identification of individual casein fractions, in this work were analyzed the different effects of denaturing solvents and solutions on the structural conformation of caseins. The caseins were obtained from skim milks by precipitation at pH 4.3, and the proteins was dissolved in: water (solution A); 8 M urea in water/acetonitrile (70:30 v/v) (solution B); 0.3% (v/v) β-mercaptoethanol in water/acetonitrile (70:30 v/v) (solution C); 8 M urea in 165 mM Tris-HCl, 44 mM sodium citrate and 0.3% β-mercaptoethanol (solution D). The chromatographic separation of caseins was performed by reversed-phase high-performance liquid chromatographic (RP-HPLC) using a C4 column and the each casein was identified by MALDI-TOF MS. The best chromatographic separation was achieved by treatment of casein powder with solution D. The CD-spectrum of caseins dissolved in this denaturing solution, showed a significant increase in the α-helix conformation and a decrease in the rate of β-sheet, while random coil conformation remained unchanged. This treatment allowed the separation of different casein sub-fractions and the identification of each component of casein portion.

Authentication of the geographical origin of Sicilian typical Citrus productions

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Abstract
Sicily is characterized by optimal pedoclimatic conditions for the production of citrus fruits, as evidenced by the wide germplasm. Blood oranges (cv. Tarocco, Moro e Sanguinello), which are mainly produced in eastern Sicily and much appreciated in Italian and foreign markets for their organoleptic and nutritional properties, obtained the recognition of the European Union as PGI (Protected Geographical Indication) ‘Arancia Rossa di Sicilia’ in 1996. Similarly, lemon fruits cv. ‘Femminello Siracusano’, grown in Siracusa district, has recently been recognized as PGI (‘Limone di Siracusa’) and has valuable market shares within Italian and foreign markets. However, Sicilian citriculture is currently involved in a marked crisis mainly related to the lack of a concrete commercial strategy along the whole chain of production. This crisis can be overcome focusing on the valorization of typical citrus productions, which have been awarded PGI status by the European Commission, through the implementation of a reliable system to verify the authenticity of these productions and the effective compliance to production regulations. The aim of this research was to classify, by a chemometric approach, Sicilian typical citrus productions whose geographical origin was authenticated by samplings specifically made in PGI or not-PGI areas. Blood orange (cv. ‘Tarocco’) and lemon (cv. ‘Femminello Siracusano’) fruits sampled in three years (2010-2012) in PGI and not-PGI areas, have been analyzed with respect to the standard quality parameters, the NIR spectral pattern, the trace element profile and the multielement stable isotopic characteristics (13C/12C, 18O/16O, 2H/1H). The collected data have been subsequently processed by a multivariate statistical approach (PCA, Principal Component Analysis and LDA, Linear Discriminant Analysis) in order to evaluate the feasibility to differentiate PGI and not-PGI productions. In conclusion, the joint use of chemical, spectral and isotopic markers has revealed that a representative database of each geographical area can be used for the development of a traceability system of such typical productions.
H, C, N, O and S stable isotope ratios of livestock from Cameroon

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Abstract
70% of the population in Cameroon is economically dependent on agriculture, essentially represented by livestock production. There are around six million cattle in Cameroon, mostly belonging to the Zebu breed. In order to improve meat and milk productivity for the Gudali zebu breed in Cameroon, international cooperation between Italy and Cameroon has recently been started up. Within the context of this cooperation, 60 samples from three different cattle species (Gudali, White and Red Fulani) from different parts of Cameroon (the Sudano-Sahelian area in the North, Guinea high savannah in the East, high plateau in the West and forests in the South) were subjected to analysis of the $^{13}$C/$^{12}$C, $^{15}$N/$^{14}$N, $^{34}$S/$^{32}$S, $^2$H/$^1$H and $^{18}$O/$^{16}$O ratios of defatted dry matter and the $^{13}$C/$^{12}$C, $^2$H/$^1$H and $^{18}$O/$^{16}$O ratios of fat, using IRMS after combustion or pyrolysis. Stable isotope ratios of bio-elements have already been shown to be capable of characterising meat products on the basis of animal diet and the geo-climatic characteristics of the area of provenance (Camin et al., 2007; Perini et al., 2009; Schmidt et al., 2011). Livestock from Cameroon are characterised by typical $\delta^{13}$C values, due to an animal diet based on C4 plants (such as Echinochloa or Panicum) and $\delta^{34}$S values.

Evaluation of stilbenes content in grapes (cv. Uvalino) during ripening and drying

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Abstract
The chemical compounds belonging to the stilbenes have recently received a particular attention due to the role they play in the plant’s physiology (phytoalexins) and for their anti-oxidant properties. The Uvalino is a red-berry grape variety typically grown in Piedmont, Italy. This cultivar is characterised by its ability to synthesise large amounts of resveratrol glucoside (trans-piceid) [ref]. This explains its high resistance to Botrytis cinerea. The aim of the work is the evaluation of stilbenes and other phenolics during ripening and drying of Uvalino grapes. After accumulation, values for trans-piceatannol and trans-pterostilbene remain constant during the whole ripening period, while the piceide isomers continue to be synthesised until vintage. At the harvest the most abundant stilbene appears to be the piceide (trans and cis) and trans-pterostilbene, while trans-piceatannol is the lowest. The amount of total stilbenes found is remarkably higher than in other Piedmontese, Italian and international wines. Due to the high levels of resveratrol also in the Uvalino wines, the study of the different stilbenes found in grapes, both in their glucoside and free forms, could be interesting for nutraceutic purposes, or alternatively, be used as a varietal marker. The determination of the stilbenes during ripening, suggests that their synthesis begins at veraison, as for antocyanins, but the stilbenes accumulation in berries happens suddenly. The high content in stilbenes and the low quantity of antocyanins are a varietal character. The amount and the evolution of stilbenes and other phenolic compounds have been monitored during drying in a drying room and over-ripening on the plant. As expected, the berries drying process appears to be more intense in grapes placed in a drying room, while all compounds are reduced when drying takes place directly on the plant, irrespective of the calculation method. During grape drying, stilbenes and flavonols show large percentual reductions, even though the formers are higher, both at vintage and after drying. When drying takes place under optimal temperature and humidity conditions, as in the drying room, the values of the main phenolics indices are higher than those found at vintage. On the other hand, on the plant, the reductions are far more important, with the exception of stilbenes and hydroxy-cynnamic acids.
Chemical composition and antioxidant activity of Algerian propolis

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Abstract

Propolis is a resinous hive product actually marketed for its claimed preventive and beneficial effects on human health. Propolis shows a composition extremely variable depending on sources, climates, season and vegetation at the site of collection. As a consequence of the chemical diversity, health properties may vary considerably. This characteristic highlights the high significance of chemical standardization to connect a particular chemical type of propolis to a specific biological activity. This paper describes a comparative analysis of fourteen Algerian propolis (AP) samples. Research was aimed to investigate the chemical composition and antioxidant activity of samples collected in different regions of North Algeria. The characterization by HPLC-DAD of chemical profiles of AP samples allowed the identification of two main representative types of Algerian propolis (AP9 and AP4) as dependent on their constituents. The main secondary metabolites of AP9 and AP4 were identified by preparative chromatographic methods and NMR and MS techniques. Four caffeate esters and six flavonoids characterized the AP9 type, whereas seven labdane and two clerodane diterpenes, together to a polymethoxyflavonol, are proper to AP4 type. Subsequently, two specific HPLC-MS/MS methods for detection of AP9 and AP4 markers were developed to study the chemical composition of AP samples coming from different Algerian regions. All of the 14 samples showed a chemical profile superimposable to AP9 and/or AP4 types suggesting a characteristic chemical composition of propolis from North Algeria. Moreover, different composition may justify the dissimilar antioxidant properties evaluated by DPPH° assay. Samples containing exclusively AP4 characteristic markers (mainly diterpenes) didn’t show free radical scavenging activity (SC 50 > 100 µg mL -1) while a significant scavenging effect (SC 50 32-82 µg mL -1) was observed for those samples with AP9 profile. The activity seems to be directly correlated to presence and percentage of caffeate esters and flavonoids; well-known antioxidant natural compounds.

HPLC-PDA-MS and NMR characterization of an extract from peel of Citrus aurantium l. var. amara with antiedematogenic activity

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Abstract

Over the last 50 years, Cuba has developed a local health system based on the extensive use of traditional herbal medicines, and the Cuban Ministry of Public Health has promoted pharmacological and toxicological evaluation of the most important and widely used phytomedicine and nutritional supplements to be included in the National Health System (Abreu et al., 2004). In this study, an extract from the peel of Citrusaurantium L. var. amara (Naranja agría, Rutaceae) fruit, developed in Cuba and used for the treatment of chronic venous insufficiency (García Mesa et al., 2002), has been studied. Our research has led to establish a HPLC-PDA-MS method to identify C- and O-glycosyl flavones, a methoxyflavone, and a series of flavanone O-neohesperidosides, commonly present in Citrus species. Furthermore, three acylated flavanones di-oxalate derivatives of neoeriocitrin, naringin, and neohesperidin, reported previously only in bergamot juice, were identified for the first time in C. aurantium. To confirm the proposed structures of the major flavonoids and to establish the coumarin content, a preparative procedure was developeled to isolation and NMR spectroscopy was used to characterization structures. Inhibitor effect of the extract on vascular hyperpermeability induced by intradermal injections of phlogistic agents was evaluated in rats. The extract inhibited significantly (p<0.05) both the histamine and dextran-induced edema in a concentration-dependent manner providing evidence for the traditional use of C. aurantium var. amara. By contrast, the extract did not cause inhibition of platelet aggregation induced by physiological agonists in human plasma, as previously reported for the extract of lime (Citrusaurantifolia) (Piccinelli et al., 2008). The authors would like to thank PON “Ricerca e Competitività 2007-2013”(Ministero dell'Istruzione, dell'Università e della Ricerca) for financial support within project Hi-LiFE
1D and 2D NMR applications on balsamic and traditional balsamic vinegar of Modena

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Abstract

In recent years it has greatly increased the interest in analytical techniques able to certify the origin and authenticity of food along the production and distribution chains starting from raw materials to commercial products. The main difficulty in the foodstuff characterization is related to the complexity of the sample, which is often a complex mixture of several compounds present in concentration ratios very different from each other. The full characterization requires supplementary information from different techniques, therefore, non-separative analytical methods, that allow to obtain molecular fingerprints of complex mixtures can be particularly useful for the characterization and quality control and foodstuff authenticity and traceability. In this regard, in recent years has greatly increased the interest in the Nuclear Magnetic Resonance (NMR) and its use as a routine method for the analysis of complex mixtures as foods, balsamic vinegar among them, thanks to the availability of instruments at high magnetic fields and the consequent improvement of analytical sensitivity (Caligiani et al., 2007; Consonni et al., 2008). The aim of the present work was to select and optimize several 1D and 2D NMR sequences (1H-NMR, 1H-1H COSY, 1H-13C HMBC) to characterize the Balsamic vinegar of Modena and the Traditional Balsamic vinegar of Modena. The application of HR-NMR techniques to the samples has generated very complicated spectra that needed to be previously processed and subsequently analyzed by chemometric methods. To reduce the inhomogeneous proton NMR chemical shift of signals along the spectra, due to small pH changes and intermolecular interactions, all spectra were aligned using the toolbox Icoshift 1.0 for Matlab (Mathworks Inc., Natick, MA) (Savorani et al., 2010). Besides, to achieve a reliable classification of the different samples, unsupervised and supervised pattern recognition procedures were applied to the NMR data obtained.

HR-NMR studies for the characterization of DOC Lambrusco wines of Modena

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Abstract

NMR spectroscopy is currently recognized as an important tool in food science and analysis for the authentication and quality control of foodstuff. The wine area is definitely one of those in which the NMR has proved most successful in recent years. High-resolution techniques were rather powerful tools for studying minor components of oenological products. Classical studies on oenological products are normally based on composition data obtained by various analytical techniques, however, since the quality and characteristics of a product are not the simple sum of individual chemical characteristics, NMR analysis with chemometric data analysis certainly is a useful tool in this regard. Over the past few years, many works based on proton NMR coupled with multivariate data analysis were been conducted concerning the study of the usability of HR-NMR as fingerprint analysis tool in oenology. Between them in particular different authors have proved the validity to use this technique as an indirect indicator of geographical traceability and demonstrated that HR-NMR is an extremely powerful method for the study of oenological product and wine in particular (Brescia et al., 2002; Viggiani and Castiglione, 2008). In this context, the present work, which is part of the extensive research project AGER (Agroalimentare e Ricerca: New analytical methodologies for geographical and varietal traceability of oenological products), aimed to use the HR-NMR techniques as molecular fingerprints in order to serve as indirect indicators of authenticity and quality control of several DOC Lambrusco wines of Modena (Lambrusco di Sorbara, Lambrusco Salamino di Santa Croce and Lambrusco Grasparossa di Castelvetro) provided by local producers joined to the research project AGER. The data obtained were coupled with chemometric analysis tools to effectively interpret the complex results collected from mono and bi-dimensional spectra (HR-1H-NMR, 1H-1H COSY, 1H-13C HMBC) acquired.
Plasticizers in tea and flavored tea by HRGC-MS

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Abstract

The plasticizer as PAEs, AAEs and SAEs, are among the most abundant contaminants in the environment and in food. The US Environmental Protection Agency (EPA) and several other agencies classified the PAEs as priority pollutants (Rios et al., 2010; Wang et al., 2010). Because of its wide use in food packaging and food containers, plasticizer has the potential to leach into foods, and widespread exposure to low levels of plasticizers in the general population has been confirmed through biomonitoring studies (Wormuth et al., 2006). In this work were determined residues of 17 phthalates, 6 adipates, bis-(2-ethylhexyl) sebacate, bis-(2-ethylhexyl) terephthalate, benzyl benzoate and bisphenol in tea and flavored tea samples. The samples were solid phase extracted with Oasis HLB glass cartridges, and residues detection and quantification were performed by gas chromatography coupled to mass spectrometry (GC-MS). The performance of the method was evaluated in terms of sensibility, linearity, accuracy and precision, achieving satisfactory results for all parameters. Residues of di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-butyl phthalate (DBP), bis-(2-ethylhexyl) adipate (DEHA) and bis-(2-ethylhexyl) phthalate (DEHP) were observed.

Carotenoids determination in some dry fruits

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Abstract

Currently attention is being draw worldwide towards exploring plant sources for molecules that provide pharmaceutical advantages to human. Carotenoids are naturally occurring phytochemicals that possess various health benefits. Amongst the carotenoids, β- and α-carotene have received much attention due to their provitamin-A activity and lutein and zeaxanthin are being studied for their possible role in the prevention of age related macular degeneration. Dry fruits are usually worldwide consumed as snack food. Here we present the carotenoids investigation on three different typical dry fruits: chickpea, almonds and hazelnuts. The carotenoids were determined by HPLC-DAD-MS methodology using a C30 column, after a liquid partition extraction of the carotenoids from the different matrices using DMF and hexane. Chickpea showed the higher carotenoids concentrations among the fruits investigated; in particular, β-carotene was the most abundant carotenoid followed by zeaxanthin and lutein. Hazelnuts showed an higher amount of zeaxanthin then β-carotene and lutein was not detected. In almonds only β-carotene was found. The results here reported have shown that the dry fruits investigated and, in particular chickpeas, can be considered as a good sources of important carotenoids and therefore their consumption should be increased.
High throughput quantitative analysis of multi mycotoxin in beer-based drinks using UHPLC-MS/MS

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Abstract
Mycotoxins often exist as contaminants in grains. Meanwhile, in response to consumer needs for food safety, food and beverage manufactures have to strictly manage the risks of such contaminants. Therefore, it is essential for the management of high-quality to rapidly determine the concentrations of mycotoxins in foods or beverages. UHPLC-MS/MS offers the best combination of selectivity, sensitivity, and speed for detection of these compounds in complex matrices. The high throughput method for the quantification of 14 mycotoxins in beer-based drinks had been developed. While high sensitivity analysis is performed, carry over becomes a problem due to the adverse effects on LC-MS/MS high-sensitivity analysis. For eliminating carry over, rinse condition of autosampler was also examined. Standards of 14 mycotoxins (patulin, nivalenol, deoxynivalenol, 4 aflatoxins, T-2 toxin, HT-2 toxin, zearalenone, 3 fumonisins and ochratoxin A) were optimized on each compound-dependent parameter and MRM transition (Q1/Q3) and then they were analyzed on LC-MS/MS condition as follows. Nexera HPLC system was connected to LCMS-8030 triple quadrupole mass spectrometer. Chromatographic separation was carried out using ODS column, TriartC18 (2.0 mm I.D., 100 mm, 1.9um) maintained at 40°C. Mobile phase consisted of: solvent A, ammonium acetate-water; and solvent B, acetic acid-methanol. And sample was applied to MS/MS with electro ion spray source, then analyzed with positive and negative MRM mode. As for Nexera autosampler, multiple rinse modes with two rinse solvents were examined to eliminate sample carry over.

Fruit quality and human health-bioactive compounds of sweet cherry (Prunus avium L.) cultivars grown in Italy

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Abstract
This research was undertaken to evaluate the fruit quality parameters (fruit weight, total soluble solids, titratable acidity, firmness and colour), phenolic compounds and antioxidant capacities of twenty four sweet cherry (Prunus avium L.) cultivars grown on the mountainsides of the Etna volcano (Sicily, Italy). High-performance liquid chromatographic methods were used to identify and quantify sugars (sucrose, glucose, fructose and sorbitol) and organic acids (malic, citric, shikimic, and fumaric acid). A total of eight phenolic compounds were identified and quantified in sweet cherry cultivars, including three hydroxycinnamic acid derivatives (neochlorogenic acid, p-coumaroylquinic acid and chlorogenic acid) and five anthocyanins (cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-rutinoside, peonidin-3-glucoside and peonidin-3-rutinoside). The relative amounts of the phenolic compounds varied widely across the cherry cultivars examined in this study. Total anthocyanin contents ranged from 4.58 to 94.20 mg of cyanidin-3-glucoside equivalents/100 g of FW, while total phenolic contents ranged from 81.62 to 166.03 mg of gallic acid equivalents/100 g of FW. The ORAC assay indicated that fruit of all genotypes possessed considerable antioxidant activity. The high level of phenolic compounds and antioxidant capacity of sweet cherry fruits studied implied that they might be sources of human health-bioactive compounds.
New triploid citrus hybrids: quality and functional properties of fruits

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Abstract
Nowadays the crossbreeding of the best existing Citrus species is an important strategy for producing new valuable hybrids. Since 1978, the CRA-Centro di Ricerca per l’Agrumicoltura e le Colture Mediterranee (CRA-ACM) has been working on a genetic improvement program to develop new seedless Citrus hybrids. The development of triploid hybrids by crossing a monoembryonic 2x female parent with a 4x male parent has been the successful breeding strategy carried out by the CRA-ACM. Within this program three new triploid hybrids have been recently obtained, namely ‘D2238’, diploid ‘Monreal’ clementine (Citrus clementina Hort. ex Tan.) x tetraploid ‘Duncan’ grapefruit (Citrus paradisi Macf.), ‘C2710’, diploid ‘Oroval’ clementine (C. clementina Hort. ex Tan.) x tetraploid ‘Tarocco’ orange (C. sinensis L. Osbeck) and ‘RC1’, diploid ‘Fantastico’ bergamot (C. bergamia Risso) x tetraploid ‘Tarocco’ orange (C. sinensis L. Osbeck). In order to investigate the heritability of traits from their parents, the fruit juices of these hybrids were analyzed to evaluate parameters related to fruit quality, as well as the content of health-promoting components such as ascorbic acid, flavanones, anthocyanins (in ‘C2710’) and hydroxycinnamic acids. In addition, the total antioxidant capacity of the juices was measured in vitro by the DPPH scavenging activity assay. Results showed that ‘D2238’ hybrid presented fruit quality characteristics intermediate to those from both parents, being morphologically similar to grapefruit, with some of the valuable characteristics of clementine (low acidity, ease of peeling, excellent juice yield) and a moderate content of naringin (~ 250 ppm), a trait inherited from the male parent. The ‘C2710’ hybrid was similar in shape and size to a large clementine with some excellent traits inherited from the ‘Tarocco’ orange, such as organoleptic properties (juiciness, sugar/acid ratio), ascorbic acid content, and a red flesh pigmentation due to the presence of anthocyanins. The ‘RC1’ fruits resembled ‘Fantastico’ bergamot respect to physico-chemical parameters (relevant peel thickness, low juice yield, high acidity) and presented a original flavanone profile with the presence of neoeriocitrin, typically present in bergamot juice but not in sweet oranges, as well as narirutin and esperidin. IC50 values (µl of juice yielding 50% inhibition of DPPH) for the three hybrids (‘RC1’>'D2238'>'C2710’) reflected the concentration of bioactive compounds in their juice. Although these three new hybrids don’t show great potentiality to be consumed as fresh fruit, they can be considered as a new valuable and original source of natural antioxidants to be exploited for processing.

Tracking wine adulteration: Identification of barbera grape in Nebbiolo wines using microsatellite DNA analysis (SSR)

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Abstract
Wine traceability/authentication is a key target for Italy, the world's second largest wine producer behind France. Several European Countries developed appellation systems, with their own unique labels and seals, to try to combat label fraud that misrepresented a wine's true origins. The fraudulent use of low-price grapes/musts/wines to adulterate high-value wines (particularly when wine is produced in purity, using a unique grape variety, like Barolo and Barbaresco DOCG wines from Piedmont, from Nebbiolo grape) has been highlighted in Italy. The fraudulent substitution of Brunello di Montalcino wine is a key example. Wine experts suspect that, as much as 5% of the wine sold in secondary markets worldwide, could be counterfeit. Despite the high number of methods for the traceability of wine using complex, time consuming and expensive techniques (e.g. SNIF-NMR, stable isotope ratio mass spectrometry, trace elements), few applications reported the use of DNA analysis in bottled wine (grape and must are largely studied). The polymorphism of SSR (Single Sequence Repeats or microsatellites, regions of tandem repeats of two to five nucleotides that are ubiquitous in eukaryotic genomes) is sufficiently stable to be used in genetic analyses. Considering the low quantity of DNA in wine (depending on the impact of processing, particularly filtration and refining steps) as well as the interference of polyphenols both during DNA extraction and DNA amplification steps, a key strategic problem is correlated with the extraction
of a pure not-degraded total genomic DNA from wine. Aims of this work were i) to perfect a microsatellite DNA-based method for the detection of Barbera grape/must/wine in Nebbiolo wine, and ii) the optimization of a high-yield/high-purity total genomic DNA extraction method, improving the detection of SSR profiles using the Micro-chip electrophoresis. No differences between the corresponding leaf and varietal must/wine profiles were noted, confirming the robustness of the method. Finally, we suggest this approach to identify the fraudulent use of Barbera wine in Nebbiolo-based wines.

P-180

Application of the SPI (saliva precipitation index) to the evaluation of red wine astringency

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Abstract
Astringency is an important sensory characteristic of food and beverages containing polyphenols. This mouthfeel is mainly due to the interactions of polyphenols with salivary proteins, causing complexes formation and their further precipitation, which leads to a reduction of the lubricating properties of saliva. As a consequence, sensations of dryness, hardness, and constriction are felt in the mouth. Wine astringency is generally estimated by tasting, but its chemical evaluation shows a high potential of interest both for researchers and winemakers. For this reason, several studies focused on methods for astringency prediction. Ovalbumin, BSA (Bovine Serum Albumin) and methylcellulose have been used as precipitation agents in different protein precipitation assays. Besides they were all correlated with sensory analysis, the utilisation of saliva seems to be the most appropriate proteic model to represent the real interactions between proteins and polyphenols involved in the mechanism of astringency during wine tasting. In this work, improvements over the existing method based on the SDS-PAGE of salivary proteins after the interaction with grape extracts were made. The optimization of the method for wine has the aim to better represent the physiological conditions during tasting. The obtained SPI (Saliva Precipitation Index) was utilised as a measure of the reactivity of selected salivary proteins towards red wine polyphenols. As resulted by Pearson’s a significant correlation between the SPI and the astringency of red wines evaluated by a trained panel test was found. Phenolic composition of red wines was also investigated. In addition, a relationship between SPI and wine phenolic content, estimated in acid gallic equivalent, may represent a easy way to universally quantify this in vitro assay for astringency evaluation.

P-181

NMR-based metabolomics of meat

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Abstract
High Resolution Magic Angle Spinning–Nuclear Magnetic Resonance (HRMAS-NMR) spectroscopy was employed in order to yield the metabolic profile of longissimus dorsi (ld) and semitendinosus (st) muscles of four different breeds: Chianina, Holstein Friesian, Maremmana and Buffalo. Multivariate data analysis, i.e. PLS-DA and OPLS-DA, was successfully used for classifying: a) muscle type according to breed, b) cattle breed according to muscle type, and c) cattle species according to muscle type. Built models gave excellent discrimination in most cases, and based on VIP (Variable Important in Projection) values we were able to identify the metabolites relevant for the different classifications. Moreover, we assessed the traceability of Chianina, by comparing IGP with non-IGP longissimus dorsi samples, and we were able to recognize the low molecular weight species useful for the determination of the place of origin. Finally, the metabolic trajectories of the aging process were measured, by elucidating some metabolic processes occurring during post-mortem. We concluded that the NMR-based metabolomics obtained by combining HRMAS-NMR and multivariate data analysis proved to be a valuable tool in the assessment of quality related parameters of meat.
A traceability study on the Moscato wine chain
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Abstract
The growing interest of consumers in the origin of foodstuffs favoured in the last years the development of powerful analytical methods to study authentication and traceability of food. Wine is without doubt the most studied item in this field, due to the fact that fraudulent practices are more common on wine than on every other food. On the other side, it is a fact that today, more than in the past, consumers tends to privilege quality wines at the expense of more ordinary wines. Therefore, it becomes an interest for wine-producers too to find ways to verify the genuinity and authenticity of wine products. It is definitely in this view that the Consorzio per la Tutela dell’Asti DOCG promoted a research project on one of its most important products, the world renowned Moscato d’Asti white wine, in order to study the traceability along the production chain. The study was performed along three years with a close collaboration of chemists, oenologists and wine-makers: complete production chains were studied by withdrawing samples after every oenological treatment; samples of soil and grapes from pilot vineyards were also included in the study. Trace and ultratrace elements were used as variables in order to study traceability, with particular reference to lanthanides. It has already been verified that the distribution of lanthanides is maintained unaltered in the passage from soil to must; here the behaviour of lanthanides distribution along the whole chain, i.e. from must to bottled wine, is studied. Elements were determined with ICP-MS after proper sample treatment. The results from the statistical elaboration of the experimental data show that the lanthanides distribution acts as a fingerprint of soil until the clarification treatment with bentonite, after which some fractionation occurs. Being verified the link between soil and must, the second part of the project involves a classification study of 120 samples of Moscato d’Asti musts in order to verify how they reflect the features of the different geographical zones where they come from, and to build a strong basis to be able to identify possible adulterations performed by addition of foreign musts. Results from ICP-MS determinations of lanthanides and multivariate analysis are promising.

Proteome analysis of post-mortem changes in bovine longissimus dorsi by two-dimensional (2-DE), P-dimensional (2-PE) electrophoresis and ranking-PCA
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Abstract
To study early post-mortem changes in muscle tissue from bovine Longissimus dorsi at 0, 12 and 26 days after slaughter we analyzed the proteome by two-dimensional (2-DE), as well as, by P-dimensional electrophoresis (2-PE). This new method, called 2-PE, takes advantage of the presence of a SDS-PAGE step with a radial electric field instead of a parallel one. Since spots with close but not equal pl are moved by diverging lines of forces, their resolution increases during the radial separation, by a factor proportional to the migration distance. Moreover reproducibility between replica gels is enhanced. Ranking principal component analysis (Ranking-PCA) was used to analyse the protein patterns obtained by 2-DE and 2-PE in order to select spots that were significantly different at the three time-points. Selected proteins were identified by nanoHPLC-Chip Ion trap MS/MS. Among the modulated proteins we have identified stress proteins members of the small heat-shock protein family (such as Hsp27 and HspB6), structural proteins (such as troponin T, fast and slow isoforms), and metabolic enzymes (such as GAPDH). Further studies will reveal the relevance of these proteins changes during post-mortem storage of bovine muscles to control better meat quality.
Influence of catechin concentration and oak chips (*Quercus alba*) on acutissimin formation in model solutions and red wine

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Abstract
During wine aging evolution of C-Glucosydic Ellagitannins occur in presence of oak wood through substitution reaction in other products as Acutissimin A and B (Saucier et al., 2006). The aim of this study was to evaluate synthesis pathway of flavano-ellagitannis in model solutions prepared adding different concentrations of catechins and oak chips (*Quercus alba*) in a hydroalcolic solutions (ethanol 12% v/v) containing 5g/L of tartaric acid. Model solutions, constituted from 100, 500 and 1000 ppm of catechin were added of 1-5 and 10% of oak chips. Time of contanct was prolonged for 42 days. Moreover, sample of commercial red wine was macerated with the same oak chips for 6 weeks. Detection of catechin and new formation molecules was carried out by HPLC-MS system coupled with diode-array detector. Presence of Acutissimin A and B was validated by comparison of mass fragmentation patterns with those of indicated by Quideau et al., 2003. Acutissimin A and B reached the maximum concentration when the model solution was composed of 10% of oak chips and 500ppm of catechin. Probably, the wood increased the expression of biosynthetic flavano-C-glucosydicellagitannins. However, normalizing the concentration of acutissimin A respect to the percentage of chips added, emerged as the best value was obtained when sample solution consisted of 500 ppm of catechin and 5% of oak chips. Low concentration of Acutissimin A and B was found in red wine aging with the same oak chips.

Frying performance of a super palm olein in comparison with olive oil

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Abstract
Deep-fat frying is an important, common and highly versatile process. During deep-fat frying, hydrolysis, oxidation and polymerization reactions cause a spectrum of physical and chemical changes, leading to the formation of decomposition products posing a direct impact damaging both the oil quality and the fried food nutritional value. The amount and type of degradation products formed in frying oils is primarily dependent on the fatty acid composition of the frying oil, so it is important to keep this in mind in selecting frying oils. Stable frying oils usually require low linolenic acid (LA<3%), increased oleic acid (OA>40%), and decreased linoleic acid (LnA<50%) (Warner & Fehr, 2008). The aim of this study was to establish the behavior of palm superolein (PSO) (OA 45%; LA 12.5%; LnA 0.2%) and olive oil (OO) (OA % 71.09; LA 12.0 %; LnA 0.60%) during repeated, discontinuous deep frying of French fries. All samples (thermo-oxidized oil samples, frying oil samples, fat samples extracted from French fries) were subjected to the following determinations: free fatty acids (FFA); peroxide value (PV); total polar compounds (TPC); fatty acids (FA) composition; volatile organic compounds (VOC). The palm superolein selected to be tested in this study may represent an alternative to olive oil as a frying medium. Although PSO presented a faster increase in some oxidation indices, such as free fatty acid (FFA) and total polar compounds (TPC), for other indicators PSO showed better behavior than olive oil (less formation of C8:0 and lower peroxide value (PV)). Among VOCs identified in two oils, several aromatic compounds, such as alkylbenzenes and alkylfurans, were detected. In particular, in the fat extracted from French fries the styrene was detected and quantified. The presence of styrene in samples fried in PSO and its absence in OO samples can be due to a possible mechanism of the formation of styrene from trans, trans-2,4-decadienal (Andrikopoulos et al., 2003).
Healthy and nutritional characterization of craft beers made with addition of Umbrian’s legumes

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Abstract
In this study we have developed techniques to get the brewing craft beers made with the addition of legumes, that are very rich in phytochemicals and in particular isoflavones (Kuhnle GG, et al., 2008; Lapcik O, et al. 1998). The project was carried out in Umbria using local spelt and barley malt for which were then added to the typical Umbrian legumes such as lentils and grass peas. In particular, the project involved the development of cooking processes and fermentation to allow the greatest transfer of active substances by the special ingredients to the finished beer, as well as the transformation of these substances in free forms most active and finally obtaining beer with a low alcohol content. From healthy and nutritional characterization of the beers emerged important results, as an interesting mineral profile and a large content of molecules with antioxidant activity like phenolic compounds in particular 50-80 mg/100 ml. It should also be noted that within the group of phenolic compounds present in these beers were also found interesting amount of isoflavones in particular genistein and daidzin, which in addition to being powerful antioxidants have beneficial effects against the body high and therefore can act in the prevention of cancer, inflammatory, cardiovascular, postmenopausal, cognitive, immune diseases (Setchell KDR, et al., 1999). Both the high content of minerals and the high content of antioxidants and isoflavones in particular, is due to use of Umbrian’s legumes as ingredients in these specialty beers obtained with a particular craft process. These results can then consider these craft beers an healthy-drink, with a low alcohol content.

Soil and cultivar influence on selected chemical components of Italian garlic (Allium savitum L.).

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Abstract
Among plant products, garlic bulbs (Allium savitum L.) are popularly used as a food dressing as well as a traditional remedy for several diseases. Due to their particular reproduction, garlic bulbs can grow in a wide range of soil textures, adapting themselves to different environmental situations. The spread of local ecotypes in Italy has produced traditional varieties well-characterized by their organoleptic properties which are very interesting from the biological and agronomic viewpoint, in regards to biodiversity protection. In order to evaluate the influence of soil and cultivar on bulbs’ chemical components, four Italian typical varieties (Rosso di Castelliri, Bianco Piacentino, Rosso di Sulmona, Rosso di Proceno) were cultivated in two different geographical areas of Lazio (Viterbo and Alvito) using the same agronomic intervention practice and afterwards were characterized for their proximate composition and micronutrients. The aim of the work was to identify potential differences among these ecotypes grown on different soils, analysing selected component: water, protein, ash and minerals. Statistical data (ANOVA factor analysis) showed a significant soil influence (p<0.05) on water content: the Alvito grown bulbs mostly had higher values than the Viterbo ones. Cultivar influence, instead, significantly affected protein content: Rosso di Castelliri had the highest value (9.83g/100g wet weight) and Bianco Piacentino showed the lowest one (6.09g/100g wet weight). Ash average content was 1.35g/100g on wet weight basis: the collected Alvito and Viterbo samples had mild variance in ash values. According to literature, potassium concentration was the highest one among minerals: Viterbo soil had a high exchangeable potassium value (421ppm) being reflected on most Viterbo cultivars values. Sodium concentration showed a wide series of values, ranging from 1.49 to 9.21mg/100g on wet weight basis. The connection between garlic characteristics and soil has great importance on agricultural production disciplinary in preserving traditional and typical products.
Effects of hot water dipping treatments (thermotherapy) on the essential oil profiles of *Citrus sinensis* cv. tarocco fruits

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Abstract

The most common and serious diseases which affect citrus fruit in Italy after harvest are incited by *Penicillium digitatum* Sacc. and *P. italicum* Weh. responsible for green and blue mold rots, respectively. Current postharvest decay control strategies are based on the application of synthetic chemical fungicides, but despite their fundamental role in postharvest disease control, chemical treatments are confronted by several problems that threaten their future potential. Therefore there is an emerging interest to develop “safer” alternative measures for decay control. Hot water dipping treatments represent a useful alternative mean to synthetic fungicides on green mold control in organically-grown citrus fruit. In this study the effectiveness of hot water treatments as alternative means to control postharvest decay on ‘Tarocco’ orange fruits and their effect on fruit quality with special regard on peel essential oil has been evaluated. The oils obtained by hydrodistillation and analyzed by a combination of gaschromatographic tools (GC-FID and GC-MS) were used as biomolecular markers to establish the preservation of qualitative traits of the orange fruits with respect to the fresh material. Treatments selected for evaluation were hot water dipping for 3 min at 52 °C and for 20 s at 56 °C. These treatments were compared with an effective-fungicide standard treatment (imazalil) applied at 1g a.i./L and an untreated control. Decay incidence, physiological disorders and weight loss was assessed after 30, 60 days of cold storage at 6 °C (85-87% R.H.), and 60 days plus 1 week of simulated marketing conditions (SMC) at 20 °C. Rheological and physicochemical parameters were also evaluated. In our tests hot water dipping at 56 °C for 20 sec. was more effective in inhibiting *P. digitatum* spore germination than hot water dipping at 52 °C for longer exposure time. Weight loss (%) after 60 days of cold storage shows the lowest value in Tarocco orange submitted to hot water treatment at 56 °C similar to imazalil treatment. Hot water treatments did not cause surface damage or color change and did not influence internal quality parameters.

Endogenous free polyamines and carotenoids content in two Sicilian apricot cultivars during ripening

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Abstract

Apricots are seasonal fruits with a fast ripening period and they are usually picked before reaching the highest organoleptic qualities. Polyamines are physiologically important compounds, produced during fruit development and senescence. Senescence in seasonal fruit usually occurs in plants when ethylene is released in relatively large quantities. On the other hand, physiological effects of ethylene in plants are known to be antagonized by treatment with polyamines, due to the metabolic connection between polyamines and ethylene through the S-adenosylmethionine (SAM). The carotenoids are the most widespread group of pigments in nature, and they are present in all photosynthetic organisms and are responsible for most of yellow to red colours of fruits and flowers (1). Ripening of the fruits involves series of complex biochemical reactions and physiological variations such the softening, the fast carbohydrate storage and the pigmentation, which lead to production of phenolic compounds, carotenoids and other phytochemical compounds (2). The objective of this paper was to study the possible relationship between endogenous polyamine contents and carotenoids production during fruit growth, of two Sicilian apricot cultivars. Fruits of two apricot (*Prunus armeniaca* L.) cultivars (*Prunus armeniaca* cv *Ninfa* and cv *Tiryntos*) were harvested from five trees in a farm of the Messina district. During fruit development, fruits were collected weekly from day 32 after anthesis (March 30) until harvest, and kept at – 60°C for analysis of polyamine and carotenoids content. Upon harvest fruit growth was expressed as fresh weight and the fruit peel colour was determined visually. The results obtained in this study put in evidence that during early development, fruit contained high polyamine concentrations that sharply declined to a low level until the ripening state, while cumulative fruit weight increased. However the quantitative and qualitative differences between carotenoids in apricot fruits during ripening depended on cultivars. Carotenene analysis showed increasing of all separated carotenoids in apricot fruits during ripening, particularly B-carotenene content, which was about 10-fold higher in mature then in immature fruits.
**Food analysis by using miniaturized chromatographic techniques: determination of polyphenols in tea samples.**

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**Abstract**

Miniaturized techniques such as nano-liquid chromatography (nano-LC) represent innovative tools useful for the analysis of food and biological samples. Capillary liquid chromatography (CLC) or nano-liquid chromatography (nano-LC) are characterized by the use of capillary column with i.d. 300-500 and 50-100 $\mu$m, respectively. These techniques can offer high efficiency, good resolution, high mass sensitivity and low costs. The last two features are due to the low flow rate (µL/min and nL/min). This reduction of flow increases the sensitivity because of the decrease of the chromatographic dilution. Furthermore the consumption of mobile phase is limited making the tools eco-friendly. In addition CLC/nano-LC can be easily coupled with the mass spectrometers (MS). Conventional stationary phases either packed or monolithic have been widely used for nano-LC separations in the field of food analysis. Recently core-shell or fused-core particles technology have been introduced in chromatography giving the advantages to improve separation efficiencies and speed without reducing particle size of stationary phases. As an example of the potentiality of nano-LC in food analysis, we report the separation of eleven polyphenols and three methylxanthines in a capillary column of 100 $\mu$m i.d.x 10 cm packed with C18 core-shell particles (2.7 $\mu$m). Detection was performed by UV-Vis detector moreover a mass spectrometer was used for the determination of analyte molecular weight. All analytes were separated in less than 15 min applying a step-gradient elution mode utilizing a laboratory assembled instrumentation. The method was validated obtaining satisfactory results concerning repeatability of retention time and peak areas, linearity and reproducibility. Afterwards the method was applied to the analysis of studied compounds present in some commercial green tea samples. Finally the nano-LC system was coupled with a ion-trap electrospray mass spectrometer for the measurement of MS and MS/MS spectra for analytes confirmation. Funded by PRIN Project 20098y822F_002

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**Endogenous free polyamines and their role in Citrus fruits ripening**

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**Abstract**

Polyamines are biological compounds of low molecular weight that are essential for cell growth and differentiation, thus their intracellular concentrations increase during periods of rapid cell proliferation (1). Putrescine, cadaverine, agmatine, spermidine and spermine universally occurring in plant organs are involved in a wide array of processes, ranging from triggering organogenesis to protecting against stress. It is evident that all types of food, whether they originate from plants (vegetables and fruits) or animals (milk, eggs and meat), contain polyamines. Fruit are rich in putrescine and spermidine, but contain little or no spermine. The main sources of spermine are bread and cereals (2). These dietary polyamines become part of body’s polyamine pool and through the systemic circulation system they should reach every tissue of body (3). Citrus fruits are very abundant in Mediterranean diet and are present for a long time in a family meal, for this reason in the present project, we evaluated the free polyamine concentrations during ripening in common lemon, orange and mandarin obtained by biological cultivars. Fresh fruits were selected and picked manually at different stages after fruit set. Free polyamines were analyzed by HPLC according to Flores et al. (4). High levels of putrescine and spermidine were found during fruit set for all species considered, as the fruit matured their levels diminished, but spermine in the pulp remained nearly costant. A correlation has been shown between high putrescine levels and rapid cell proliferation in the early stages of fruit growth, suggesting their direct involvement in cell division and proliferation. Polyamine concentrations were higher in immature mandarin than in other examined fruits, and decreased by ripening.
Effects of storage on biogenic amines development and bacterial flora contribution in some Mediterranean fish

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Abstract
Seafood quality is affected by handling, gutting, processing and storage temperature. The deteriorative changes that take place under refrigerated or iced conditions are primarily due to bacterial and endogenous enzymes. Endogenous product is insignificant when compared to the exogenous pathway. The result of microbial attack involves the degradation of protein and amino acids of fish muscle - which is rich in free amino acids, and whose content may increase even further post mortem - to their corresponding amines (1). Hazardous levels of histamine and other toxic end products, such as additional biogenic amines, can also be present in seafood products as result of bacterial activity. Strong evidence suggest that biogenic amines such as putrescine, cadaverine, spermine and spermidine in fish tissue can potentiate the toxic effect of histamine by inhibiting intestinal histamine-metabolizing enzymes (2). Recent research has focused on using histamine and other biogenic amines as indicators of product decomposition because these compounds are almost undetectable in fresh fish, and their formation is usually associated with bacterial spoilage (3). For the seafood industry, the monitoring of indicator compounds such as biogenic amines, associated with seafood safety is as important as quality assurance. The fishes used in this study were caught off the coast of Messina and the Eolian Island. Fish samples were filleted and skinned and packed in polythene bags, and a) stored at room temperature (23 ± 2°C) for 10 h, b) kept in a refrigerator with controlled temperature (4 ± 1°C) for the same time, or c) frozen and kept at -20°C until analysis. Fillet sample were used to determine polyamine contents and bacterial loads. The results obtained in this study put in evidence how polyamine levels can be influenced by the storage conditions and the biogenic amines producing bacteria (capable of growing at wide temperature range), quite common in the marine environment and naturally occurring in the fishes gills, skin and within the gut.

Solid phase extraction followed by gas chromatography and ion-trap mass spectrometry detection for determining phthalate esters at trace levels in light alcoholic beverages and soft drinks

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Abstract
An analytical method based on Solid-Phase Extraction (SPE) with XAD-2 for the enrichment of Phthalate Esters (PAEs; dimethyl phthalate DMP; diethyl phthalate DEP; di-isobutyl phthalate DIBP; n-dibutyl phthalate DBP; butyl cyclohexyl phthalate BcEP; butyl benzyl phthalate BBP; bis-(2-ethylhexyl)phthalate DEHP) in alcoholic food beverages (Alcohol by Volume below 6% alc vol-1) and in soft drinks has been set up whereas the analysis was performed by means of gas chromatography coupled with an ion trap mass spectrometer detector (GC/IT-MS). The instrumental analytical protocol was found to yield a linear calibration in the range 1-1000 ng mL-1 with r² values ≥ 0.912; the Limits of Detection (LOD) vary between 0.2 pg mL-1 and 20 pg mL-1 (RSD ≤ 6.2) whereas the Limits of Quantification (LOQ) range between 0.5 pg mL-1 and 40 pg mL-1 (RSD ≤ 8.5. This simple, reliable, reproducible and not expensive analytical method developed, has been applied to several commercial light alcoholic samples such as beers, and soft drinks. Presence at ng mL-1 levels of DEP (0.1-1.0 ng mL-1), DIBP(0.2-2.5 ng mL-1), DBP(1.9-4.4 ng mL-1), BBP(0.08-0.8 ng mL-1), DEHP (3.6-101 ng mL-1) is found in every sample analyzed whereas DMP (1.9 ng mL-1) and BcEP (0.08 ng mL-1) are found only in 1 sample of beer. Finally, a statistical approach was performed to find out some correlations about a correlation among the different PAEs.
Rapid determination of acrylamide in conventional cereal-based foods and potato chips through conversion to 3-[bis(trifluoroethanoyl)amino]-3-oxopropyl trifluoroacetate by gas chromatography coupled with electron capture and ion trap mass spectrometry detectors

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Abstract
A new, simple, and fully validated method based on gas chromatography coupled with Electron Capture and Ion Trap Mass Spectrometry detectors (GC-ECD and GC-IT/MS) is presented for quantitative analysis of acrylamide (AM) contaminant in conventional cereal-based foods and potato chips. Before analysis AM was efficiently derivatized with trifluoroacetic anhydride (TFA). The role of the temperature, reaction time and catalyst on the acylation reaction is evaluated. The chromatographic analysis is performed on the SE-54 capillary column; good retention and peak response of acrylamide derivative are achieved under the optimal conditions, The analytical method was fully validated by assessment on the following parameters: limits of detection (0.1 ng g⁻¹ with a Relative Standard Deviation, RSD, below 4.5), linearity (R² above 0.9988 in the range 0.1-200 ng g⁻¹) and extraction recovery (ranging between 91-98% with RSD below 3.5 for acrylamide spiked at levels of 1, 20, 50 and 100 ng g⁻¹). Furthermore, the method proposed shows no clean-up step of acrylamide derivative to be performed prior to the injection. The developed method has been successfully applied to determine acrylamide in different commercial cereal-based foods and potato chips.

The molecular basis of working mechanism of natural polyphenolic antioxidants

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Abstract
In this communication, a summary of the research work performed so far using high accuracy quantum chemical methods on polyphenolic antioxidant compounds will be reviewed. Particular emphasis will be given to the different groups of polyphenols, which mostly belong to the Mediterranean food culture, i.e. phenolic acids, flavonoids and stilbenes. The three main proposed mechanisms through which the antioxidants may play their protective role, which is the H atom transfer, the single electron transfer and the metals chelation, will be analysed and discussed in details [Lepoldini et al., 2010 and 2011]. This work represents a further important contribution to the elucidation of the beneficial effects on health of these substances.
Biogenic amines as freshness index of meat and fish wrapped in an innovative active packaging system

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Abstract
Biogenic amines (BAs) are basic nitrogenous compounds present in living organisms and, hence, in foods (C. Ruiz-Capillas et al., 2004). They are produced by decarboxylation of free aminoacids, by amination or transamination of aldehydes and ketones, by normal cellular metabolism of tissues. Due to the importance of their toxicological aspects, BAs are considered an important indicator of freshness and quality of food. Fresh food as meat and fish, that are known for their high nutritional values, contain significant levels of BAs. For enhancing the shelf life of these perishable foods, various packaging systems were developed during years by researchers and food industries. In particular, active packaging (AP) is an innovative concept in which the package, the food and the internal environment interact among them to enhance the shelf-life and sensory properties of wrapped food (Suppakul et al., 2003). Natural extracts and essential oils of plants are extraordinary sources of bioactive molecules mixtures having important antioxidant and antimicrobial actions. The aim of this work was to develop a new packaging system that, incorporating essential oils such as those of rosemary (Rosmarinus officinalis) and lemon (Citrus limon), enhances the shelf life of fresh foods as meat and fish by inhibiting the growth of BAs during storage. BAs were analyzed by using HPLC-DAD and HPLC-MS methods developed in our lab. The level of BAs in food, stored in the new AP system at 4°C for 1 week, was monitored after 2, 4, 7 days in comparison with the same food wrapped in a non-AP system. A significant growth inhibition of BAs was observed for all monitored period. Other chemical and microbiological parameters of food freshness as color, pH, hexanal (marker of lipidic oxidation) and selected groups of microorganisms were monitored during this time. Results confirmed the positive action of new packaging for enhancing the shelf life of analyzed food.

Separation of monoacylglycerols by cellulosic chiral stationary phases: preliminary data

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Abstract
The separation of sn-1 and sn-3 enantiomers of monoacylglycerols can be useful for the stereospecific analysis of fatty acids in lipids. Four different types of cellulosic Chiral Stationary Phases (CSPs) were tested to assess the possibility to discriminate 1(3)-palmitoylglycerol, 1(3)-stearoylglycerol, 1(3)-oleoylglycerol and 1(3)-linoleoylglycerol. The phases consisted of cellcellose tris(3,5-dimethylphenylcarbamate), cellulose tris(3-chloro-4-methylphenylcarbamate), cellulose tris(4-methylbenzoate) and cellulose tris(4-chloro-3-methylphenylcarbamate); all the columns were 250x4.6 mm, the mobile phase used were n-hexane: 2-propanol in various proportions and the detector employed was an Evaporative Light Scattering (ELSD). Cellulose tris(4-methylbenzoate) CSP was not able to separate the racemic monoacylglycerols; all the other CSPs reached in the discrimination of individual racemes with very good resolutions, significantly higher than the literature values (Deng et al., 2007; Deng et al., 2008). Two CSPs could separate mixtures of 1(3)-palmitoylglycerol and 1(3)-stearoylglycerol, while none were able to discriminate mixtures containing 1(3)-palmitoylglycerol and 1(3)-oleoylglycerol.
Analysis of triacylglycerols in *Brevoortia tyrannus* (menhaden) oil by non-aqueous reversed phase liquid chromatography in combination with mass spectrometry

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**Abstract**

Triacylglycerol (TAG) separation in fish oils represents a challenging and cumbersome task due their high complexity. Such samples contain a variety of different fatty acids including long-chain polyunsaturated components, such as 20:5(ω-3) and 22:6(ω-3). In this work, a non-aqueous reversed phase high performance liquid chromatography method was developed, and optimized for triacylglycerol analysis in a *Brevoortia tyrannus* (menhaden) oil sample. Four columns were serially coupled to tackle such a task, for a total length of 60 cm of shell-packed stationary phase, and operated under ultra high pressure conditions. As detection, positive-ion atmospheric pressure chemical ionization mass spectrometry was used to attain identification of the analyzed sample components. A number of 137 triacylglycerols containing up to 19 fatty acids, with 14 to 22 carbon atom alkyl chain length and 0 to 6 double bonds, were positively identified in the complex lipidic sample. This is the first work that reports an extensive characterization of the triacylglycerol fraction of menhaden oil.

Inorganic anions differences in Italian donkey milk

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**Abstract**

The analysis of inorganic anions is very important because they may be indicative of the quality and safety of milk (Heeschen et al., 1991). Different methods to determine the concentration of cow milk anions have been described in literature (Cataldi et al., 2003; Gaucheron et al., 1999), but the simultaneous determination of inorganic anions in milk has always represented a difficult challenge due to the complexity of this matrix. In this work the simultaneous content of chlorides, nitrites, nitrates, phosphates and sulphates was used to classify 45 donkey milk samples collected from different Italian regions. The anions concentrations were easily determined by Suppressed Ion Chromatography (SIC) following fast milk pre-treatment. The data set was subdivided into three groups according to origin region of milk, and was statistically evaluated by analysis of variance (ANOVA). In a first discriminant analysis procedure, functions based on linear combinations of the loge-transformed element concentrations of anions were generated to classify donkey milk samples from different regions. In an alternative approach, a three-step discriminant analysis procedure to classify a milk sample was tested. The correct classification of the milk samples into one of the group varied from 91.1 to 97.8% depending on the statistical investigation adopted, showing results very satisfactory. The procedure used has proved to be very simple so it could be used as a method for valuating traceability of donkey milk in order to preserve this peculiar product against frauds or commercial disputes.
Technological and permeation properties of a microencapsulated soy isoflavones extract

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Abstract

Functional extracts from Soybean have numerous health benefits deriving from their content in isoflavones, natural antioxidant molecules which fight damages caused by free radicals, mimic the estrogen (ES) in chemical structure and, therefore, have the ability to bind to ES receptors. The interest for isoflavones in skin care is not surprising because ES therapy improves skin parameters such as elasticity, moisturizing, pigmentation and vascularity. As matter of fact, the cosmetic industry use of isoflavones appears significant in anti-wrinkle and skin lightening field because the ability to protect the skin from UV damage, photo- and physiological aging (Iovine et al., 2011). In combination with topical application, there is emerging interest in the potential for their dietary use to improve skin health. In this research we studied a soy isoflavones extract (Iso) which is unique because its highest concentration of these powerful compounds, especially genistein and daidzein. Unfortunately, Iso has a low water solubility giving practical difficulties for the successive manufacturing and reducing its bioavailability. This paper reports on the encapsulation of Iso by spray-drying in a Sodium carboxymethylcellulose coating/swelling matrix. Physicochemical and technological characteristics of the produced powder (Iso-I) such as encapsulation efficiency, particle size (LLS analysis), solid state (DSC) and morphology (SEM, FM), were examined. During 6 months, under accelerated storage conditions, stability of Iso-I, evaluated by analyzing extract content (HPLC), DSC profile as well as antioxidant activity (DPPH test) (Sansone et al., 2011), was kept constan. Interestingly, in vitro dissolution and basic permeation profiles, studied through dissolution apparatus and Franz cell equipment, respectively, showed an enhancement of the dissolution rate in water (80% in 30 min) and a higher permeation (20.6 µg/cm² in 180 min) of Iso-I with respect to Iso (9% dissolution rate and 8.5 µg/cm² of permeation, at the same times). The process led to a handling powder with improved technological characteristics, bioavailability, quality and safety of use, suitable as an ingredient for cosmetic or nutraceutical products.

Ready to use therapeutic food (RUTF): New promising nutraceuticals

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Abstract

Therapeutic foodstuff represent a new promising challenge in the use of food and functional food ingredients for the therapy of different pathologies. Ready to Use Therapeutic Food (RUTF) is the name given to a recently developed mixture of nutrients that has been designed and primarily destined to the therapy of the severe acute malnutrition of babies aged between zero and five years. In the last years the RUTF made possible to intervene locally and establish the right therapy for the severe malnutrition in children in areas where there is need, e.g. Africa or South-East of Asia. The main ingredients of the formulation are powdered milk, peanut butter, vegetable oil, sugar, and a mix of vitamins, salts, and minerals. The potentiality of this food lies both on the low percentage content of water, and on the high energy and nutritional density. The incorporation of active ingredients in the RUTF formulations could allow to develop new foodstuff that can be targeted to specific pathologies or addressed to specific nutritional conditions or health problems. Although the efforts directed to the information enhancement and to the export management, the high cost of the powdered milk, and the food safety problems connected to the possible onset of toxigenic moulds mainly on the peanut butter used in the formulations, slowed down considerably the widespread and homogenous diffusion of the RUTF. The aim of the proposed paper is to add information, present the state of the art on the RUTF, review the different already known formulations, and suggest some possible new formulations.
Aromatic and sensory profiles of blended wines from different percentage of Nero d’Avola, Sagrantino and Barbarossa grape varieties

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Abstract
In this study we evaluated the contribution of different aromatic percentage of Sagrantino and Barbarossa blended with Nero d’Avola: (1) Nero d’Avola 50% Sagrantino 15% and Barbarossa 35%, (2) Nero d’Avola 50% Sagrantino 25% and Barbarossa 25%, (3) Nero d’Avola 50% Sagrantino 35% and Barbarossa 15%. We carried out chemico-physical, gas chromatographic and sensory analyses on the wine samples (vintage 2008). In the volatile fraction we identified 45 components belonging to different classes of substances such as esters, alcohols, acids and terpenes. In all samples analyzed, esters were the most represented class of substances, in particular ethyl octanoate (fruity) and ethyl decanoate (grape); among alcohols, isoamyl alcohol (pungent, alcohol) and beta-phenylethyl alcohol (sweet, floral) were the most represented compounds. Interesting was the content of terpenes, such as hydrocarbon and oxygenated monoterpenes and sesquiterpenes, in charge of floral notes. Comparing the samples, it was observed that the content of esters increased gradually with increasing of the percentage of Sagrantino. A higher percentage of Barbarossa brought greater amount of linalool (fresh, lavender) and cis-rose oxide (floral, pink). From the point of view of the volatile compounds responsible for the aroma, assembled wines, especially the 50% Nero d’Avola, Barbarossa, 35%, 15% Sagrantino may be of great interest. A panel of 12 judges described the sensory profile of wines for characterizing the contribution of each different blending could determine.

Tocopherol content of different legume crops

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Abstract
The health promoting properties of vegetables are strongly related to the presence of bioactive phytochemicals, such as tocopherols; they act as antioxidants by trapping radical intermediates, reducing the risk of chronic diseases, such as cardiovascular diseases and cancer (Bramley et al., 2000). Tocopherols (T) can exist in four different regioisomers: alpha-T, beta-T, gamma-T, and delta-T, which differ for the methylation pattern of the phenolic ring. They have different activities: alpha-T affects health aspects, while gamma-T has a relevant role in protecting the fatty acids in the seed (Cho et al., 2007). In this work, seeds from the main legume crops, i.e. soybean, lupin, chickpea, lentil, bean, pea, and bean, were analysed by HPLC coupled with fluorimetric detector. Gamma-T is the most abundant isomer in all samples, whereas beta-T has never been detected. In general, alpha-T and delta-T are very sensitive to each specific sample, for example alpha-T is absent in common beans, whereas delta-T is absent in narrow-leaf lupin. In soybeans, the total tocopherol content is about 14 mg/100 g, in Albanian peas it falls in the range between 10 and 14 mg/100 g, in Italian peas and broad beans is about 6 mg/100 g, in Italian common beans is about 3 mg/100 g, and in chickpeas is about 11 mg/100 g, in good agreement with literature values (Wyatt et al., 1998; Yoshida et al., 2007).
An HPLC method for the evaluation of the ACE inhibitory activity of plant proteins hydrolysates

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Abstract
Recently in vitro and in vivo studies have demonstrated that peptides obtained from hydrolysis of plant and animal proteins have a direct action on the Angiotensin Converting Enzyme (ACE), the key enzyme involved in blood pressure regulation in humans, competing with angiotensin I for the active site. Food ACE-inhibitory peptides from milk and dairy products, egg, wheat and legumes may be of interest in lowering human pressure maintaining health (Fujita et al., 2000; Vermeirssen et al., 2004). The evaluation of the ACE-inhibitory activity is mainly performed by spectrophotometric methods, based on the use of the model tripeptide hippuryl-histidyl-leucine, HHL (Cushman and Cheung, 1971). However, these techniques show low specificity and sensitivity when applied to complex peptide mixtures obtained from food, containing several interferences. It was thus necessary to develop an innovative strategy based on more accurate methods. For this reason a method based on HPLC coupled with a DAD detector was developed. The optimized method was tested on three drugs (captopril, enalapril, and lisinopril) commonly used in the treatment of hypertension and two tripeptides (IPP and VPP) responsible of ACE inhibitory activity in milk hydrolyzates. Then the method was applied to enzymatic mixtures obtained from digestion of proteins from the main legumes: soybean, pea, lupin, bean, lentil, chickpea.

In-house validation of chromatographic speciation methods for arsenic in fishery products

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Abstract
The relatively high concentrations of arsenic found in shellfish in recent years have contributed to raise the threshold of attention of European Union, in terms of food security. Among the various factors that influence the toxicity of arsenic, the chemical form is of particular significance, given the high toxicity of the inorganic form in respect of the organic that frequently contaminates fishery products. The Expert Committee of FAO / WHO defined a provisional tolerable weekly intake (PTWI) of 15 mg/kg b.w. only for the inorganic form and, in view of definition of residual limits for arsenic in fish, it becomes necessary to have analytical methods able to differentiate inorganic form from the organic ones (e.g. monomethyl- and dimethylarsenic acid, arsenobetaine, arsenocholine). The purpose of this study was to optimize and validate an analytical method for the speciation of arsenic in fishery products, that could identify and quantify the organic forms of arsenic. Screening of organic forms of arsenic was carried out using HPLC (high performance liquid chromatography) coupled to a tandem mass spectrometry detector, while determination of total arsenic was carried out using the atomic absorption spectroscopy. The validation procedure was conducted according to the requirements of the European Community to allow the use of the present method by the Official Control laboratories. The matrices considered for method optimization and validation have been fish, molluscs and crustaceans from the coasts of southern Italy. Obtained results allowed the method to enter within the routinely activities of the laboratory and require method accreditation.
Eating habits and human blood levels of dioxins (PCDD/FS) and PCBs in Campania

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Abstract
The PCDD/Fs (dioxins) and PCBs are polychlorinated organic compounds highly stable and extremely persistent. The main sources are anthropogenic processes as incineration of chlorine-containing wastes, industrial emissions, cement and paper production; they are ubiquitous environmental contaminants and bio-accumulate along the food chain. Dioxins and PCBs exposition for the general population is over 90% through food. As regards the recent Campania crisis, data obtained from Italian food and environmental monitoring plans showed marked differences between some areas within the Region in terms of contamination. The most used food bio-indicator for the evaluation of human exposition to dioxins was milk, although blood levels for Campania’s population are poorly known, and are generally unknown for humans. The aim of the project was then to determine blood dioxins levels in subjects living in the areas at high risk of environmental contamination of Naples province, and, for comparison, residents in areas where contamination is lower. Subjects were divided into normal weight, overweight and obese according to respective body mass indexes; the dietary habits were also investigated due to the strict correlation with dioxins and PCBs accumulation in fatty tissues. The constant lipid mobilization can make it bio-available lipophilic contaminants, resulting in an increase in blood concentrations of dioxins and PCBs and therefore an increased health risk. The results of the project allowed to obtain the knowledge of the actual blood levels of dioxins and PCBs in eleven territories of the Campania region and to assess the effect of a dietary habit on blood levels of such lipophilic contaminants.

Antioxidant activity of Parmigiano Reggiano cheese at different ageing time

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Abstract
Parmigiano Reggiano is a typical Italian cheese, included in the food list Protected Designation of Origin (PDO, EU regulation 2081/92). Parmigiano Reggiano is an hard cheese made from cow’s milk and is characterized by a long maturation period, of at least 12 months of ripening. In the ripening period, several biochemical reactions occur. Among them, proteolysis has an important role concerning the development of flavour and texture. The proteolysis is catalyzed by enzymes from milk, coagulant agents and proteinases from microflora starter and non starter, and yields free amino acids and different sized-peptides as final products. As a consequence, the composition of the aminoacidic and peptide fraction in cheeses is constantly changing during the ageing time. (Sousa et al., 2001; Gatti et al., 2008). Peptides and free amino acids can be characterized by antioxidant activity (Sarmadi et al., 2010; Tsopmo et al., 2011, Li et al., 2011). In order to determine the antioxidant activity of Parmigiano Reggiano cheeses, in this study ABTS assays of water soluble extracts of Parmigiano Reggiano cheese (WSEs) at different months of ripening were performed. The WSEs of Parmigiano Reggiano cheese were also fractionated by semipreparative HPLC-UV and the radical scavenging capacity for each subfractions was also measured. In order to correlate the radical scavenging capacity to the molecular composition, the WSEs and each subfraction were analyzed by LC/ESI-MS. All the extracts were found to have a good antioxidant activity, nearly unaffected by the ripening time, which was mainly associated to the free aminoacidic fraction, and in particular to tyrosine, tryptophan and methionine. In vitro simulated gastro-intestinal digestions of WSE were also performed. The digestion only caused a slight decrease in the measured antioxidant acitivity.
Common wheat determination in durum wheat samples through LC/MS analysis of gluten peptides

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Abstract
Cereals are widely harvested and wheat is one of the most important crop in the world, especially in Western countries (Leff et al., 2004). The main species for human consumption are *Triticum aestivum* (common wheat, usually employed for bread or other baked products) and *Triticum turgidum* spp *durum* (durum wheat, usually employed for pasta production) (Liu et al., 1996). In Italy, pasta must be made exclusively from durum wheat, and a maximum of 3% of common wheat contamination is allowed in durum wheat flour (DPR 187/01). Since durum wheat price is quite 25% higher than those of common wheat (ISMEA) useful tools for the detection of the adulteration of durum wheat flour with *T. aestivum* are required. In the present work, a method for identifying and quantifying the adulteration of durum wheat with common wheat was developed, analyzing peptic/chymotryptic digests of wheat flour by LC/ESI-MS. Among the peptides identified, one was found to be present only in common wheat, due to the absence in durum wheat of DD genome, where the sequence coding for that particular isoforms of gliadin typical of common wheat was located. In order to relate the amount of common wheat to the total wheat content of the sample, a peptide present in all wheat samples was taken as an internal reference. The marker peptides were identified by the mass spectra obtained from MS/MS experiments. A calibration curve was obtained, calculating the ratio between the chromatographic areas of the two marker peptides for samples at known composition. The method was blindly tested with samples at different levels of contamination and showed a good accuracy, even when blending different wheat varieties. Finally, a survey made analyzing the durum wheat flour brands in the Italian market outlined that common wheat contamination is commonplace.

Non proteolytic aminoacyl derivatives in cheeses

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Abstract
Cheeses are complex mixtures of aminoacids, peptides and proteins, mostly derived from the proteolysis of the caseinic fraction precipitated in the curd during cheese production (McSweeney, 2004). In the last years several studies have demonstrated that small aminoacyl dipeptide-like derivatives of non proteolytic origins are present in not negligible amount in several cheeses, like Parmigiano-Reggiano, Grana Padano and Asiago. These compounds were identified as γ-glutamyl-amino acids, lactoyl-lamino acids and pyroglutamyl-amino acids, and collectively named Non Proteolytic Aminoacyl Derivatives (NPAD). NPADs were found to be only formed by L-glutamic, lactic and pyroglutamic acids (although in cheese their D-counterparts are abundant) linked to lipohylic aminoacids (mostly Phe, Leu, Ile), suggesting a common enzymatic origin, and their amount was found to increase with the ageing time (Sforza et al., 2009). By using LC/MS technologies, a deep investigation on their origin was undertaken. The cheeses where their amount was found to be higher (up to 50 mg /100 g of cheese) where those with a long ageing time and a consistent presence of lactic acid bacteria. Several strains of lactic acid bacteria, both starters and non starters, isolated from Parmigiano-Reggiano cheese, were found to have the ability to produce γ-Glu-Phe e Lac-Phe. Experiments with Parmigiano-Reggiano extracts and isotopically labelled precursors indicated the presence in the cheese of an enzymatic activity able to produce these compounds starting from glutamic and lactic acid. Further investigations are now in progress in order to identify and isolate the producing enzyme(s). These compounds demonstrate that the peptidic fraction of Parmigiano-Reggiano is more complex than usually thought, and that enzymatic activities on proteins are not only reponsible for their hydrolysis, but starting from free amino acids can also form new unusual aminoacyl derivatives.
Towards a new generation of food additives based on the covalent bond principle

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Abstract
Oxidative stress and inflammatory processes are thought to be the key factors in functional degenerations such as those related to age, and an efficient prevention might be achieved using antioxidant and anti-inflammatory substances, possibly coming from natural fresh sources such as fruit and vegetables. Unfortunately, despite their performances in vitro, the activity of these micronutrients in vivo is scarce, and this is mainly due to their poor bioavailability and metabolism within the body. The concept underlying this work, which is taken from Nature itself, is that bioactivity can be enhanced through pre-organization at molecular level; the idea is to bind around a central and inert multifunctional scaffold such as a PAMAM dendrimer platform a series of bioactive metabolites (namely polyphenols) from natural sources, with the aim to build up a pre-organized and multifunctional biomolecular device. The use of these multifunctional scaffolds would rise two objectives: bring the bioactive molecules all together at the target, and exploit, if any, their synergistic effects. The main aim is to create a new food line meeting the particular nutrition requirements of specific people (such as the elderly). This approach is new in this area, although it has been successfully used in latest generation pharmacological preparations. An expected scientific impact of this work concerns the connections of the results with the developments in the nutritional genomic, chemogenomic and proteomic. In fact, according to the recent studies on nutritional genomics (nutrigenomics and nutrigenetics) “in the long term, a ‘personalized nutrition’ approach, whereby food and nutrient intake can be manipulated/optimized based on an individual’s genetic profile, may be used to promote health and quality of life”.

Levels of nitrates and nitrites in baby foods marketed in Italy

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Abstract
Recently EU Commission asked the European Food Safety Authority (EFSA) to update an opinion on the risk posed by human exposure to nitrates particularly for infants and children consuming foods containing leafy green vegetables. The opinion was not entirely reassuring, especially for young children, in relation to the ingestion of spinach. For infants and children is important to define limits of daily exposure, that can exclude a risk of methemoglobinemia. Aim of this study was to examine the levels of nitrates and nitrites in baby foods, after considering the data on the consumption of these products in the DONALD study in the period of weaning in order to evaluate if they could exceed the ADI and ARfD to small consumers. N. 53 samples of baby food, (n.20 of animal origin, n.18 of vegetable origin and n.15 mixed) were collected on Italian market and analyzed using AOAC official method (1995). No sample exceeded the limit set by EC Regulation 1881/2006. In addition, in all samples nitrite levels were lower than the nitrates ones. The highest nitrate values were found in infant foods of vegetable origin. High nitrite levels were detected in baby food of animal origin. The results show that commercial baby food might represent some risk. In order to reduce the risks arising from their consumption manufacturers of baby food produced with raw vegetable materials from areas with high levels of nitrates in the soil should monitor the amount of nitrate / nitrite in the final products.
Nutritional aspects of white lupin seeds consumed as a snack

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Abstract
White lupin (Lupinus albus L.) is a typical product of the Mediterranean area which has recently been a renewed interest in the lupin in relation to its interesting nutritional properties and potential health benefits. Towards this end, this paper presents the chemical characterization of two landraces (GEN1 and GEN2) and four varieties (‘Aster’, ‘Lublanc’, ‘Lutteur’, ‘Multitalia’) of white lupin. Protein, starch and elements were determined on the whole flour obtained with a rotary mill fitted. Resistant starch fraction was determined on seeds before and after soaking and cooking. The macro- (N, P and K), meso- (Ca and Mg) and micro-elements (Mn, Fe, Zn, B, and Cu) were quantified on the extracts obtained by acid digestion of the sample and successive analytical determination by ICP- AES. The results showed a wide range of variability of protein content from 27.12% (cv ‘Multitalia’) to 45.34% d.m. (cv ‘Lublanc’) with mean value 35.97% d.m.. The mean values of macro-elements such as phosphorus and potassium were 5306 mg/Kg and 4081 mg/Kg, respectively on dry weight basis (d.m.). On average, the calcium and magnesium contents of the white lupin were found to be 1478 mg/Kg and 983 mg/Kg, respectively on d.m.. A high content of manganese (644.68 mg/Kg) was evidenced among the considered micro-elements. This result had been already pointed out in previous reports of the XXth century. The mean iron and copper contents for the samples were 47.62 mg/Kg and 15.79 mg/Kg, respectively on d.m.. The mean value of resistant starch fraction was 0.24% d.m.). Following the study objectives, the lupin seeds were also soaked (12 h) and cooked (10 and 20 minutes) in water and a significant increase of the resistant starch fraction was observed after soaking and cooking. Information on nutritional potential of white lupin genotypes is a useful tool for breeders, food industries and consumers.

Quality of pseudocereals cultivated in Sicily for ‘gluten-free’ foods

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Abstract
The need to provide people with gluten intolerance and celiac disease has increased the requests for gluten-free foods with good nutritional values as well as with sensory and technological quality. There are amaranth, buckwheat and quinoa among the species suitable for obtaining gluten-free flours. In this study, we compared three species of amaranth (Amaranthus caudatus, A. cruentus, A. hypocondriacus), three varieties of buckwheat (Fagopyrum esculentum Moench., cvv ‘Rana’, ‘Siva’ and ‘Veronica’) and one genotypes of quinoa (Chenopodium quinoa Willd.) cultivated according a randomized block design in South-Eastern Sicily. The seeds were ground and on the whole flour, protein content by Kjeldahl method and ash were determined. Micro-elements (Mn, Fe, Zn, Cu) were quantified on the extracts obtained by acid digestion of the sample and subsequent analytical determination by ICP-AES. The colorimetric parameters (L* a* b*) of whole flours were determined. The results were compared with three genotypes of durum wheat such as ‘Russello’, a local landrace cultivated in the South-Eastern of Sicily; ‘Simeto’ and ‘Mongibello’, that is old and new varieties respectively, both selected in Sicily. As for the protein content, the highest values were recorded for amaranth (15.91% d.m.) and quinoa (15.31% d.m.). The buckwheat cv ‘Rana’ showed a high iron content (527.42 and 313.35 mg/kg, respectively). The highest content of copper was found in the genotypes of buckwheat (6.54 mg/Kg). Quinoa has recorded the highest values of zinc (24.73 mg/Kg) and manganese (40.89 mg/Kg). The different species of pseudocereals, compared to genotypes of durum wheat, showed a highest average ash content (3.68%). The Amaranthus spp. showed a good index yellow of the whole flour (13.57 b*), a feature that ensures color of the processed products similar to the one given by durum wheat.
Analysis of triacylglycerols in *Brevoortia tyrannus* (menhaden) oil by non-aqueous reversed phase liquid chromatography in combination with mass spectrometry

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Abstract

Triacylglycerol (TAG) separation in fish oils represents a challenging and cumbersome task due their high complexity. Such samples contain a variety of different fatty acids including long-chain polyunsaturated components, such as 20:5(ω-3) and 22:6(ω-3). In this work, a non-aqueous reversed phase high performance liquid chromatography method was developed, and optimized for triacylglycerol analysis in a *Brevoortia tyrannus* (menhaden) oil sample. Four columns were serially coupled to tackle such a task, for a total length of 60 cm of shell-packed stationary phase, and operated under ultra high pressure conditions. As detection, positive-ion atmospheric pressure chemical ionization mass spectrometry was used to attain identification of the analyzed sample components. A number of 137 triacylglycerols containing up to 19 fatty acids, with 14 to 22 carbon atom alkyl chain length and 0 to 6 double bonds, were positively identified in the complex lipidic sample. This is the first work that reports an extensive characterization of the triacylglycerol fraction of menhaden oil.

Varietal characterization of autochthonous Maltese grapes

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Abstract

The phenolic composition of the two most important maltese grape varieties, Gellewza (red berries) and Girgentina (white berries), has been studied. The Gellewza features a prevalence of trioxigenated anthocyanins, a medium-high content of acylated anthocyanins and a acetates to p-cumarates ratio lower than 1. Among flavonols, quercetin glycosides are more abundant than myricetin glycosides, even though the glucosides of both are present at comparable levels. Among HTCAs the caftaric acid is more abundant than p-coutaric and fertaric ones. This cultivar is characterized by berries with an average weight of 2.13g; anthocyanin content reached 2029 g/Kg, 780mg/Kg of which were easily extractable. The Girgentina has rather large berries (4g approx). Similarly to all other white grapes quercetin glycosides are more prevalent compared to glycosides of other flavonols, caftaric acid of other HTCAs as well. Vinifications of the two cultivar were performed taking into account grape composition. The ProMed Project is funded by the Operational Programme Italy-Malta 2007-2013
P-216

**Authenticity studies of Parmigiano-Reggiano cheese by LC/ESI-MS and NMR analysis**

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**Abstract**

Parmigiano Reggiano is a well known Italian hard cheese, long ripened, made from raw and partially skimmed cows’ milk. It is included in the list of Italian cheeses bearing the Protected Designation of Origin (PDO, EU regulation 2081/92). This definition includes technological characteristics and geographic restrictions. During the ripening time casein degradation occurs, catalyzed by proteolytic enzymes with different specificities. On the other hand, during the last few a great interest has been grown for the protection of typical food products, as Parmigiano–Reggiano, from adulteration, sofistication and falsification. In this work we have been focused on the development of methodologies able to characterize Parmigiano-Reggiano from other cheese fakes. In particular we performed parallel analyses by with two different techniques: NMR and LC-ESI-MS, which are frequently used as technological platforms for metabolomics applications. Water extracts coming from different ages of ripening of Parmigiano-Reggiano and other cheese fakes were analyzed with both the techniques giving rise to a “molecular map” made by characteristic peptides, amino acids and other metabolites. Multivariate statistical analysis (PCA) was applied on the integrated data in order to evaluate significant variations between the different groups of cheese samples. Statistical data elaboration will be presented and discriminant molecular markers assignment will be discussed. Some initial HR-MAS-NMR studies on the same cheese samples will be also showed and results will be compared to aqueous extracted analyses.

P-217

**Antioxidant properties and anti-neoplastic effects of purple-fleshed potatoes**

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**Abstract**

*Solanum tuberosa* L. var *vitelotte* is a purple-fleshed potato widely used for human consumption and well appreciated for its good nutritional characteristics. The pigments responsible for the attractive colour of these potatoes varieties belong to the class of anthocyanins, whose concentration vary in large ranges and contribute to the degree of pigmentation in potato flesh. A good correlation between the total anthocyanins and the biological activities of the tubers was found. The objectives of this study were to characterize and measure the concentration of anthocyanin pigments in purple-fleshed potatoes and to evaluate their antioxidant effect and anti-cancer potential. The preparative isolation of the pigments was carried out by means of HPLC instrument coupled to photodiode array detector and triple quadruple mass spectrometer for the anthocyanin characterization. Antioxidant activity was evaluated using chemical assays such as DPPH and FRAPS methods. To characterize the anti-neoplastic action of the tubers, we studied morpholgy of our cells, cell cycle characteristics and apoptosis. Immunostaining, differentiation and western blot analyses were also performed. Results show that anthocyanins extracted by *S. tuberosa vitelotte* were characterized by a good radical-scavenging and reducing capacity and were able to exert anti-cancer activity on cultures in vitro and ex vivo.
Antioxidant profile and in vitro antiproliferative and cardioprotective properties of typical red and white wines from vineyards cultivated in Scafati (Salerno, Italy)

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Abstract
The polyphenolic composition and antioxidant properties of red (Aglianico) and white (Falanghina) wines were evaluated. Samples showed higher values than those reported elsewhere for the same variety of wines with a different geographical origin and other more conventional wines. Lyophilised samples demonstrated to store their antioxidant profile and were subjected to in vitro assays. The best results were exhibited by white wine samples which revealed a good antiproliferative activity on intestinal neoplastic cells and an appreciable radical scavenging activity on cardiomyocytes. Results highlighted the wine samples and their processed form as products with high added nutritional value and therapeutic potential.

Antioxidant profile and in vitro cardiac radical-scavenging vs pro-oxidant effects of commercial red grape juices (Vitis vinifera L. cv. Aglianico N.)

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Abstract
Several works have reported on the antioxidant and cardioprotective properties of grape products, mainly grape seed extracts. The present study represents the first investigation on whole commercial red grape juices (Vitis vinifera L. cv. Aglianico N.) before and after lyophilisation. The results obtained revealed that the juice samples were characterised by a valuable antioxidant profile in comparison with other red grape products and a good stability to the freeze-drying process. Experiments on cardiomyocytes showed an appreciable direct radical scavenging activity at low sample doses while pro-oxidant properties at higher concentrations were detected. A combination of doxorubicin and lyophilised juice was tested on cardiomyocytes: an appreciable cardioprotective effect at low juice sample doses was revealed. Our data suggest that both juice and its formulation for food supplements could be able to favor a reduction of cardiovascular disease risk both in normal subjects and in patients undergoing doxorubicin therapy.
Volatile and sensory profile of wines produced without Sulphur Dioxide

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Abstract
Aroma is one of the main attributes of wine and its characteristics depend on grape variety, winemaking and storage conditions. Sulphur dioxide (SO₂) is the most important preservative, antiseptic and antioxidant used in winemaking for the protection of wine from alterations. However, sulphur dioxide can increase to various problems in people with asthma or allergy as well as create discomfort in healthy individuals (Vally, 2001). In the last years there has been a growing interest in alternative compounds to SO₂ (i.e. ascorbic acid or tannins), especially to protect wine aroma during storage (Lambropoulosa, 2007; Roussis, 2007). The aim of this study was to evaluate the reliability of wines from four different grape varieties (Chardonnay, Pinot, Greco and Montepulciano), produced without adding SO₂ in the winemaking process. GC-MS analyses together with sensory analyses were performed to compare aroma of wines obtained with and without adding SO₂. A total of 54 volatile compounds were quantified and their concentration appeared significantly influenced by SO₂. In particular, flavour compounds increased in Pinot wine without SO₂. Conversely, the volatiles content of Montepulciano and Greco wines were negatively affected by the absence of SO₂, that was probably due to the presence of wild yeasts, that inhibited the development of the selected yeast (Tamborra, 1993). However, they showed higher organoleptic quality by sensory analysis. Finally, no significant differences in total volatile contents were found in Chardonnay wines. Although adding SO₂ is still a widespread practice in winemaking process, gathered results are major arguments in favour of the hypothesis to obtain wines of good sensorial quality without using hazardous chemical additives.

New inflammatory testing in milk cattle based on immunosensor for IGG determination

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Abstract
We have developed a “direct” measurement immunosensor for IgG determination in milk based on surface plasmon resonance (SPR) transduction [Campanella et al., 2010]. The analytical features of this new device were compared with those of a classical “competitive” amperometric immunosensor using peroxidase as marker and hydrogen peroxide transducer. The present study is aimed at testing suitable immunosensor methods for the measurement of immunoglobulin G (IgG) in several milk samples from dairy farms in the pontine area (Lazio, Italy). The immunoglobulin G concentration of different samples ranges form about 675 mg L⁻¹ to 2,690 mg L⁻¹. This wide range may be only in part justified on the basis of data in literature, in which several authors observed as IgG concentration in cow and in buffalo milk can change considerably during the lactation period depending on different factors, such as the season, the animal’s physiological status and feeding [Hurley et al., 2011], but can depend also from typical inflammatory condition occurring in the cattle, such as mastitis [Harmon et al., 1976]. Therefore studied immunosensors can be proposed as an useful method for a rapid test to check inflammatory diseases in cattle, thus safeguarding also the health qualities of cow’s milk available on the market.
Multidimensional liquid-gas (LC-GC) chromatography for the rapid determination of saturated hydrocarbon contamination in baby food

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Abstract
The present research is directed toward the use of a rapid heart-cutting LC-GC method for the analysis of mineral oil saturated hydrocarbons, contained in a series of baby foods (vegetable, meat, fruit). The automated LC-GC experiments were carried out by using a system equipped with a syringe type interface, capable of both heart-cutting and comprehensive two-dimensional analysis. The first dimension separation was achieved on a silica column, operated under isocratic conditions (hexane). The heart-cuts were transferred to a programmed temperature vaporizer. After the large volume injection, the target analytes were separated in a rapid manner (~ 9 min) using a micro-bore GC capillary. The overall LC-GC run time enabled the analysis of circa 4 samples/hour. Several commercial samples were subjected to analysis, with various degrees of contamination found in all samples.

Bioethanol production from pineapple wastes

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Abstract
Bioethanol at industrial scale is currently produced from sugar and starch which, coming from the vegetal world, creates concerns regarding competition with food and feed supplies. Alternative substrates such as lignocellulosics from agricultural wastes, woody biomasses, and lignocellulosic energy crops, are targeted as the new “second generation” feedstocks for bioethanol production. This may reduce competition with food production as well as providing a potential additional income stream. Pineapple wastes are produced in large quantities by canning industries throughout the world. These wastes are rich in sugars and plant cell walls. The purpose of this study has been to investigate the potential for transforming such residues into bioethanol by saccharification of the plant cell walls, and fermentation of available sugars using yeast (Saccharomyces cerevisiae). Pineapple wastes, comprising fruit skin and core, were homogenized in a fruit blender. The resulting homogenate was immediately thermally treated at 100°C to inactivate endogenous enzymes and to reduce microbial spoilage. The homogenate (1.5 l) with a dry matter content of 9% (w/w) was saccharified at in a 2.5 l fermenter using commercially-available cocktails of cell-wall degrading enzymes at 50°C, pH 5 for 2-4 h with constant stirring. At this stage, the digestate was cooled to 30°C and active yeast inoculum was added (approximately 107 cells per ml). Fermentation was carried out at 30°C, pH 4.5 with constant stirring. CO2 evolution was measured during the fermentation and representative samples of the fermentate were taken at regular intervals. These were then evaluated for ethanol concentration, soluble sugars and undegraded polysaccharides using GC and HPLC methods. The initial results show that the main sugars in the biomass were glucose, uronic acid, xylose, galactose, arabinose and mannose. All the hexoses are suitable for bioethanol production by S. cerevisiae. The yield of ethanol will be discussed and compared with successful field trials of a commercial pilot plant facility carried out during 2009/10 by PBI S.A., Costa Rica.
Comprehensive analysis of vitamin E isoforms in pistachio (Pistacia vera l.) by liquid chromatography tandem mass spectrometry

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Abstract
The eight naturally occurring lipophilic compounds α-, β-, γ- and δ-tocopherols (T) and the respective tocotrienols (T3) belong to vitamin E family. In addition to the vitaminic activity, they exert other biological activities due to their antioxidative property and prevention capability in the lipid peroxidation propagation. Concerning the analysis of vitamin E, chromatographic separation of β- and γ- isomers is generally carried out by NP-HPLC despite of hazardous solvents use and the weakness of MS detection sensitivity. Therefore, it is more desirable the employment of RP-HPLC. The main aim of the study was to develop an extremely simple, rapid and sensitive LC-MS/MS method for eight Vitamin E isoforms simultaneous determination in raw Bronte Pistachio (Pistacia Vera L.) nuts samples. Chromatographic separation was carried out on a pentafluorophenyl (PFP) column and was achieved by an isocratic elution with MeOH/H2O acidified with TCA. MS detection was performed on a QQQ instrument interfaced with APCI source operating in positive mode. A multiple reaction monitoring method was employed to increase sensitivity. Remarkable features of the method were: low detection (1-3 ngmL⁻¹) and quantification (4-10 ngmL⁻¹) limits, wide linear ranges (5-15000 ngmL⁻¹) and shortness of analysis time (only 15 min). All vitamin E isoforms were found in raw Bronte Pistachio nuts: γ- and α- isoforms were the predominant ones (average content values: γ-T = 9.10±1.04 mg/100g, γ-T3 = 1.08±0.15 mg/100g; α-T = 0.31±0.05 mg/100g and α-T3 = 0.05±0.01 mg/100g). The proposed LC–MS/MS method is suitable to expand the composition knowledge of the comprehensive vitamin E constituents in foodstuffs.

Traceability of PGI “Pachino cherry types” Through ICP-MS analysis of rees

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Abstract
In recent years, traceability has raised interest because of the impact on economy and on quality appearance. Free trade has encouraged a highly competitive economy where food quality comes after lower prices. In this context, food authentication is of paramount importance to consumers and producers. This aspect is more important when the products are protected by trademarks such as PDO and PGI, in accordance with the European Union policy aimed at protecting agricultural products and identifiable food in respect of their geographical origin (CEE Regulation 2081/92). Our study aims to outline the multielemental profile, through the use of ICP-MS, to establish the traceability of the Pachino Tomato, a product typified with the PGI label (Protected Geographic Indication). This method allows a rapid analysis of most chemical elements, reaching quantification limits (LOQ) in the order of ng/g and providing a wide linear dynamic range. In our research, 24 samples of Pachino tomatoes were analyzed, 15 samples were of Pachino origin and 9 of non-Pachino origin. The study was focused on the distribution of some elements belonging to the lanthanide group, called “rare earth elements” or REEs. Because of their great chemical similarity, these elements may not be subject to selective subdivisions in the distribution of concentration from soil to food, thus making a correlation between food and soil of origin possible. Before ICP-MS analysis, “cherry types” samples undergone a process of preparation characterized by the following stages: freezing, lyophilization, homogenization, digestion with microwave system, filtration and preparation of a volumetric solution (Bettinelli et al., 2005; Spalla et al., 2009). Analytical data were, then, studied by multivariate statistical analysis to extract the information contained in the data set to highlight the differences between samples of different origins without a priori knowledge of true sample origin. The results of this study indicate that it is possible to classify a sample of unknown origin as Pachino or non-Pachino, with a high percentage of correctness, on the basis of the lanthanide concentration.
Discrimination of donkey milk according to geographical origin by ICP-MS and ICP-OES

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Abstract
In this work we applied the results carried out on 45 samples of milk of a donkey belonging to racial Ragusa, 15 forage samples and 15 feed samples, all taken between 2009 and 2010 from 3 different farms in Sicily to the PCA (Principal Component Analysis). The application of multivariate chemical data has provided additional applications that have allowed to address different questions (Merengo et al., 1991; Favretto et al., 1989). In the samples analyzed by ICP-OES and ICP-MS, were investigated following elements: As, Cd, Cr, Hg, Ni, Se, Sb, Pb, Cu, Fe and Zn. Thanks to the processing of test results using the statistical technique of PCA has been able to assess whether the concentrations of certain mineral elements could allow to discriminate the geographical origin of milk, and see which one could observe correlation between the concentrations of elements in milk, forages and feed. So it was possible to discriminate between samples of donkey's milk from different farms based on the content of toxic and essential metals. These results may be of particular importance for the production of a donkey milk quality, which has an adequate content of essential metals and which respects the values for toxic metals of the guidelines proposed by various agencies of pediatric and European Community Legislation.

Characterization of secondary metabolites in Saffron from Cascia (Umbria)

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Abstract
Saffron spice, the dried stigmas of Crocus sativus L., is characterized by the presence of biologically active secondary metabolites. The major bioactive compounds in saffron are crocins, a family of red-colored and water-soluble carotenoids, picrocrocin, a monoterpene glycoside and safranal, a monoterpene aldehyde. These secondary metabolites contribute not only to saffron sensory properties, color, taste and aroma but also to the health-promoting properties. It has been reported that saffron metabolites have positive effects on gastric disorders, cardiovascular disease, insulin resistance, depression, insomnia, and anxiety (Melnyk et al., 2010). In vitro and in vivo studies have also demonstrated that saffron extract show anticancer properties (Tavakkol-Afshari et al., 2008). Saffron’s quality depends on the concentration of the secondary metabolites, which is influenced by many factors such as soil, climate, rainfall, harvest time, and post-harvest treatments. Dehydration not only is important to the preservation of saffron but is actually critical in the release of safranal from picrocrocin via enzymatic activity (Del Campo, 2010). While it is accepted that the dehydration procedure is responsible for saffron properties, the best conditions to carry out this post-harvest treatment remain difficult to establish. The aim of the present research was to study the secondary metabolites of saffron produced in the area of Cascia, in central Italy. In particular, changes in secondary metabolites as a result of different dehydration conditions have been evaluated, with the objective to standardize the drying conditions for high quality saffron obtaining. Water soluble crocins and picrocrocin have been analyzed by high-performance liquid chromatography (HPLC) - diode array detection (DAD) while solid-phase microextraction high resolution gas chromatography-mass spectrometry (SPME-HRGC-MS) has been used for volatile compound analysis.
P-228

Coeliac disease and oats: Molecular assessment of their suitability for coeliac’s diet
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Abstract
Coeliac Disease (CD) is an immune-mediated enteropathy that develops in genetically predisposed subjects following the ingestion of gluten, specially gliadin, the alcohol-soluble protein present in wheat, or similar proteins such as secalin in rye and hordein in barley. In CD subjects, the ingestion of gluten causes an inappropriate small intestinal immune response characterized by villous atrophy, crypt hyperplasia and increasing intraepithelial lymphocytes infiltration (Fasano and Catassi, 2001). The issue whether oats can be considered safe for coeliac patients has been debated for a long time, and only recently EU (EU Regulation 41/2009) included oats among gluten-free ingredients, when the gluten content does not exceed 20 ppm. The aim of this study was the evaluation of the protein pattern of 36 different oat cultivars and their safety for coeliac patients. The cross-reactivity between prolamins of oats and gliadins was evaluated by using both immunoelectrophoretic techniques (SDS-PAGE/ immunoblotting) and a commercial ELISA kit. The protein patterns of oat varieties were both qualitatively and quantitatively different and showed different binding affinities for specific anti-gliadin polyclonal antibodies in immunoblotting. In most oat samples, the content of “gluten-like proteins”, measured by ELISA, was below the established limit of 20 ppm for gluten-free products; on the contrary, two samples presented a gluten-like immunoreactivity (contamination must be considered negligible) above the upper limit of the method (80 ppm). Even though the biochemical characteristics of most oats analyzed in this study allow to confirm its safe use for CD patients, we suggest a careful choice among those oat varieties associated with the lowest gluten-like immunoreactivity.

P-229

Involvement of maillard reaction in Fontina cheese with brown discoloration
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Abstract
Brown discoloration was observed in many cheeses but, currently, there are few research documents available on the discoloration of Italian cheese and very few on Fontina cheese. Fontina is an Italian semi cooked and semi hard cheese made from raw milk and typically produced in Aosta Valley, PDO labeled. The discoloration of some Italian cheeses was associated with the nondialyzable fat-free fraction; the color was found to be covalently bounded to the protein or was the water-insoluble portion of the casein, which has been changed through oxidation. In Manchego cheese with brown discoloration it was found an accumulation of residual sugar, in particular galactose, during ripening. Accumulation of reducing sugar, accompanied by the normal proteolysis that occurs in cheese, creates the potential for the Maillard browning reaction. A sensitive indicator for the assessment of Maillard browning reaction in food, in dairy products in particular, is Furosine [Nε-(furoylmethyl)-L-lysine] formed during the hydrolysis of early Maillard products of proteins. Aim of this study was to investigate, in Fontina cheese with brown discoloration, the presence of residual reducing sugar by using an HPLC method and study the involvement of Maillard reaction through the determination of Furosine by liquid chromatography-electrospray tandem mass spectrometry method. Fontina cheese without defect was used as control. The brown band, observed in Fontina cheese, started at approximately 2 cm below the surface and spread throughout the entire cheese. Fontina cheese with brown discoloration showed a significant accumulation of glucose in comparison with control (0.13± 0.09 g/Kg cheese vs 0.06 ± 0.06 g/Kg cheese; p<0.05) while some, but not significant, residual galactose was present in both (0.29±0.18 vs 0.27±0.14 g/Kg cheese; p=0.639). No residual lactose was found. Furosine content was significantly higher in cheese with defect than in control (29.61 μg/g protein vs 16.86 μg/g protein; p=0.00). These results suggest the hypothesis that the presence of residual sugar during proteolysis, occurring in cheese throughout ripening, creates the potential for the formation of glycation compounds in particular furosine that accumulates causing brown discoloration in cheese. The present study describes some chemical investigations carried out to determine the origin of brown discoloration in Fontina cheese, but it remain to investigate microbiological and biochemical mechanisms involved in residual sugar accumulation.
Dihydroasparagusic acid, a strong antioxidant natural product from *Asparagus spp.*

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Abstract

Dihydroasparagusic acid (DHAA) is the first dimercaptanic substance isolated from a natural source [Jansen, 1948]: *Asparagus spp.* (Liliaceae) [Zangheri, 1976] a vegetable widely distributed and also cultivated in the Mediterranean area. This vegetable is largely used in traditional medicine considering its diuretic and depurative effects, but it is also an important ingredient of Italian cooking and generally in Mediterranean diet. Recently this substance was investigated to provide a preventive and/or therapeutic medicine for vascular disease, having both vasodilator and antioxidant action [Adachi, 2009]. Therefore we decided to evaluate the antioxidant capability of DHAA which was not previously quantified, using a synthetic sample of DHAA. Even if this substance is known since 1948, only few synthesis are reported in the literature [Schotte, 1956; Yanagawa, 1973; Basinger, 1981; Singh, 1990]. In this work we improved the existing synthetic procedures, obtaining a final yield of 60% respect to 30% reported in the literature. The in vitro antioxidant activity of DHAA was tested in comparison to BHA using DPPH method [Brand-Williams, 1995; Faudale, 2008]. We found an IC<sub>50</sub> = 3.7 µg/mL. The chelating ability toward Fe<sup>2+</sup> was also tested indicating a 33% of inhibition compared with EDTA. These results confirm a notable antioxidant activity of this compound which was compared also with glutathione [Mazor, 2006], an important endogenous antioxidant. In conclusion DHAA demonstrates to have a significant antioxidant activity which may confirms the protective action on vascular disease and may be considered as an active ingredient for the preparation of functional foods.

Resveratrol: From diet to model for novel therapeutic applications

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Abstract

Resveratrol (trans-3,4′,5′-trihydroxystilbene), a polyphenolic phytoalexin present in grapes, peanuts and mulberry, has attracted increasing interest of the biomedical researchers due to its relatively simple structure and the number of beneficial physiological effects it produces, ending in a number of health food preparations. Although animal experimental models support the beneficial effects of resveratrol, data on intake at high doses in humans are still lacking. In the course of our studies on chemical derivatisations of the parent compound to improve its chemopreventive properties, we have selectively introduced in the original molecule a number of lipophylic chains in the di-hydroxylated ring. The prenyl derivatives are more potent than resveratrol in tumor anti-initiating mechanisms. In terms of antitumor promoting activities, they are less efficient in Cox-1 inhibition, but more potent in inhibiting iNOS induction. They potently inhibit aromatase activity with IC<sub>50</sub> values below 7.5 µM, whereas resveratrol was inactive at 50 µM concentration. They potently induce alkaline phosphatase activity in Ishikawa cells, indicative of estrogenic activity. One derivative with an EC<sub>50</sub> of 60 pM was more effective than -estradiol also in a rat in vivo uterotropy model, opening possible applications in bone regenerating medicine.
Designing chemopreventive nutraceuticals based on natural polyphenols

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Abstract
Polyphenols, are molecules present in foods of plant origin, this important class of compounds is of high nutritional interest, but with a wide margins of variations among activities in different reports. Besides the antioxidant activity which has so far been attributed to their phenolic structure, experimental data show that polyphenols also determine a reinforcement of endogenous antioxidant defences and that this enhancement is achieved through the activation of antioxidant response elements (ARE), involved in the induction of antioxidant enzymes and detoxification. Thus, a very important role is played on the regulation of DNA synthesis. The role of epigenetic alterations in several chronic human diseases has gained increasing attention and led to a paradigm shift in our understanding of disease susceptibility. In the field of cancer research, for example, genetic abnormalities / mutations were historically seen as the main underlying causes, however, the epigenetic mechanisms that alter gene expression without changing the DNA sequence are now recognized as of importance equal to or greater to oncogenesis. DNA methylation, histone modification, and interference of microRNA (miRNA) collectively constitute a framework of epigenetic factors disregulated in cancer. Targeting the epigenome with compounds that modulate DNA methylation, histone markers and miRNA profiles represents an evolution strategy for chemoprevention of cancer, and these approaches are beginning to show significant results in clinical trials. Essential micronutrients such as tea polyphenols are among a growing list of agents that affect epigenetic events such as new mechanisms of chemoprevention. Conducting structure-activity studies (SAR) could considerably improve the understanding of the mechanistic aspects and the discovery of potential anticancer agents and cancer prevention. We here report the results of our investigations carried out on olive extracts components and in particular on Verbascoside, a phenylpropanoid glycoside known for its antioxidant, anti-inflammatory and photoprotective actions. The study molecules were investigated for their antioxidant activity by different techniques (PCL, ORAC, DPPH, FRAP) and in vitro on their differentiation inducing properties on immortalized K562 cell lines.

Analytical characterization of italian red wines for commercial valorization

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Abstract
The qualification of the wine business is based traditionally more on the enhancement of links between product and territory, than on the intrinsic characteristics of the wine itself. This strategy, although certainly proved to be successful for many wines, does not appear, however, able to produce the same effects for the entire Italian viticulture, that cannot always offer products with highly identifiable areas and wine producers of particular image and tradition (DOC, DOCG, IGT) (Hussain et al, 2007; Thach et al, 2007). Moreover, the growing competition from new producers countries makes it necessary to implement more articulate strategies of enhancement, not based only on geographical origin, but also on highlight other qualifying parameters related not only to the territory, but to other important aspects, more closely linked to the characteristics of the product. In order to determine these intrinsic characteristics of the wine, the identification, analysis and evaluation of some markers substances become a priority together with the choice and optimization of cheaper, faster, simpler but reliable analytical methods. Wine is a water-alcohol solution whose chemical composition depends on many
factors. Among the hundreds of substances identified, polyphenols and hydroxyl acids are of particular interest as a marker of quality wine (Šeruga et al, 2011; Vinci et al, 2008). These compounds, in fact, appear to influence the organoleptic properties of red wine such as color, body, bitterness and astringency, and they have also well-documented positive effects on human health due to their antioxidant capacity (Lakshman et al, 2010). As regards to the risk indicators, among others, biogenic amines and sulphites must be considered. The determination of biogenic amines content, is important both for the toxicological risk linked to their assumption, both because it is a parameter for evaluating hygienic-sanitary conditions occurring during the winemaking process (Galgano et al., 2009). Sulfites are added for their antibacterial properties, their concentration is regulated by legislation, as they can cause health problems. These parameters will be evaluated along with other chemical characteristics (alcohol content, color and pH, etc) in 60 representative commercial Italian red wines, divided into three retail price segments to identify a possible correlation between the markers and the quality of the wines in examination. The wines analyses will be carried out by chromatographic and spectrophotometric methods, in particular liquid chromatography with UV-visible, Fluorescence and Light Scattering detectors and innovative chromatographic columns (UPLC). Data of all the parameters examined will be subject of chemometric treatment.

Determination of glucotropaeolin and ecdysterone in Maca products

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Abstract

Lepidium meyenii (Maca) is a Peruvian plant of the Brassicaceae, which grows exclusively in the central Andes between 4000 and 4500m altitude. Maca is used as a food supplement and also in medical care: it has nutritional properties, and it acts on sexual dysfunctions, benign prostatic hyperplasia, osteoporosis, memory and learning. Nowadays there is an increasing interest in Maca products; Maca is a plant with a great potential as adaptogen and appears to be promising as a nutraceutical in the prevention of several diseases. It contains high amounts of vitamins, amino acids, carbohydrates, protein, fiber and minerals; it also contains several aromatic glucosinolates, like glucotropaeolin, and its degradation product, benzylisothiocyanate, which has caught scientists’ attention because of its biological activity. Maca-based products in commerce are used for the presence of ecdysterone, a natural hormone that has aroused particular interest because of the anabolic activity. In this study we analysed glucotropaeolin and ecdysterone extracted from several Lepidium meyenii supplements. Afterwards glucotropaeolin was extracted with methanol and analysed by HPLC as desulphoglucosinolate after reaction with sulfatase; benzylisothiocyanate were extracted with ethyl acetate, dried, filtered and analysed by Gas-Chromatography. The relationship between the levels of the two compounds allows a qualitative analysis of the plant used. The levels of ecdysterone were investigated by HPLC after extraction with methanol and compared with those reported on the commercialized products.

Rapid and highly sensitive quantitative analysis and screening of aflatoxins in foods using liquid chromatography triple quadrupole mass spectrometry

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Abstract

Aflatoxins (AFs) are the most harmful mycotoxins which the fungi Aspergillus flavus and Aspergillus parasiticus produce and can contaminate foods such as cereals and nuts. To reduce the risk of the ingestion from foods, analyses of the AFs are carried out in many countries. It is necessary to quantitate the total aflatoxin (B1, B2, G1, G2) in foods by the regulation in JAPAN. The total analysis time of the conventional LC/MS method proposed by the Ministry of Health, Labour and Welfare of Japan had taken 30 minutes. In this study, we examined two high-throughput LC-MS/MS methods, one is sensitive quantification method, and the other is rapid screening method, using UHPLC for the purpose of speeding up of a work. A standard mixture of aflatoxin B1, B2, G1, G2 was obtained from Biopure (Austria). In order to examine a matrix effect, the matrix solution was prepared. The roast peanut matrix were extracted using a water/methanol=1/4(v/v) and cleaned up by immunoaffinity columns (IAC). The standard solution was spiked into the matrix solution. UHPLC separation was performed using the Shimadzu Nexera system with the Shim-pack XR-OBS column and was applied an isocratic elution using ammonium acetate water and methanol. The rapid MRM measurement was carried out on a Shimadzu LCMS-8030 triple quadrupole mass spectrometer using positive electrospray ionization (ESI).
Detection of free fatty acid alkyl esters in olive oils by direct oil thermodesorption and GC-MS analysis

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Abstract

Esterification of free fatty acids (FFA) with low molecular alcohol, such as methanol and ethanol, can easily take place in an acid medium. The first order reaction strongly depends on the reagent contents and temperature [Perez-Camino et al., 2008]. In olive fruits the content of free fatty acid alkyl esters (FFAEs) is highly related to olive health conditions and is obviously enhanced if olives undergo hydrolytic and fermentative processes. A “soft deodorization” employing temperature below 100 °C easily corrects the negative sensorial feature of low quality virgin olive oils obtained from fermented olives, but invalidates the analytical method generally used to detect refined oils [Perez-Camino et al., 2008]. Thus, in order to detect the presence of “soft deodorized” oils in virgin olive oils, the European Union (EU) has recently adopted [EU Regulation 2011] the method for FFAEs determination previously proposed by International Olive Council (COI) [COI, 2010]. This analytical method appears time-consuming and has sometimes shown low repeatability. Thus a faster and simpler analytical method allowing a rapid quantification of methyl and ethyl esters of fatty acids in the crude oil by TDS-CIS-GC-MS, avoiding any sample preparation, is proposed. The analytical conditions were optimized by Design of Experiment (DOE) techniques. In the first step the influence of 10 variables related to TDS (3 variables), CIS (3 variables) and GC (4 variables) conditions were explored by a Plackett Burmann design (1 dummy variable). Then the 3 most significant variables, i.e TDS final temperature, column gas flow and oven final temperature, were studied by a full factorial design. Separation was optimized in TIC mode but in order to avoid possible residual interference of FFAs, SIM quantification of FFAEs was then employed. Since the numerical results obtained by MS detection may be slightly different from those obtained by FID detector, a validation of the method by comparison with the official one is in progress.

Study on the effects of cocoa seeds processing on the content of water-soluble protein, tryptophan and its metabolites

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Abstract

Plant seeds accumulate large amounts of storage proteins that serve as sources of nitrogen, sulphur and carbon compounds during seed germination. The major seed proteins can be classified on the basis of their solubility into four classes: albumin (water-soluble), globulin (salt-soluble), prolamin (alcohol-soluble) and glutelin (soluble in dilute acids or alkali). The seeds of Theobroma cacao have been reported to contain albumins, globulins, prolamins and glutelins, the albumin being the major seed protein fraction. During cocoa processing procedures such as fermentation, drying and roasting, structural and chemical changes occur in seeds. Proteolysis, for example, is referred to produce peptides and amino acids, that contribute to the flavour of cocoa, being involved, together with carbohydrates, to Maillard reaction. In previous investigations MALDI/MS proved to be a valid analytical tool in the characterization of protein profiles of coffee and grape seeds (Procida G et al 2003, Pesavento I.C. et al 2008), and the same approach was used in the present study. The protein profiles, obtained by MALDI/MS, of defatted and powdered fresh, dried and toasted cocoa beans have been compared, in order to distinguish among the possible processing-induced differences of the specific peptides in the morphologically different parts of the beans. During fermentation, microbiological and enzymatic reactions produce an extensive digestion of cocoa proteins with an increase of peptides, free aminoacids and biogenic amines. Recently, we found that tryptophan is also present as non-protein tryptophan in most of the common foods and in cocoa beans (Bertazzo A., et al 2011). Therefore we investigated the effect of seed processing on the levels of non-protein tryptophan, 5-OH-tryptophan, serotonin and tryptamine (both amines deriving from tryptophan) in cocoa beans
Compensatory growth in Nero Siciliano pigs: effect on performance and fat quality

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Abstract
The influence of nutritional level on growth performances, carcass characteristics and fatty acids profile of subcutaneous backfat was studied. The Nero Siciliano pig is an autochthonous breed traditionally reared in free-range conditions to produce high-quality meat and meat products but, as the natural resources are not always available, the aim of this study was to evaluate feeding strategies to optimize growing pig performances. Forty Nero Siciliano pigs, 20 males and 20 females, were employed. Pigs were divided into two groups balanced for weight (29.6±0.14 kg) and age (6 months old). During the growing period, a group was fed with 90 g feed/kg live weight⁰.⁷⁵ (LW⁰.⁷⁵)/day (high feeding level, group H) and the other group was fed with 70 g feed /kg live weight⁰.⁷⁵ (LW⁰.⁷⁵)/day (moderate low feeding level, group ML). Ultrasonic measurements of backfat were recorded for all pigs every month and, at the beginning of the fattening periods, backfat biopsy samples were taken for the FA determination. At the end of growing period the two groups were divided into four subgroups: two groups were fed under Outdoor free-range conditions with acorn and grass fully available (HO and MLO groups) and the other two groups were fed Indoor with a commercial diet (HI and MLI groups). During the growing period, feeding level had significant effect on body weight and inner backfat layer thickness. The subcutaneous backfat from H pigs had a significantly higher proportion of C18:1 n-9 and MUFA than those from ML group, while the ML pigs showed a higher proportion of C18:2 n-6, C18:3 n-3 and PUFA than H group. At slaughter, a compensatory growth during the fattening period reduced differences between groups for weight and backfat thickness. No significant differences were found in monounsaturated (MUFA), polyunsaturated (PUFA) and saturated fatty acids (SFA), while the high nutritional level during the growing period increased the proportion of n-3 and decreased the n-6 concentration. In conclusion, as feeding level affect pig performances and fatty acid composition, it is possible to develop different feeding strategies to produce fresh meat or dry products.

Characterization of unifloral honey markers identified through an NMR-based fingerprinting approach

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Abstract
The importance of honey has been recently upgraded because of its nutrient and therapeutic effect. In parallel, the adulteration of honey has increased exponentially in terms of both geographic and/or botanical origin. Honey contains many different substances, mainly sugars but also minor components that strongly depend on the botanical origin of honey. Each honey has a unique and individual organoleptic character on the basis of the nature, amount, and combination of the various components. The phytochemical constituents of honey have been extensively studied to discriminate unifloral honeys. Finding reliable marker compounds is crucial not only to characterize a certain type of honey, but also to expose possible adulterations. Our previous works were focused on the NMR analysis of chloroform extracts of polyfloral honeys and of six different unifloral honeys. Principal Component Analysis (PCA) and O2PLS-DA (orthogonal partial least-squares-discriminant analysis) of the processed ¹H NMR data revealed clear differences among the botanical origins and allowed us to highlight important resonances belonging to specific markers. In this study, silica gel chromatography was used to purify compounds in honey samples from different floral origin. Different elution conditions were optimized for each specific compound. Several two-dimensional (2D)-NMR techniques and mass spectrometry were employed, leading to the identification of metabolites responsible for the botanical discrimination. We identified molecules belong to several classes, i.e., terpenes, organic acids, flavonoids, and others. Moreover, in this work was identified and characterized for the first time in honey, a diacylglycerylether, a compound present in each type of honey analyzed. On the basis of these results, the distribution of different metabolites in the different honeys is presented.
Modification of membrane fluidity in response to variable contents of cholesterol and PUFA: Role in beta-amyloid aggregation

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Abstract

Cholesterol and polyunsaturated fatty acids (PUFAs), normal constituents of dietary food, play a crucial role in the modulation of cell membrane fluidity. Specifically, they are proved responsible for the increase and the reduction of membrane fluidity. Modification of cell membrane fluidity is related to the etiopathogenesis of important pathologies such as neurodegenerative diseases. A large amount of evidences show that the deposition of amyloid fibrils as a consequence of amyloid peptide aggregation, is related to membrane fluidity modification in Alzheimer disease. Here we present a biophysical characterization based on electronic paramagnetic resonance (EPR) and atomic force microscopy (AFM) of amyloid peptide in membrane models containing variable quantities of cholesterol and PUFAs. Our data puts in evidence the tendency of beta amyloid peptide to follow distinct aggregation pathways in response to qualitatively and quantitatively different content of lipids.

Application of DNA-based methods for the cultivar identification of wines of Vitis vinifera

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Abstract

The quality of wines is highly dependent on different factors among which the grapevine variety is of primary importance. The cultivar used is essential in the case of monovarietal wines or in wines identified by an appellation of origin (DOC) that include more than one variety in a specific ratio. Sometimes, the irregular addition of wines derived from other grapevine varieties is used with the aim of enhancing the sensory characteristics of the final product and/or to decrease the production cost. Therefore technical approaches and legislative guidelines have been developed for grape, must, and wine traceability in order to guarantee product origin and detect fraud. DNA-based methodologies are expected to become the gold standard for the characterization of varieties in grape musts and wines; other methods such as must profiling of proteins, anthocyanins, amino acids, aromatic compounds, and chemical elements, are indeed more time-consuming and can be affected by various parameters such as soil composition, weather conditions, winemaking methodologies, and wine aging. DNA extraction from commercial wines was reported in few papers while PCR based molecular markers were successfully applied to the analysis of grape juice, must, fermenting must and unprocessed wines. Nevertheless, efficient DNA extraction and amplification from must and wine samples remains difficult. The aim of our project is the improvement of molecular tools for wine traceability through the optimization of DNA isolation protocols and the development of molecular probes useful not only to identify the grapevine cultivar, but also potentially applicable to the quantification of the relative amount of different varieties that may be present in a wine.
Chemical composition and biological activity of essential oil from Mentha suaveolens
ssp. insularis

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Abstract

Mentha suaveolens is currently divided into two subspecies: suaveolens and insularis. The two subspecies are botanically
close, but various morphological characters allow the differentiation of the two subspecies. Mentha suaveolens
ssp. insularis (Req.) Greuter is an endemic species to the islands of the occidental Mediterranean Sea: Corsica, Sardinia,
Balearic Islands. It grows mainly wetlands [1] (1983a, 1993a). It reaches up to one meter and half high and
is characterized by a strong aromatic smell considered, by some people, not very pleasant. Starting from plant material
collected in Sardinia, chemical composition of the essential oil and the biological activity have been studied.

The main components of the essential oil, obtained with a yield of 0.22% (v / w), were -as expected- pulegone (42.4%),
piperitenone (7.4%) and cis-cis-p-menthenolide (27.3%) which correspond to the 77% of the total of the oil composition.

Biological activity was tested against several bacterial strains of interest food. In particular, the antimicrobial activity of the
essential oil has been tested using the technique of diffusion on Agar, against five Lactobacillus species (L. rhamnosum, L.
acidophilus, L. paracasei subsp. Paracasei, L. casei, L. plantarum) and a strain of Staphylococcus xilosus. Only growth of
Staphylococcus xilosus has been inhibited by essential oil (10 ± 0.4 mm halo) whereas the minimum inhibitory concentration
(MIC), estimated using the technique of Broth microdilution method, is found to have a value of 0.34% (v / v); at this
concentration was not observed growth over 24 hours. Anyway, starting from a concentration of 0.16%, the growth rate is
halved compared with the control.

Pharmacokinetics of crocetin-monogentobiosylesters in rats

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Abstract

The orange-yellow pigments in Crocus sativus L. (saffron) and Gardenia jasminoides E. consist of the water soluble
carotenoids crocetin and various crocetin-glycosyl esters (see Figure 1). The most abundant crocetin-glycosyl esters are
crocetin- digentobiosyl esters (Crocin) and crocetin- monogentobiosylesters (Crocetin-MGE). Saffron and gardenia share a
long tradition of use as natural food colorants. In addition, published studies suggest that pigment extracts from saffron or
gardenia, crocetin and crocin exert a series of biological effects. The pharmacokinetics of crocetin and crocin is well known.
Crocetin is absorbed unchanged after oral administration from the intestine. However, crocin is not absorbed after oral
administration, but metabolized in the intestine to crocetin which is then absorbed. Considering the difference in the
physiochemical properties of crocetin and crocin it was speculated that crocetin-MGE are suited for direct absorption. In the
current study the pharmacokinetics of crocetin-MGE was investigated and compared to crocetin. Crocetin-MGE were
obtained from gardenia extract by optimized hydrolysis and fractionated precipitation. Crocetin or Crocetin-MGE were
administered orally to five rats as single dose. Plasma concentrations of crocetin and crocetin-MGE were determined by
HPLC/MS. The test results showed that (a) crocetin-MGE are absorbed directly, (b) crocetin-MGE are quickly converted to
crocetin and (c) the bioavailability of crocetin-MGE is almost the same as of crocetin if the plasma concentrations of
crocetin-MGE and crocetin observed after administration of crocetin-MGE are combined (see Figure 2). The current study
results indicate that other crocetin-monoglycosylesters (i.e. -β-D-glucosyl, -β-D-neapolitanosyl) may have the potential to
even improve the bioavailability of crocetin.
Bioactivation of dietary phytochemicals by intestinal microbiota and probiotic bacteria

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The human health is affected by both fermentation metabolites and enzymatic activities of intestinal microbiota. The microbial enzymes of colonic bacteria transform a wide spectrum of molecules that enter with the diet into harmful compounds or into molecules which exert healthy benefits. In particular, edible plants are dietary sources of hundreds of non-nutritional phytochemicals that are typically secondary metabolites, and occur in small quantities in plant tissues and plant-derived foods. The level of phytochemicals within the body is largely determined by diverse phenomena, such as the digestive transformations of native compounds, the absorption in the intestine, the hepatic activity, and the biliary or urinary elimination. The phytochemicals which are not absorbed in the small intestine reach the colon, where they may undergo extensive biotransformation by the resident microbiota. Polyphenols represent a major group of bioactive phytochemicals, whose health benefits are due to their ability to inactivate reactive oxygen species. Phytoestrogens are a broad group of non-steroidal compounds of different structure, falling into the three main classes of isoflavones, coumestans and lignans that mimic the action of estrogens on target organs and exert many health benefits against hormone dependent diseases. Microbial transformation of phytochemicals includes the hydrolysis of glycosides into the respective aglycones, and may lead to the degradation of the compounds. Besides, the specific conversion of diverse molecules into bioactive metabolites can be accomplished by the microbiota, such as the conversion of lignans into enterolactone and enterodiol and the conversion of soy isoflavones into S-equol. An important target for the development of novel probiotics is the selection of strains that combine the intrinsic beneficial properties with specific healthy activities, such as the activation of phytochemicals. In this perspective, the identification of Bifidobacterium strains capable of activating phytochemicals is quite attractive. Among probiotic bacteria, bifidobacteria exert an important role in the hydrolysis of isoflavone and lignan glyco-conjugates, although their involvement in the reduction of daidzein toward S-equol and in reactions required for the transformation of the aglycone secoisolariciresinol into the powerful compounds enterolactone and enterodiol can be excluded. Furthermore, selected strains of bifidobacteria are active in bioactivation of chlorogenic acid and hesperidin, with release of caffeic acid and hesperetin, respectively, which present antioxidant property, inhibition of NO production, and inhibition of inflammatory enzymes.
### Author Index

<table>
<thead>
<tr>
<th>COGNOME</th>
<th>NOME</th>
<th>SIGLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abruzzo</td>
<td>Provvivenda Maria</td>
<td>P-116</td>
</tr>
<tr>
<td>Aceto</td>
<td>Maurizio</td>
<td>CO-6; P-182</td>
</tr>
<tr>
<td>Acquastucci</td>
<td>Rita</td>
<td>P-1; P-134</td>
</tr>
<tr>
<td>Acuti</td>
<td>Gabriele</td>
<td>P-113</td>
</tr>
<tr>
<td>Addeo</td>
<td>Francesco</td>
<td>P-78</td>
</tr>
<tr>
<td>Adobati</td>
<td>A.</td>
<td>CO-5</td>
</tr>
<tr>
<td>Afanador</td>
<td>German</td>
<td>CO-51</td>
</tr>
<tr>
<td>Agostiano</td>
<td>Angela</td>
<td>P-105</td>
</tr>
<tr>
<td>Aguzzo</td>
<td>Pasquale</td>
<td>P-19; P-68</td>
</tr>
<tr>
<td>Alesci</td>
<td>Alessio</td>
<td>P-155; P-156; P-157</td>
</tr>
<tr>
<td>Aldini</td>
<td>Antonio</td>
<td>CO-9</td>
</tr>
<tr>
<td>Aldini</td>
<td>Giancarlo</td>
<td>CO-39</td>
</tr>
<tr>
<td>Aielli</td>
<td>Riccardo</td>
<td>P-123</td>
</tr>
<tr>
<td>Aielli</td>
<td>Alessia</td>
<td>P-155; P-156; P-157</td>
</tr>
<tr>
<td>Alfa</td>
<td>Maria</td>
<td>P-199</td>
</tr>
<tr>
<td>Alfieri</td>
<td>Ilaria</td>
<td>CO-59</td>
</tr>
<tr>
<td>Aloisi</td>
<td>Viviana</td>
<td>P-188</td>
</tr>
<tr>
<td>Altucci</td>
<td>Lucia</td>
<td>CO-4</td>
</tr>
<tr>
<td>Amore</td>
<td>Gabriele</td>
<td>P-92</td>
</tr>
<tr>
<td>Amoreano</td>
<td>Claudia</td>
<td>CO-24; P-176</td>
</tr>
<tr>
<td>Amoroso</td>
<td>Alessandra</td>
<td>CO-3</td>
</tr>
<tr>
<td>Anastasio</td>
<td>Aniello</td>
<td>P-126; P-211</td>
</tr>
<tr>
<td>Angioni</td>
<td>Alberto</td>
<td>CO-4; P-14; P-15</td>
</tr>
<tr>
<td>Antraversi</td>
<td>Fabrizio</td>
<td>P-61; P-62</td>
</tr>
<tr>
<td>Antonia</td>
<td>Riccardo</td>
<td>CO-55; P-16; P-233</td>
</tr>
<tr>
<td>Antonacci</td>
<td>Donato</td>
<td>CO-44; CO-56; P-46; P-58; P-70; P-220</td>
</tr>
<tr>
<td>Antonello</td>
<td>Marta Letizia</td>
<td>P-233</td>
</tr>
<tr>
<td>Appendino</td>
<td>Giovanni</td>
<td>PL-9</td>
</tr>
<tr>
<td>Aquino</td>
<td>Rita Patrizia</td>
<td>P-170; P-171; P-200</td>
</tr>
<tr>
<td>Arace</td>
<td>O.</td>
<td>P-74</td>
</tr>
<tr>
<td>Aragon</td>
<td>Marcela</td>
<td>P-13</td>
</tr>
<tr>
<td>Arakawa</td>
<td>Kyomi</td>
<td>P-7; P-146</td>
</tr>
<tr>
<td>Arduini</td>
<td>Fabiona</td>
<td>P-144</td>
</tr>
<tr>
<td>Aresta</td>
<td>Antonella</td>
<td>CO-48</td>
</tr>
<tr>
<td>Arfelli</td>
<td>Giuseppe</td>
<td>P-17</td>
</tr>
<tr>
<td>Argentieri</td>
<td>Maria Pia</td>
<td>P-4</td>
</tr>
<tr>
<td>Ariza</td>
<td>Claudia</td>
<td>CO-51; P-13</td>
</tr>
<tr>
<td>Ariza</td>
<td>Magnolia</td>
<td>P-13</td>
</tr>
<tr>
<td>Arolli</td>
<td>Marco</td>
<td>CO-38; P-35; P-36; P-128; P-179</td>
</tr>
<tr>
<td>Armano</td>
<td>Paolo</td>
<td>P-158</td>
</tr>
<tr>
<td>Armenteros</td>
<td>Dulce Maria</td>
<td>P-171</td>
</tr>
<tr>
<td>Arnoldi</td>
<td>Anna</td>
<td>CO-64; P-18; P-37; P-204</td>
</tr>
<tr>
<td>Austriti</td>
<td>Zeneb</td>
<td>P-190</td>
</tr>
<tr>
<td>Avato</td>
<td>Pinarsa</td>
<td>P-4</td>
</tr>
<tr>
<td>Avelone</td>
<td>Giuseppe</td>
<td>P-19; P-68</td>
</tr>
<tr>
<td>Avondo</td>
<td>Riccardo</td>
<td>P-59</td>
</tr>
<tr>
<td>Avino</td>
<td>Pasquale</td>
<td>P-193; P-194</td>
</tr>
<tr>
<td>Avola</td>
<td>Giovanni</td>
<td>CO-7</td>
</tr>
<tr>
<td>Azzini</td>
<td>Elena</td>
<td>P-20; P-72; CO-54</td>
</tr>
<tr>
<td>Baldeserotti</td>
<td>Anna</td>
<td>P-232</td>
</tr>
<tr>
<td>Ballecchi</td>
<td>Chiara</td>
<td>P-21; P-22; P-111; P-186</td>
</tr>
<tr>
<td>Ballabio</td>
<td>Cinzia</td>
<td>P-228</td>
</tr>
<tr>
<td>Ballini</td>
<td>Roberto</td>
<td>P-12; P-80</td>
</tr>
<tr>
<td>Ballistreri</td>
<td>Gabriele</td>
<td>P-177</td>
</tr>
<tr>
<td>Barbera</td>
<td>Daniela</td>
<td>P-85; P-92; P-202</td>
</tr>
<tr>
<td>Barberi</td>
<td>Andrea</td>
<td>P-18</td>
</tr>
<tr>
<td>Barile</td>
<td>Daniela</td>
<td>CO-38</td>
</tr>
<tr>
<td>Barmaz</td>
<td>A.</td>
<td>P-229</td>
</tr>
<tr>
<td>Barone</td>
<td>Eleonora</td>
<td>P-92</td>
</tr>
<tr>
<td>Barreca</td>
<td>Davide</td>
<td>P-102</td>
</tr>
<tr>
<td>Bartolomeo</td>
<td>Giovanni</td>
<td>CO-29; P-225</td>
</tr>
<tr>
<td>Baschieri</td>
<td>Carlo</td>
<td>CO-14; P-25</td>
</tr>
<tr>
<td>Basile</td>
<td>Adriana</td>
<td>CO-45</td>
</tr>
<tr>
<td>Basile</td>
<td>Teodora</td>
<td>P-58</td>
</tr>
<tr>
<td>Bastianini</td>
<td>Maria</td>
<td>CO-57</td>
</tr>
<tr>
<td>Battista</td>
<td>Fabio G.</td>
<td>CO-27</td>
</tr>
</tbody>
</table>

Beccaria M. P-198; P-214
Beghelli Daniela P-113
Bellante Simona P-44
Bellucco Elsa P-102; P-191
Bellumori Maria P-148; P-150
Bencivenni Mariangela P-208
Bencucci Ilaria P-23; P-75; P-104
Benzi Micaela P-24
Berenato Daniele P-51
Bergamo Luca P-114
Bertacchini Lucia CO-14; P-25; P-173
Bertazzoni Adele P-234
Bertelli Davide CO-14; CO-15; P-26; P-27; P-99; P-100; P-172; P-173
Bertoldi Daniela P-114
Bertolone Eleonora P-169
Bertuccio C. P-156
Bevilacqua Marta P-131
Bezo Guido P-182
Bianchi Giulia P-32
Bianco Giuliana P-27
Bianco Armandododionaro P-230
Bicchi Omar CO-57; P-54; P-103
Bifulco Elsa P-204
Bignardi Chiara CO-21; P-28; P-96
Bilancia Maria T. P-163
Biondi Pietro Antonio P-89
Bisi Arriavaldco P-11
Bisignano Giuseppe P-71
Bianchini Giacomo P-151
Blasi Francesca CO-57; CO-62; P-55; P-227
Boban Mladen CO-40
Bobba Fabrizio P-240
Boggia Raffaella CO-31; P-32; P-236
Bolognese Adele P-115
Bonacorsia Ivana L. CO-19; P-33
Bonetti Gianpietro CO-8; P-26
Bonificci Gabriele P-182
Boniglia Concetta P-46; P-45
Bono Monina Grazia P-29
Bontempi Luana P-34; P-43
Bontempi Paola P-217
Bordiga Matteo CO-38; P-35; P-36
Borgogni Cristina P-236
Borsa Daniela P-169
Bottolin Emanuela P-6
Boschin Giovanna CO-64; P-37; P-203; P-204
Botè Francesco P-38
Botta Bruno P-49
Bottesini Chiara P-207; P-209; P-216
Brandolini Ada P-39
Brandolini Vincenzo P-26; CO-8
Bravo Laura CO-43
Breveglieri Giuliia P-232
Bruno Claudio CO-47
Bruno Franco P-7; P-241
Bruno Maurizio P-40; P-51
Bruno T. P-74
Bucco Remo P-131
Budimir Danijela P-40
Bufo Sabino A. CO-27
Bugaroli Francesca P-116
Bugamelli Francesca P-93; P-94; P-95
Burini G. P-133
Calabrese Gianni CO-63
Caliari Pierluigi CO-4; P-15; P-86
Cacciafrasco P-198; P-214
Caglieri Cecilia P-54
Calabrese Massimo P-8
Calabrese Antonella P-8; P-41

Hotel Continental Terme, Ischia (NA), Italy, June 03-07, 2012

<table>
<thead>
<tr>
<th>Name</th>
<th>Paper Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Salvador Roberto</td>
<td>P-80</td>
</tr>
<tr>
<td>De Simone Bruna Clara</td>
<td>P-63</td>
</tr>
<tr>
<td>De Simone Carmela</td>
<td>P-79</td>
</tr>
<tr>
<td>De Siino Paola</td>
<td>P-158</td>
</tr>
<tr>
<td>De Tito Stefano</td>
<td>CO-24; P-176</td>
</tr>
<tr>
<td>De Tommasi Nunziatina</td>
<td>P-59; P-137</td>
</tr>
<tr>
<td>Dedola Fabrizio</td>
<td>P-14</td>
</tr>
<tr>
<td>Del Balzo Valeria</td>
<td>P-109</td>
</tr>
<tr>
<td>Del Carlo Michele</td>
<td>CO-34; P-53; P-60</td>
</tr>
<tr>
<td>Del Castillo Maria Luisa Ruiz</td>
<td>P-151</td>
</tr>
<tr>
<td>Del Frate Viviana</td>
<td>P-212</td>
</tr>
<tr>
<td>Del Roza-Delgado Begolita</td>
<td>P-123</td>
</tr>
<tr>
<td>Delline Sebastiano</td>
<td>P-40</td>
</tr>
<tr>
<td>Delfini Maurizio</td>
<td>P-90; P-117; P-119</td>
</tr>
<tr>
<td>Deibbato Elisabetta</td>
<td>P-61; P-62</td>
</tr>
<tr>
<td>Demmevò Katefina</td>
<td>P-136</td>
</tr>
<tr>
<td>Di Bella Giuseppa</td>
<td>CO-29; P-50; P-65; P-174; P-197; P-226</td>
</tr>
<tr>
<td>De Cesare Luigi Francesco</td>
<td>P-69; P-106</td>
</tr>
<tr>
<td>Di Cozzo Maria Enrica</td>
<td>P-49; P-117</td>
</tr>
<tr>
<td>Di Costanzo Maria Gabriella</td>
<td>P-66</td>
</tr>
<tr>
<td>Di Costanzo Maria Gabriella</td>
<td>P-66; P-120</td>
</tr>
<tr>
<td>Di Ferdinando Sandra</td>
<td>P-83</td>
</tr>
<tr>
<td>Di Lorenzo Chiara</td>
<td>CO-50; P-228</td>
</tr>
<tr>
<td>Di Luccia Aldo</td>
<td>P-70; P-78</td>
</tr>
<tr>
<td>Di Maio Sabina</td>
<td>P-85; P-92</td>
</tr>
<tr>
<td>Di Marino Sara</td>
<td>P-240</td>
</tr>
<tr>
<td>Di Martino Massimo</td>
<td>P-53</td>
</tr>
<tr>
<td>Di Natale Corrado</td>
<td>P-53</td>
</tr>
<tr>
<td>Di Rosa Ambra</td>
<td>P-67; P-238</td>
</tr>
<tr>
<td>Di Sanzo Rosa</td>
<td>CO-8</td>
</tr>
<tr>
<td>Di Stefano Rocco</td>
<td>P-182</td>
</tr>
<tr>
<td>Di Stefano Vita</td>
<td>P-19; P-68</td>
</tr>
<tr>
<td>Di Taranto Aurelia</td>
<td>P-101</td>
</tr>
<tr>
<td>Diletti G.</td>
<td>P-206</td>
</tr>
<tr>
<td>Dolci Paola</td>
<td>P-79</td>
</tr>
<tr>
<td>Dominici Luca</td>
<td>CO-53</td>
</tr>
<tr>
<td>Donarziki James A.</td>
<td>P-118</td>
</tr>
<tr>
<td>Donato Paola</td>
<td>P-9; P-145; P-198; P-214</td>
</tr>
<tr>
<td>Donato Rosa</td>
<td>P-148; P-150</td>
</tr>
<tr>
<td>Dos Santos Ariana</td>
<td>CO-50</td>
</tr>
<tr>
<td>Dossera Arnaldo</td>
<td>CO-2; P-207; P-208; P-209; P-216</td>
</tr>
<tr>
<td>Dragone Roberto</td>
<td>CO-35</td>
</tr>
<tr>
<td>Dugo Giacomo</td>
<td>CO-26; CO-29; CO-61; P-3; P-5; P-9; P-50; P-51; P-65; P-71; P-96; P-157; P-174; P-175; P-197; P-198; P-199; P-214; P-222; P-226</td>
</tr>
<tr>
<td>Dugo Paola</td>
<td>CO-19; CO-61; P-9; P-33; P-51; P-96; P-145; P-190; P-198; P-214; P-222;</td>
</tr>
<tr>
<td>Dugo Giovanni</td>
<td>Opening lecture; CO-19; P-222</td>
</tr>
<tr>
<td>Durante Caterina</td>
<td>CO-14; CO-15; P-25; P-172; P-173</td>
</tr>
<tr>
<td>Durante Viviana</td>
<td>P-98</td>
</tr>
<tr>
<td>Durazzo Alessandro</td>
<td>CO-54; P-20; P-72</td>
</tr>
<tr>
<td>D’Urso Anna Maria</td>
<td>P-240</td>
</tr>
<tr>
<td>Eidenberger Thomas</td>
<td>P-243</td>
</tr>
<tr>
<td>Emanuele Maria Carmela</td>
<td>P-49</td>
</tr>
<tr>
<td>Esposito Mauro</td>
<td>CO-34; P-60; P-73; P-74; P-206</td>
</tr>
<tr>
<td>Esti Marco</td>
<td>P-104</td>
</tr>
<tr>
<td>Euterpio Maria Anna</td>
<td>CO-28; P-76</td>
</tr>
<tr>
<td>Fabroni Simona</td>
<td>P-167; P-173; P-178</td>
</tr>
<tr>
<td>Facchin Chiara</td>
<td>P-139; P-239</td>
</tr>
<tr>
<td>Faccia Michele</td>
<td>CO-23</td>
</tr>
<tr>
<td>Failla Sebastiana</td>
<td>P-181</td>
</tr>
<tr>
<td>Fancchi Chiara</td>
<td>P-180</td>
</tr>
<tr>
<td>Fancelllo Francesco</td>
<td>P-242</td>
</tr>
<tr>
<td>Fang F.</td>
<td>CO-42</td>
</tr>
<tr>
<td>Fasolato Luca</td>
<td>P-183</td>
</tr>
<tr>
<td>Fasoli Elisa</td>
<td>PL-8</td>
</tr>
<tr>
<td>Favaro Giada</td>
<td>P-24</td>
</tr>
<tr>
<td>Favero Gabriele</td>
<td>P-221</td>
</tr>
<tr>
<td>Fawzy N.</td>
<td>P-198; P-214</td>
</tr>
<tr>
<td>Fazio Alessia</td>
<td>P-77</td>
</tr>
<tr>
<td>Fernández Mercedes Campo</td>
<td>P-135</td>
</tr>
<tr>
<td>Fernández-Franzón Monica</td>
<td>P-96</td>
</tr>
<tr>
<td>Ferrantelli Vincenzo</td>
<td>CO-26; P-225</td>
</tr>
<tr>
<td>Ferranti Anna</td>
<td>P-49</td>
</tr>
<tr>
<td>Ferranti Pasquale</td>
<td>P-78; P-79</td>
</tr>
<tr>
<td>Ferrari Carlotta</td>
<td>P-81</td>
</tr>
<tr>
<td>Ferrari Davide</td>
<td>CO-9</td>
</tr>
<tr>
<td>Ferrari Valenito</td>
<td>P-60</td>
</tr>
<tr>
<td>Ferreri Carla</td>
<td>P-116</td>
</tr>
<tr>
<td>Ferrini Monica</td>
<td>P-112</td>
</tr>
<tr>
<td>Filizzola Felce</td>
<td>P-19; P-68</td>
</tr>
<tr>
<td>Fioreni Dennis</td>
<td>P-12; P-80</td>
</tr>
<tr>
<td>Foca Giorgia</td>
<td>P-81; P-82</td>
</tr>
<tr>
<td>Fochi Igor</td>
<td>CO-22</td>
</tr>
<tr>
<td>Foddaia Stella</td>
<td>P-20</td>
</tr>
<tr>
<td>Font Guillermina</td>
<td>CO-60; P-86</td>
</tr>
<tr>
<td>Formisano Carmen</td>
<td>P-40; P-217</td>
</tr>
<tr>
<td>Fortuna Filippo</td>
<td>P-116</td>
</tr>
<tr>
<td>Fria Vincenzo</td>
<td>P-197</td>
</tr>
<tr>
<td>Francesca Nicola</td>
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<td>P-7; P-146; P-241</td>
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<td>Furfaro Maria Elena</td>
<td>P-67; P-238</td>
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<td>Gabrielle Bartolo</td>
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<td>P-42; P-83; P-107; P-124; P-187; P-224</td>
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<td>CO-2; CO-52; P-52; P-143; P-207; P-208; P-209</td>
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<td>P-23; P-75; P-104</td>
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<td>P-130; P-161; P-162</td>
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<td>P-58</td>
</tr>
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<td>Genna Giuseppe</td>
<td>P-202; P-215</td>
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<tr>
<td>Genesio E.</td>
<td>P-88; P-91; P-152</td>
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<td>Gentile Alessandra</td>
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<td>P-102; P-199</td>
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<td>CO-13; P-34; P-168</td>
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<td>CO-28; CO-30; P-135;76; 170; 171</td>
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<td>P-167; P-177; P-178;188; 212</td>
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<td>CO-8; P-14; P-115;P-218; P-219</td>
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<td>P-193; P-194</td>
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<td>P-63; P-195</td>
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<td>P-212; P-213</td>
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<td>P-160</td>
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<td>P-73; P-205; P-206</td>
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</tr>
<tr>
<td>Squadrato Margherita</td>
<td>P-92; P-215</td>
</tr>
<tr>
<td>Srafini Mauro</td>
<td>P-110</td>
</tr>
<tr>
<td>Stancher Bruno</td>
<td>P-47</td>
</tr>
<tr>
<td>Statti Giancarlo A.</td>
<td>P-10; P-30; P-125</td>
</tr>
<tr>
<td>Stauder Monica</td>
<td>P-161</td>
</tr>
<tr>
<td>Stinso Paolo</td>
<td>CO-45; P-218; P-219</td>
</tr>
<tr>
<td>Stocchero Matteo</td>
<td>CO-37</td>
</tr>
<tr>
<td>Strano Maria C.</td>
<td>P-188</td>
</tr>
<tr>
<td>Strano Tania</td>
<td>P-188</td>
</tr>
<tr>
<td>Summo Carmine</td>
<td>P-98; P-163; P-164; P-165</td>
</tr>
<tr>
<td>Surzi Giovanna</td>
<td>P-17</td>
</tr>
<tr>
<td>Tabilio Maria Rosaria</td>
<td>P-80</td>
</tr>
<tr>
<td>Tamborra Pasquale</td>
<td>P-220</td>
</tr>
<tr>
<td>Sublitti Saponetti Mattele</td>
<td>P-240</td>
</tr>
<tr>
<td>Tamura Masayoshi</td>
<td>P-176</td>
</tr>
<tr>
<td>Tardagno Maria</td>
<td>CO-41</td>
</tr>
<tr>
<td>Tasca Federico</td>
<td>CO-55</td>
</tr>
<tr>
<td>Tedeschi Paolo</td>
<td>CO-8; P-26</td>
</tr>
<tr>
<td>Tedeschi Tullka</td>
<td>P-208; P-216</td>
</tr>
<tr>
<td>Tega Luigi</td>
<td>P-110</td>
</tr>
<tr>
<td>Temperini Olindo</td>
<td>P-20</td>
</tr>
<tr>
<td>Tenore Gian Carlo</td>
<td>CO-45; P-115;166;217; 218; 219</td>
</tr>
<tr>
<td>Tieri Alessandra</td>
<td>P-38</td>
</tr>
<tr>
<td>Tirillini Bruno</td>
<td>CO-53</td>
</tr>
<tr>
<td>Toci Aline T.</td>
<td>P-58; P-220</td>
</tr>
<tr>
<td>Todini Luca</td>
<td>P-113</td>
</tr>
<tr>
<td>Tomás-Barberín Francisco A.</td>
<td>Closing lecture</td>
</tr>
<tr>
<td>Tomassetti Mauro</td>
<td>CO-35; P-221</td>
</tr>
<tr>
<td>Tommassini Alberta</td>
<td>P-117; P-119</td>
</tr>
<tr>
<td>Tonelli Alessandro</td>
<td>CO-2</td>
</tr>
<tr>
<td>Torre Germania</td>
<td>CO-61; P-96; P-145</td>
</tr>
<tr>
<td>Torregiani Elisabetta</td>
<td>CO-60</td>
</tr>
<tr>
<td>Torres Kalina Bermudez</td>
<td>P-37</td>
</tr>
<tr>
<td>Torricelli Alessandro</td>
<td>CO-32</td>
</tr>
<tr>
<td>Toscano M.</td>
<td>P-195</td>
</tr>
<tr>
<td>Tranchida Peter Q.</td>
<td>P-9; P-33; P-198; P-214; P-222</td>
</tr>
<tr>
<td>Trapani</td>
<td>A.</td>
</tr>
<tr>
<td>Travaglia Fabiano</td>
<td>CO-38; P-35; P-128; P-179</td>
</tr>
<tr>
<td>Trimmerco Bruno</td>
<td>PL-7</td>
</tr>
<tr>
<td>Tropea Alessia</td>
<td>CO-24; P-223</td>
</tr>
<tr>
<td>Trota Roberta</td>
<td>CO-24; P-176</td>
</tr>
<tr>
<td>Troy-Davis Peter J.</td>
<td>P-223</td>
</tr>
<tr>
<td>Tsokakis H.</td>
<td>P-88</td>
</tr>
<tr>
<td>Tuberoso Carlo I.G.</td>
<td>CO-40</td>
</tr>
<tr>
<td>Tufi Sara</td>
<td>P-64; P-83; P-107; P-108,P-224</td>
</tr>
<tr>
<td>Tundis Rosa</td>
<td>P-125</td>
</tr>
<tr>
<td>Tursani Valeria</td>
<td>CO-54; P-72</td>
</tr>
<tr>
<td>Uberti Francesca</td>
<td>CO-50; P-228</td>
</tr>
<tr>
<td>Ubiali Daniela</td>
<td>P-141</td>
</tr>
<tr>
<td>Ulrici Alessandro</td>
<td>P-81; P-82</td>
</tr>
<tr>
<td>Urbani Eleonora</td>
<td>P-227</td>
</tr>
<tr>
<td>Urbani V.</td>
<td>P-73</td>
</tr>
<tr>
<td>Vannini Samuele</td>
<td>P-55</td>
</tr>
<tr>
<td>Vanoli Mariestella</td>
<td>CO-32</td>
</tr>
<tr>
<td>Vecchio Stefano</td>
<td>CO-35</td>
</tr>
<tr>
<td>Venditti Alessandro</td>
<td>P-230</td>
</tr>
<tr>
<td>Vennari Eugenia</td>
<td>P-20</td>
</tr>
<tr>
<td>Veredelli Maria Cristina</td>
<td>CO-58</td>
</tr>
<tr>
<td>Vero Stefania</td>
<td>CO-36; P-122</td>
</tr>
<tr>
<td>Verotta Luissella</td>
<td>CO-43; P-231</td>
</tr>
<tr>
<td>Vertuani Silvia</td>
<td>P-116; P-232</td>
</tr>
<tr>
<td>Vezzella Antonella</td>
<td>P-202</td>
</tr>
<tr>
<td>Viari Simone</td>
<td>P-38; P-233</td>
</tr>
<tr>
<td>Vignali Valentina</td>
<td>P-232</td>
</tr>
<tr>
<td>Villa Carla</td>
<td>P-32</td>
</tr>
<tr>
<td>Villarini Milena</td>
<td>CO-53; P-55</td>
</tr>
<tr>
<td>Vincicu Giuliana</td>
<td>P-16; P-38; P-233</td>
</tr>
<tr>
<td>Visai Livia</td>
<td>CO-9</td>
</tr>
<tr>
<td>Visconti Angelo</td>
<td>CO-10; P-105</td>
</tr>
<tr>
<td>Vist Silvia</td>
<td>P-49</td>
</tr>
<tr>
<td>Vita Patricia</td>
<td>P-139</td>
</tr>
<tr>
<td>Vitale Marcello</td>
<td>P-49</td>
</tr>
<tr>
<td>Vitello Giusepppe</td>
<td>P-240</td>
</tr>
<tr>
<td>Vitiello Simona</td>
<td>P-185</td>
</tr>
<tr>
<td>Vittori Sauro</td>
<td>CO-60; P-196</td>
</tr>
<tr>
<td>Vollano Lucia</td>
<td>P-126; P-211</td>
</tr>
<tr>
<td>Volpe Giulia</td>
<td>CO-33; P-61; P-62</td>
</tr>
<tr>
<td>Volpe Luisa Antonella</td>
<td>P-82</td>
</tr>
<tr>
<td>Waldron Keith W.</td>
<td>P-223</td>
</tr>
<tr>
<td>Watanabe Jun</td>
<td>P-176; P-235</td>
</tr>
<tr>
<td>Watanabe Kyoko</td>
<td>P-235</td>
</tr>
<tr>
<td>Wilson David</td>
<td>P-223</td>
</tr>
<tr>
<td>Wilson Michael</td>
<td>P-161</td>
</tr>
<tr>
<td>Yamaki Satoshi</td>
<td>P-235</td>
</tr>
<tr>
<td>Yeum Kyun-Jin</td>
<td>CO-39</td>
</tr>
<tr>
<td>Zacchigna Marina</td>
<td>P-47</td>
</tr>
<tr>
<td>Zacone Daniele</td>
<td>P-2; P-3; P-157</td>
</tr>
<tr>
<td>Zambonin Carlo</td>
<td>CO-23; CO-48</td>
</tr>
<tr>
<td>Zacanato Mirella</td>
<td>P-234</td>
</tr>
<tr>
<td>Zanoli Riccardo</td>
<td>P-112; P-186</td>
</tr>
<tr>
<td>Zanonl Bruno</td>
<td>P-48</td>
</tr>
<tr>
<td>Zara Severino</td>
<td>P-242</td>
</tr>
<tr>
<td>Zara Stefano</td>
<td>P-235</td>
</tr>
<tr>
<td>Zaza Stefano</td>
<td>P-235</td>
</tr>
<tr>
<td>Zucchi Marioisimone</td>
<td>P-222</td>
</tr>
<tr>
<td>Zumbi Alessandro</td>
<td>P-67; P-238</td>
</tr>
<tr>
<td>Zunin Paola</td>
<td>CO-31; P-32; P-236</td>
</tr>
</tbody>
</table>
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