1st FCUB ERA Workshop

Food Safety and Health Effects of Food

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FCUB ERA

SEVENTH FRAMEWORK PROGRAMME

1st FCUB ERA Workshop

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Content:

Session 1: Clustering research activities in WB region and EU neighboring countries

Food Research and Molecular Biotechnology at FCUB, Belgrade, Serbia

Tanja Ćirković Veličković

University of Belgrade, Faculty of Chemistry, Serbia

Faculty of Chemistry, University of Belgrade (FCUB) represents a unique, internationally recognized, centre in the region working actively on various aspects of molecular food research. A multidisciplinary research background of more than 20 senior researchers working within the FCUB ensures a flexible platform able to cope with complex research tasks in the field of food research aiming at improving safety and quality of food. The aim of the FCUB ERA project is reinforcement of research potential and facilities for molecular food research through upgrading of present facilities for molecular biotechnology and food research and mobility and training of the research staff. The objectives will be achieved by exchanging know-how and experience with institutions of broadly recognized reputation in the field (KI, Sweden, VTT, Finland, University of Aachen, Germany, RCB, Germany, INRA, France, NHR IBRB, Greece, University of Plovdiv, Bulgaria, IRAS, the Netherlands) through mutual research visits; upgrade of already existing facilities for biomolecules analysis and molecular biotechnology to enable future cutting-edge research; establishment of a Proteomics centre in Serbia; training visits of young and senior scientist to EU institutions to improve specific skills in molecular biotechnology, food (bio)processing and managerial skills; knowledge dissemination through web portal, organization of two workshops and a conference, as well as meetings with the EU partners in order to gradually form an efficient network for future collaborative research projects. The action plan will provide rising of the research potential of the FCUB as a centre of excellence in food research and molecular biotechnology, it will enable its sustainable development by establishing a network of collaboration with outstanding research centres in the EU and make basis for future highest quality research in molecular biotechnology and food research.

Research potential of the Faculty of Technology in food safety and emerging risks

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CEFSER is the FP7-REGPOT-2008-1 project dedicated to the reinforcement of research potential of the Laboratory for Chemical Contaminants in Food and the Environment at the Faculty of Technology, University of Novi Sad, towards the establishment of a unique Western Balkan Country (WBC) Centre of Excellence in Food Safety and Emerging Risks (CEFSER) with infrastructure and activities in food safety and emerging pollutants. It is integrated with advanced and well experienced institutions from EU: Institute of Environmental Assessment and Water Research, Barcelona, Spain, Institute of Chemical Technology from Prague, Czech Republic, Institute for Environmental Studies, Vrije University, Amsterdam, The Netherlands, and with CHIRON AS, Trondheim, Norway, a private company for fine chemicals and reference materials. Through postulated general objectives such as: capital investments in a highly sophisticated analytical instrument, upgrading of the existing equipments, reinforcement of the human resources (hiring, mobility, etc), and networking with advanced EU institutions, CEFSER integrates the Faculty of Technology within the European Research Area, contributing to the regional capacities towards the general harmonization of R&D within the food safety research.

This presentation deals with the CEFSER project structure and outcomes with an aim to emphasize the opportunities of the FP7-REGPOT calls to the Convergence regions for their successful engagement in the main research streams.

Valorization of natural vegetable substances at the High School of Food Industries

Nabiha Bouzouita

Ecole Supérieure des Industries Alimentaires, 58 Avenue Alain Savary, 1003 Tunis, Tunisia

The High School of Food Industries (ESIAT) of Tunis is a public corporation creates in 1976 and whose vocation is higher education, research and continuing education in the field of food industries. L' ESIAT is subjected to cotutelle ministry of agriculture and of the hydraulic resources and the ministry of higher education. This school ensures a single training in industries food to allow the graduates: to direct line productions, to control and optimize quality and to design new product. The formations we have in our school are licence applied in industries and food processes, engineer, master and PhD. The ESIAT counts three research units: "Valorization of natural substances of vegetable origin", "valorization and conservation of the food products" and "sciences and Technology of food".

The valorization of natural substances of vegetable origin research group led by Professor Nabiha Bouzouita and Professor Mohamed Moncef Chaabouni includes/understands 20 researchers, 6 PhD and 14 masters. Tunisia presents a ground of predilection to the development of the aromatic and medicinal plants of share its geographical position, Tunisia includes/understands a rich and diversified spontaneous flora and pedoclimatic potentialities extremely interesting and very favorable to the intensive development of culture of aromatic and medicinal plants. The valorization of these vegetable natural resources which can be spontaneous or cultivated passes primarily by the extraction of their essential oils. These last are products with strong added value, used in the drug companies, cosmetics and agroalimentary. The study of the activities biological and biotechnological of the extracts of plants is of a great interest. Our objective consists in developing an extremely interesting vegetable inheritance by making biological and chemical studies of some plants investigated in our laboratory. During this work, studies of the chemical composition, antimicrobial and antioxidant activities of the essential oils extracted from these plants were carried out.

We were interested in other share to galactomannans, macromolecular glucidic compounds extracted from the endosperm of leguminous plant seeds. The carob shrub seeds of the Mediterranean coastline provide aqueous solutions of galactomannanes which give, even to weak concentration solutions with very strong viscosity one thus employs them largely in food industry for their remarkable thickening properties.

The development of the process of purification by microfiltration, ultra filtration and drying by atomization and freeze-drying of the anthocyans of Tunisian origin (starting from the grape marc) give us an added natural dye with great value which answers the international standards: E163. The betalains are nitrogenized pigments responsible for the red or yellow color in certain plants such as red beet. Replacing the pigments anthocyanins, they are endowed with an important antioxidant capacity and do not have any toxicity on the human health. Different extracts from Myrtle berries were obtained using alcohol-water mixtures as extraction medium in the range 60–90 % (v/v) to study the extraction efficiency in the preparation of myrtle liqueur.

We work within the framework of research projects as well national and international (we have a project with the Walloon Center of Industrial Biology in Liege in Belgium and a project with the University of Tsukuba to Japan).

Essential oils, extracts of plants and active molecules isolated from these extracts can be used for the protection of greasy substances from oxidation, the protection of food from the micro-organisms and the conservation of food. The carob gum, the natural dyes are food additives very required by the consumer who seeks all that is in relation to the naturalness, to guarantee a good food for health, a food safety and quality.

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Somaia A. Nada

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National Research Centre (NRC) is the largest multidisciplinary R&D centre in Egypt devoted to basic and applied research within the major fields of interest. ***NRC*** possesses an impressive scientific & technological infrastructure and man power resources of **4847** research staff. ***NRC*** Consists of **14** divisions and **111** departments covering the major areas of industry, health, environment, agriculture, basic sciences and engineering. ***NRC*** is headed by a president with ministerial status, assisted by two vice presidents, one for research and the other for technical affairs. The minister of state for Scientific Research is the higher president of ***NRC***.

NRC* Vision:** the ***NRC has to correspond to the country's key production and services sectors through the research conducted in different areas of science and technology, scientific consultation and training as well.

The ***NRC*** mission is to conduct basic and applied research within the major fields of interest in order to develop production and services sectors.

Brief History: ***NRC*** was established as an independent public organization in **1956**, with the aim "to foster basic and applied scientific research, particularly in industry, agriculture, public health and other sectors of national economy". It is the largest of all institutions affiliated to the ministry of Scientific Research and employs about **60%** of all scientists working in these institutions.

Between the sixties and eighties of the last century six divisions of ***NRC*** developed into independent research institutes: the national institute of standards, petroleum research, central metallurgical research, Theodore Bilharz, ophthalmology research, electronic research institutes.

Since its establishment, the ***NRC*** has passed through three evolutionary stages: the initial stage extending to 1968 focused on basic sciences research and capacity building, the second stage (1968-1973) was characterized by a growing interaction with the production and service sectors, and the third stage (1973 till now) concentrating on customer oriented research to serve specific needs of end users.

National projects: industrial modernization R&D program, contracts with the academy of scientific research, projects funded from STDF (science, technology & development fund) and contracts with the private sector.

International projects: scientists from ***NRC*** and foreign scientists, mainly from developed countries, cooperate in undertaking joint research work of mutual interest to both countries. Such cooperation strengthens the link between local and foreign scientists, stimulates research and urges the display of capabilities and thoughts on the international level. The programs are entirely designed to establish working relation between ***NRC*** scientists and their co-partners in many countries such as European Union, NATO, Canada, England, Norway, Belgium, Italy, Holland, Finland, Portugal, Australia, Bulgaria, Austria, Korea, Ukraine, Poland, China, Japan, Korea, France, Spain, South Africa, Commonwealth Republics, India, Tunisia, and Pakistan. Most of the foreign cooperation is in the field of textile and chemical industries, agriculture, environment, food industry, medical research, biotechnology and mineral resources.

International Agreements: 1) Memorandum of Understanding (**MOU**) Between China Science and Technology Exchange Center (**CSTEC**) and National Research Centre; **2)**

Agreement Between University of Bonn and National Research Centre for Establishing a new Programme of Master of Science in Organic Farming in Egypt (**NPMSOFE**) with the Education, Audiovisual and Cultural Executive Agency; **3)** Agreement Between ***NRC*** and **INRA**, France; **4)** Project consortium agreement "Sustainable use of irrigation water in the Mediterranean region", **FP7; 5)** Evenor- Tech Company, Spain; and **6)** Institut National des Sciences et Technologies de la Mer (**INSTM**), Tunisia.

NRC Scientific Journals: Journal of Egyptian Agricultural Research; Journal of Applied Veterinary Sciences; Journal of Genetic Engineering and Biotechnology
Journal of Egyptian Pharmacy; Egyptian Medical Journal of the NRC; NRC Newsletter.

The pharmacology department at *NRC* has the necessary equipments to performing the experimental research work in biological evaluation and pharmacological properties of new drugs, synthetic compounds and natural products. The pharmacology research group is interested in nutraceutical agents, prebiotic and probiotics for food safety toward better health for human and animals. The teamwork contains from expertise of different subject areas; the leader is Somaia A. Nada (Pharmacology dept.), Omar M.E. Abdelsalam (Toxicology and Narcotics dept.), Enayat A.Omara (Pathology Dept.), Sayed A. El-Toumy (Chemistry of Tannins dept.), and 10 PhD students. This research group performing the biological evaluation studies in the National Mega project entitled “Food nanotechnology” (STDF) April 2010- until now.

Session 2: Health effects of food

Invited lecture: **Neutraceuticals as therapeutic options for the treatment of Alzheimer's disease**

S Kirubakaran, ML Steele, A Rahmadi, L Bennett, G Münch

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In many chronic neurodegenerative diseases including Alzheimer's disease (AD), chronic activation of microglia and astroglia can be observed. Activated microglia secrete a variety of cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor as well as reactive oxygen and nitrogen species (ROS/RNS). Since ROS act as signaling molecules in pro-inflammatory redox-active signal transduction pathways, it is likely that membrane-permeable antioxidants that act intracellularly, including polyphenols, scavenge these "signaling" reactive oxygen species, and via this mechanism, act as anti-inflammatory drugs. We have tested a variety of pure polyphenolic compounds and samples from the CSIRO food and plant library for their ability to attenuate NO and TNF production in macrophages and microglia. Among the pure flavonoids, apigenin and diosmetin were most potent, and exhibited EC_{50} values < 10 µM. Among the plant library samples, cinnamon and cloves were the most potent anti-inflammatory candidates.

Another promising target in AD might be activated astroglia, which transform from a basal to a reactive state, and appear to neglect their neurosupportive functions, particularly the release of glutathione. Apigenin and the thiol antioxidant lipoic acid were the most potent compounds increasing GSH production in both activated and non-activated astroglia. In conclusion, we suggest that neutraceutical, including plant-derived polyphenols, might offer therapeutic opportunities to delay the progression of neuro-inflammatory diseases including Alzheimer's disease.

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Invited lecture: **Animal-free tests to evaluate health effects of food compounds**

Raymond Pieters, Jean Paul Ten Klooster, Marc Teunis, Cyrille Krul

Research Centre Technology and Innovation, Utrecht University of Applied Sciences, The Netherlands

The interest in consumer healthcare products and medicinal products derived from natural (e.g. plants) and food resources, i.e. natural products, is increasing. Presently, it remains unclear how safety and efficacy of such products, particularly extracts or purified ingredients, can be most efficiently assessed. Importantly, European legislation to ensure scientific validation of health claims has been enforced recently (The EFSA Journal 2008, 644, 1-44) and requires human studies as gold standard. But compounds need to be tested prior to these human studies, preferentially without animal testing and in a translational manner (from cell to organism and from bench-to-bedside).

Recently, we have proposed an alternative tiered testing strategy to screen for both safety and efficacy of natural products in similar test systems. Outcomes of such testing may support and guide subsequent human studies. In this strategy initial focus would be on testing the likelihood that these products will be metabolically converted. In the next phase, mutagenic or carcinogenic as well as sensitizing potency need to be evaluated. Subsequent tests should allow assessment of efficacy. The type of tests and cells to be used depends on specific applications of the product, such as the route of exposure, i.e. cells may range from intestinal epithelial cells to keratinocytes or skin explants. For target analyses, cell types are more diverse, and may vary from e.g. immune cells to liver cells and adipocytes as well as combinations of these. Reporter systems for instance for various cytokines and chemokines may allow high throughput screening of products for potentially beneficial or adverse effects. The concept that both types of effects can be assessed in similar test systems relies on the idea that the ultimate effect is concentration (or dose)-dependent.

Importantly, tests of a strategy as proposed need to be robust, transferable and validated. Recently, we have prevalidated a human T cell activation test (for immunotoxicity) as one of the possible tests and we are currently evaluating a two-tiered test strategy for contact sensitization.

Invited lecture: **Utility of rodent models for evaluating protein allergenicity**

Christal C. Bowman and MaryJane K. Selgrade

U.S. Environmental Protection Agency

Animal models are needed to assess novel proteins produced through biotechnology for potential dietary allergenicity. Food allergy may be mimicked by administering antigen with cholera toxin, which helps to elicit IgE responses. While the IgE response in such a model is driven by cholera toxin, the properties of the antigen itself may be influential. We hypothesized that giving a panel of allergenic and non-allergenic food extracts orally with cholera toxin would elicit IgE responses to allergens but not to non-allergens. Additionally, due to the implied lack of oral tolerance to the offending antigen in food allergy, we hypothesized that food allergens may be less subject to oral tolerance. Oral exposure using cholera toxin resulted in IgE to allergens (peanut, Brazil nut, and egg white) but not to non-allergens (spinach and turkey), provided that the dose and exposures were limited. Including sodium bicarbonate to limit digestion during oral exposure enhanced IgE responses to non-allergenic foods. Data from *in vitro* digestibility assays demonstrated that peanut, Brazil nut, and egg white contain pepsin (stomach) resistant proteins, while spinach and turkey proteins are highly labile. This observation, coupled with the very low IgE response to spinach or turkey when administered with cholera toxin, implies that the lability of these non-allergenic foods limits their availability for immunologic presentation within the digestive tract.

Our results also implicate digestive stability in oral tolerance induction. Mice were exposed orally to food extracts (without adjuvant) and subsequently challenged intraperitoneally. Reduction of antigen-specific serum IgE relative to appropriate controls was used to indicate tolerance. Foods associated with persistent, severe allergy (peanut, Brazil nut) and non-allergens (turkey, spinach) were less tolerizing than foods associated with frequently resolving allergy (egg white). *In vitro* digestion indicated that pepsin-resistant peanut and Brazil nut proteins were sensitive to trypsin, whereas egg proteins resisted both stomach and intestinal enzymes. We speculate that once through the stomach, only proteins resistant to intestinal enzymes induced tolerance. Our results suggest that oral exposure under the appropriate experimental conditions will result in differential allergic and tolerance responses in accordance with known allergenicity. Moreover, those foods containing pepsin-resistant proteins elicit IgE in the oral sensitization model, supporting the current use of pepsin resistance for potential allergenicity assessment. The complementary oral tolerance model suggests that resistance to both pepsin and trypsin is required for tolerance induction, which may facilitate allergenic characterization based on biochemical features.

Session 3: Biochemistry and Molecular Biology of Food Allergens

Invited lecture: **Overview of peanut allergens**

Stef J. Koppelman

HAL Allergy

Oral 3.1. **Thermal and non-thermal effects of ultrasound treatment on β-lactoglobulin structure**

Dragana Stanić-Vučinić [1], Marija Stojadinović [1], Ana I. Sancho [2], Marina Atanasković-Marković [3,4], E.N. Clare Mills [2] and Tanja Ćirković Veličković [1]

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In the past two decades, ultrasonic cavitation has seen diverse applications in food biotechnology for preservation and quality retention of foods. β-Lactoglobulin (BLG) is one of the most abundant proteins in milk, and due to its compact globular structure BLG is a poor substrate for digestive enyzmes and a potent food allergen. In this study, thermal and non-thermal effects of ultrasound (20 kHz, 10 W) on BLG secondary and tertiary structure were analyzed by far and near UV CD spectrometry. Short time sonication of BLG induced marked alternation of its secondary structure, but it refolds with a tertiary structure similar to the native conformation. In contrast, prolonged exposure caused fewer changes in the secondary structure with marked non-rigid side-chain packing, indicative of a stable molten globule state formation. Due to this both thermal and non-thermal effect, sonication induced polymerisation of BLG to dimers, trimers and higher polymers. Non-thermal ultrasound effects alone generated only small quantity of dimers, with BLG variant B being more prone to dimerisation than variant A. Compared to untreated BLG, sonicated BLG showed better digestibility by pepsin in simulated gastric fluid. BLG Structural changes by ultrasound caused neither significant differences in specific CD3/CD4 + cell proliferation in PBMCs culture from milk allergic donors, nor significant differences in cytokine profiles in culture supernatants of PBMCs. However, ultrasound treated BLG showed less ability to activate degranulation of basophiles from some milk allergic patients, compared to native BLG. By inducing fine structural changes, ultrasound treatment could have the potential to generate hypoallergenic foods with improved digestibility and thus higher nutritional value.

Oral 3.2. **Actinidin enzyme activity levels differ in kiwifruit extracts depending on the method of extract preparation**

Milica Grozdanović, Milica Popović, Marija Gavrović-Jankulović

Faculty of Chemistry, Dept. Biochemistry, Belgrade, Serbia

Background: Actinidin is a kiwifruit cystein protease with a molecular weight of 30 kDa and a pH optimum of around 5. In ripe kiwifruit actinidin is the most abundant soluble fruit protein and has been identified as its major allergen. Crude protein extracts are used in the diagnosis of food allergy, and their protein composition, and therefore allergenic activity, are known to differ depending on the source of the allergenic material and the method of extract preparation. The aim of this study was to explore levels of actinidin enzymatic activity depending on the method of kiwifruit extract preparation.
Methods: Three different kiwifruit protein extracts were prepared from a single kiwifruit. Equal amounts of the pulp of the fresh fruit were homogenized in three different extraction buffers: 100 mM potassium phosphate buffer, pH 7.0; 100 mM ammonium bicarbonate buffer, pH 9.3; and a 100 mM sodium citrate buffer, pH 5.0. The proteolytic activity of actinidin in the extracts was determined using the protease enzymatic assay with casein as a substrate. An additional fourth extract, commercially used for skin prick tests, was included in analysis.
Results: The casein digestion enzyme assay showed varying levels of actinidin proteolytical activity in the different kiwi extract preparations. The basic kiwi extract (pH 9.3) possessed the highest enzyme activity, followed by the acidic (pH 5.0) and neutral (7.0) kiwi extracts, respectively. The commercial extract had the lowest measured enzyme activity.
Conclusion: We have shown a significant difference in the enzyme activity of the most abundant kiwifruit protein and major allergen, depending on the method of crude protein extract preparation. Further studies will determine the possible correlation of actinidin enzyme activity levels with its allergenic potential.

Session 4: Probiotics and Prebiotics

Invited lecture: **Pectin and the prebiotic activity of its ologosacchardies**

Estelle Bonnin

INRA, UR1268 Biopolymères Interactions Assemblages, F-44300 Nantes, France

Pectins are natural constituents of terrestrial plants and therefore form part of our diet as components of fruits and vegetables. They are a complex family of heteropolysaccharides that arise from the primary cell walls surrounding each cell in higher plants. After extraction, they are widely used as food ingredients and additives because of their gelling, thickening, stabilising and emulsifying properties.

Pectin molecules are mainly composed of four different structural elements: homogalacturonans (HGs), type I rhamnogalacturonans (RGs-I), xylogalacturonans (XGAs), and type II rhamnogalacturonans (RGs-II). Due to their structural complexity, a large panel of enzymes is needed for the enzymatic degradation of pectins. As far as the enzymatic mode of action is known in details, enzymes can be used as tools to elucidate the fine structure of the polymer. They can also be used to produce oligosaccharides that are now recognised as having biological properties. The biological effects of pectin have been studied extensively and they are reported to be highly fermentable dietary fibres. As pectic molecules are complex and heterogeneous, different regions of pectin may exert different health supporting functions among which prebiotic potential.

In this talk, pectin structure and pectin degrading enzymes will be presented to introduce the production of specific oligosaccharides. In the second part, fermentation selectivity of the pectic oligosaccharides will be shown.

Oral 4.1. **Effect of Prebiotic Infant Formula on the Infant's Gut Microbial Composition and Anthropometric Factors: Clinical Study**

Olga B. Martinov[1], Snežana D. Spasić[1], Nikoleta M. Lugonja, Gordana Đ. Gojgić-Cvijović[1] and Miroslav M. Vrvić[1, 2]

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Human breast milk is the best dietary choice for a newborn baby, and as it is considered as a gold standard, all the manufacturers of infant formula (substitutes for the breast milk) aim to produce these products with composition very similar to that of human breast milk. Current trends in the composition of infant milk formula include products supplemented with non digestible oligosaccharides and inulin to obtain a bifidogenic effect comparable to that of breast milk. The aim of a bifidogenic effect on the infant's intestinal flora is to counteract the current rise of allergic diseases and to enhance protection of the infant against gastrointestinal infections. The aim of this study was to investigate the influence of an infant formula containing added inulin and fructooligosaccharides (FOS) on the gut microflora, as well as on the growth development and development of infants, compared with infants who were exclusively breastfed.

The 28-day study enrolled 21 healthy, term born infants of both sexes, divided into two groups: formula fed group and control- breast milk fed group. Fecal samples were obtained before (day 0), and during formula administration (14 and 28 day) and level of infant's gut colonization was investigated. The anthropometric factors (weight, height) were measured every day during this study period. There were no significant differences in the fecal numbers of lactobacilli, total aerobes, anaerobes or yeasts and fungi between these groups. In contrast, the bifidobacteria number in the stools increased significantly during the study in the infants receiving the supplemented formula. The body weight and length of the infants in both study groups increased at similar rates during the study period and were within the normal framework for the postnatal period. All infants exhibited normal growth during the study.

This clinical study showed that the infant formula containing additional prebiotics (inulin and FOS) and mature breast milk have similar gut microflora and bifidogenic effect, also prebiotic supplemented infant formula favorably influences the growth and development of infants in a manner similar to that of mature breast milk.

Keywords: inulin, fructooligosaccharides, infant formula, anthropometric factor, bifidobacteria

Oral 4.2. **Comparative Clinical Study and *In Vitro* Test of Bifidogenic Effect of Two Infant Formulas Supplemented With Inulin and Fos**

Nikoleta M. Lugonja[1], Olga B. Martinov[2], Snežana D. Spasić[2], Gordana Đ. Gojgić-Cvijović[2] and Miroslav M. Vrvić[1,2]

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Development stimulation of bifidobacteria in the intestinal tract by the effect of food ingredients is called bifidogenic effect. Bifidogenic effect is one of the most important indicators for quality of the infant's food, in addition to nutritive and biological values.The aim of this study was to compare bifidogenic effect of infant formulas supplemented with inulin and fructooligosaccharides *in vitro* and clinically, and to compare that of mature breast milk.

Healthy, term born infants, younger than 6 months and babies 6-12 months old, were enrolled in 28-day study. According to the type of feeding, infants were divided in groups – formula fed and breast milk fed (control) group. Fecal samples were obtained before (day 0) and during formula administration (14 and 28 day), stool specimens were quantitatively cultured and evaluated count of bifidobacteria and biochemical parameters. *In vitro* test examined microbiological and biochemical changes in two infant formulas and mature breast milk, induced by the action of bifidobacteria isolated from newborn's feces.

The bifidobacteria number in the stools increased significantly during the study in both infant groups receiving the supplemented formula as well as in both breast milk groups. The comparative *in vitro* test showed that the bifidogenic effect was similar for infant formulas and mature breast milk in terms of the number of bifidobacteria. There were no statistically significant differences in biochemical parameters between groups. Consumption of infant formula with added inulin and fructooligosaccharides stimulated the bifidogenic effect, both clinically and *in vitro.*

The *in vitro* test can quickly and objectively determine the bifidogenic effect of infant formula and indicate its quality. However, a clinical test is necessary to determine the acceptance and biological value of infant formula.

Key words: Bifidogenic effect, *in vitro* test, clinical study, bifidobacteria, inulin

Session 5: Proteomics and metabolomics

Invited lecture: **Application of proteomics in food safety: detection of bacterial protein toxins**

Gabriella Pocsfalvi

Mass Spectrometry & Proteomics Group, Institute of Protein Biochemistry, CNR, Napoli, Italy

Intoxications related to the consumption of food colonized by *Staphylococcus aureus* is one of the most frequent causes of food borne diseases in many countries. Pathogenic bacteria secrete a variety of virulence factors into extracellular medium and to the cell surface which have essential roles in the colonization and insurrection of the host cells, and thus reflect the degree of bacterial pathogenicity.

The currently available immunochemical assays address only the detection of a few known staphylococcal enterotoxins (SEs) and typically do not allow multiplex analysis. Recently we have shown the utility of proteomics for the identification and the simultaneous monitoring of staphylococcal virulence factors linked to pathogenesis.(*1*) Secretomes of different *Staphylococcus aureus* strains were analyzed using gel-based bottom-up proteomic approach. A total of 119 distinct proteins were identified for the enterotoxin gene cluster (*egc*) negative and *seb* gene positive *S. aureus* ATCC 14458 strain by the use of 1DE and 2-DE based proteomics. Detailed analysis of enterotoxin region of the 2-D map confirmed, beside the highly expressed staphylococcal enterotoxin B (SEB), the presence of enterotoxin-like proteins SElK and SElQ.

Solid phased immunoaffinity capture provides an efficient way to enrich and purify a wide range of proteins from complex mixtures. We have shown that SEs can be efficiently enriched by means of magnetic immunocapture using antibody functionalized paramagnetic beads.(*2*) The method can be successfully interfaced by the on-beads and off-beads detection using MALDI-TOF-MS at the protein level and by the off-beads nano-ESI-MS/MS detection at the enzyme digests level enabling thus the unambiguous identification of the toxin. The method is applicable to any bacterial toxin which has available antibody. The usefulness of the method is demonstrated for the detection of selected SEs both in culture broths and in dairy products.

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Invited lecture: **Structural analysis of underivatized oligosaccharides by mass spectrometry**

Bernard Quemener

INRA, Nantes, France

The structure of various oligosaccharides has been investigated in the last decade using ^{1}H- and ^{13}C NMR spectroscopy, and numerous studies have been reported on the structural characterization of oligosaccharides obtained after enzymatic digestion of polysaccharides. Although, NMR sensitivity has considerably improved with the introduction of nanoprobe equipments and very high field magnets, this approach still requires large amounts of high purity samples. As an alternative to NMR spectroscopy, FABMS and ESIMS have been used for oligosaccharide characterization. The main advantages of tandem MS are its sensitivity and its capability to assign linkage, sequence and branching or substituent pattern informations upon collision-induced dissociation (CID) of underivatized oligosaccharides. Moreover, derivatization e.g. permethylation or reducing-end derivatization in combination with CID proved to be very useful to clearly identify complex branching patterns of oligosaccharides. Some authors have also reported that sequence and branching patterns of underivatized oligosaccharides can however be obtained using negative CID-ESIMS/MS. The main advantage of negative CID MS/MS is to produce series of C-type glycosidic cleavage ions which produce in turn series of related A-type cross-ring cleavage ions carrying linkage information.

In this presentation common ionization methods used for oligosaccharides as well as the main characteristics of tandem MS of underivatized oligosaccharides are discussed in term of negative and positive ionization modes. We will detail the nomenclature of Domon and Costello[1] commonly used for the identification of glycosidic and cross-ring cleavage ions produced by oligosaccharide fragmentation throughout tandem or multistage tandem MS. Some examples dealing with disaccharides and trisaccharides analysis will specifically address the capability of CID MS/MS to assign anomericity and linkage. Recently, we have applied negative CIDMS^{n} to the assignment of acetyl groups to O-2 and/or O-3 positions of pectic oligogalacturonides. These results will be shown and discussed as they offer a good illustration of the interest of mass spectrometry in determination of enzyme specificity. Finally, we will shown an example to highlight the capability of MALDI-TOF MS approach to screen cell wall mutants and how this technology is suitable to give insights into the structural features of cell wall polysacharides in plants.

Oral 5.1. **The Influence of Soybean Storage Protein Genotypic Differences on Tofu Quality**

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Tofu is known as soybean curd. Coagulation properties of soymilk are critical to achieving high yields and desired texture of tofu. Yield and quality of tofu are affected by several factors, such as tofu-processing method, growing environment and soybean cultivar. Soybean varieties differ in chemical components, including proteins, lipids, and minerals, that may influence yield and quality of tofu.

The objective of this study was to investigate the effect of soybean genotype and processing method on the quality of tofu. The contribution of main soybean storage proteins, namely glycinin (11S), which is high in sulfur-containing amino acids, and β-conglycinin (7S) and their respective subunits to tofu yield and gel texture was studied. High temperature/pressure for soymilk extraction and commercial chymosin-pepsin rennet were used for tofu processing. They had effects on tofu storage protein composition determined by SDS-PAGE, on the yield of tofu and on the rheological properties of the gel. The examined genotypes are characterized by high total protein content in soy flour and respective tofu, good tofu yield and texture. For all soybean varieties, there was approximately a 90-98% protein recovery rate of tofu. This would be an indication of processing method efficiency in protein extraction and subsequent coagulation. Soybean variety and the new production method had effects on the 7S and 11S protein content and 11S/7S protein ratio of tofu. Significant differences in tofu firmness among soybean genotypes have been found. These results are in agreement with smooth and uniform microstructure of tofu. Registered tofu firmness showed a very strong positive correlation with 11S/7S protein ratio of soybeans. Soybeans β'-subunit of 7S protein showed very strong negative correlation with tofu extractable soluble protein content and with tofu firmness leading to a strong negative correlation of both β subunits with these properties. Also, a very strong negative correlation exists between proteins 11S/7S ratio in tofu and tofu protein extractability as well as between 11S/7S ratio and tofu extractable soluble protein content. Moreover, strong positive correlation is found between tofu protein extractability and β-conglycinin concentration and very strong positive correlation between tofu protein extractability and α´+α - 7S subunits. These facts indicate that tofu containing higher level of β-conglycinin α subunits would have a higher extractability whereas 11S protein is firmly integrated into gel matrix. This is probably due to differences in protein structure of glycinin and β-conglycinin. Glycinin is a hexameric protein with compact quaternary structure, wheraes β-conglycinin is a trimeric protein. β-Conglycinin is glycoprotein with about 5% carbohydrate moieties. It is known that the carbohydrate moieties contribute to solubility. Furthermore, β-conglycinin has lower surface hydrophobicity than glycinin.

Evaluation of protein quality in soybeans, based on subunits composition of their protein, assessment of the genetic variability of soybean genotypes and its correlation with properties of the resulting protein product may serve as very effective tool for assisting plant breeders in their selection of high quality genotypes and in further development of new soy-food of improved quality.

Acknowledgement: The authors are indebted to Serbian Ministry of Science and Technological Development for providing grant for this study.

Oral 5.2. **Classification of Serbian honey according to their sugar profiles and physico-chemical characteristics**

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Despite the long tradition of Serbian honey production, the issues of physical, chemical and sensory properties of Serbian honey have not, insofar been covered by scientific publications, contrary to the large number of foreign papers dealing with these issues. Although Serbia is not a big country, differences in climate, soil and plants provide a solid base for the production of different types of honey, such as those most often produced: acacia (*Robinia pseudoacacia*), sunflower (*Helianthus annuus*) and linden (*Tilia cordata*) honey.

In scope of this work a total of 350 different honeys from three botanical origins (acacia, sunflower, and linden), as well as polyfloral honey have been characterized and classified by applying Principal Component Analysis to their sugar profile and physicochemical parameters. Besides the two main constituents, the monosaccharaides glucose and fructose, different polysaccharides (trehalose, isomaltose, melezitose, gentiobiose, turanose, maltose, maltotriose, sucrose, isomaltotriose) were determined by means of ion chromatography. In addition, five physicochemical parameters were analyzed following the Harmonized Methods of the International Honey Commission (water, ash content, moisture, free acidity and optical rotation).

The Principal Component Analysis has been performed on the entire data set, in order to reveal the most important factors influencing the grouping pattern among the several honey species. The first three principal components capture 62.00% of the total cumulative variance, while the first PC component explains 27.00% and the second one 13.61%. The three different types of honey: acacia, sunflower, and polyfloral honey have been well separated according to the score values of the first two principal components. The first two exhibit higher and positive values of PC2 scores, while the last one has significantly decreased and negative values of PC2 scores. In addition it was found that the positive values of PC1 scores are typical for polyfloral honey, while the other two types have significant negative values.

Free acidity, electrical conductance, and water percentage as well as trehalose and glucose content, having higher values in both sunflower and polyfloral types of honeys, and, pH and sucrose and maltose content found higher in *Robinia pseudoacacia* honey, were identified as the most influential factors leading to the best discrimination among investigated types of Serbian honey.

Oral 5.3. **Co-occurrence of aflatoxins and ochratoxin A in red paprika spice powder in Serbia: Occurrence and evaluation of a multimycotoxin analytical method**

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Mycotoxins are a group of chemical substances, produced by some fungal species, which can cause illness or even death. There is a growing concern regarding mycotoxin contamination in foods and feeds, since they have been found in a wide range of commodities, including cereals, spices, dried fruits, apple products, wine and coffee. Poor hygienic conditions and deficient water activity control during processing of peppers can lead to fungal growth, which can result in mycotoxin accumulation in paprika. The European Commission (EC) set a legal limit for the first time in 2002 for aflatoxins (AFs), and just recently in 2010 for ochratoxin A (OTA). Contrary, the Serbian regulation on food safety does not specify the mycotoxin limits for paprika. Contamination of red pepper spice by AFs and OTA may occur at any stage of production, from preharvest to drying and storage. The co-occurrence of different mycotoxins in paprika increases the probability of interactions, such as additive or synergistic effects, which may increase the risk to human health. Thus, there is increasing need for multimycotoxin analysis enabling simultaneous determination of various toxins with the employment of simple and time-effective preparation methods.

This paper describes a simple preparation method for simultaneous determination of AFs and OTA in paprika using ultra-high performance liquid chromatography/heated electrospray ionization coupled to tandem mass spectrometry (U-HPLC/HESI-MS/MS, Accela- TSQ Vantage, Thermo Fisher Scientific, US). The method is based on a single extraction step using acetonitrile/water mixure followed by analysis of the diluted crude extract. The method validation was performed in accordance to latest EC directive 401/2006 on the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. The method was applied on red paprika spice powder attended for the meat industry revealing that the samples were in compliance with the limits imposed by the EC Directive. The prepared paprika extracts were also analyzed by injecting the samples into the high-resolution MS (HRMS) system patented with Orbitrap technology (Accela-Exactive, Thermo Fisher Scientific, US) in order to confirm the results obtained by U-HPLC/ESI-MS/MS. The screening with HRMS indicated that the samples also contained mycotoxins other than AFs and OTA, like zearalenone, HT-2 and T-2 toxin. So far there have been only a few studies that describe the simultaneous occurrence of mycotoxins in paprika powder, and this is the first coming from Serbia.

Acknowledgement. The results presented in this work have been obtained in the Laboratory for Mass Spectrometry of the Center of Excellence in Food Safety and Emerging Risk established at the Faculty of Technology, University of Novi Sad, during the FP7 project CEFSER, GA 229629 and supported by the Ministry of Science and Technological Development of the Republic of Serbia.

Session 6: Nutrition and immunology

Invited lecture: **The interplay between innate and adaptive immunity in food allergy**

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Food allergy affects approximately 5 % of children and is the leading cause of hospitalization for anaphylactic reactions in westernized countries. Animals models have proven to be crucial to elucidate the mechanisms behind food allergic responses. Remarkably, immune cells in the intestine itself are among the least studied cells in the process of (food) allergic sensitisation. Therefore, we focused on the changes in different leukocyte populations the intestine and draining lymph nodes during allergic sensitization with peanut proteins. Interestingly, local allergic sensitisation induced a shift in dendritic cell (DC) subsets in the intestine. Mucosal tolerogenic (CD103+) DC were decreased in number, but conventional inflammatory (CD11b+) DC were increased. In addition to changes in DC subsets, we observed a decrease in γδ T cells in the intestine upon exposure to CT. As both CD103+ DC and γδ T cells contribute to immune homeostasis and regulation in the intestine, decreased numbers will render the intestine more susceptible to immune activation. Next studies investigated the functional role of earlier mentioned DC subsets and γδ T cells. Results demonstrate that expansion of DC numbers and especially plasmacytoid DC inhibite the establishment of allergic manifestations in the intestine. In addition, we demonstrate a unique regulatory role for γδ T cells. These data suggests that targeting local intestinal leukocyte populations contributes to strategies to prevent and possibly treat food allergy.

Invited lecture: **The role of selenium and selenoproteins in human health and nutrition**

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Selenium, the only genetically encoded dietary micronutrient, is essential for human health and survival. Selenium deficiency and mutations in selenoprotein genes lead to numerous pathologies, and there is strong evidence that selenium is important in preventing various types of cancer. Biochemical, nutritional and clinical studies have shown that there is an inverse correlation between selenium intake and cancer, cancer-related and overall mortalities, viral infections and inflammation. The emerging evidence suggests that the accuracy and efficiency of the selenium incorporation into the amino acid selenocysteine, and subsequently into human selenoproteins, is an essential biological process that may be regulated at multiple levels. In humans, six enzymes, selenocysteine tRNA and a specialized structure in the mRNA coordinate both synthesis and insertion of selenocysteine into selenoproteins. Here, the most relevant findings in the field of selenium biology will be summarized and the mechanistic details on selenocysteine formation in humans will be presented.

Oral 6.1. **Absorption and metabolism of chokeberry anthocyanins in humans**

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After food consumption, the phytochemicals undergoes complex processes of biotransformation. Finally, a number of metabolites possessing uncertain biological activity occur in biological fluids and affect homeostasis of human organism. Unfortunately, many of these compounds remaining unidentified.

Anthocyanins are red, orange, blue or purple water soluble pigments occurring in fruit and vegetables. As components of plant food products anthocyanins are consumed by humans in the amounts which may be significant from the physiological point of view. The chokeberry is rich in anthocyanins such as cyanidin-3-galactoside. This compound comprised 66% of total anthocyanins found in chokeberry.

The absorption and metabolism of anthocyanins from chokeberry juice was studied on 13 subjects after formal approval by the local Bioethical Committee. Following 3-day anthocyanins free diet and overnight fast volunteers were given a dose of 0.8 mg anthocyanins/kg. The blood and urine were collected before and within 24 h after consumption of chokeberry juice. Chokeberry anthocyanins and their metabolites were measured with the HPLC-DAD-MS method.

Apart from the native anthocyanins the presence of their methylated and glucuronided metabolites was found. Anthocyanins appeared in plasma within 30 min after dosing. The highest anthocyanins plasma concentration was observed one hour after the challenge when native anthocyanins dominated. Intensive metabolism resulted in domination of anthocyanins metabolites in the plasma collected one hour later. The peonidin monoglucuronide was the predominant metabolite. The cumulative concentration of total anthocyanins detected in the plasma (0-24h) was 109.36 nmol/L x h. The urine excretion rate was the highest within the first two hours after the juice intake. Totally, during 24 h 0.3% of the ingested dose was excreted with urine: 70% as metabolites and 30% as native compounds.

Acknowledgement: The research was supported by the Polish State Committee for Scientific Research project PBZ-KBN-094/P06/2003.

Oral 6.2. **Iodine Intake And Status And Their Relationship To Developmental Outcome In Children**

Danijela Ristić-Medić, Vesna Vučić, Gordana Petrović-Oggiano, Tamara Popović, Marija Glibetić, Mirjana Gurinović

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Background: Iodine deficiency (ID) is the leading cause of preventable mental retardation worldwide. Biomarkers of iodine status are required to study ID in diferent parts of the world and to effect fortifications strategies. The EURRECA (EURopean micronutrient RECommendations Aligned) Network of Excellence (www.eurreca.org) is working towards aligned micronutrient recommendations and carried out the systematic reviews focussed on the relationships between dietary micronutrient intake, status markers (of exposure or body stores) and health outcomes.
Objectives:. In this systematic report we provided an overview of randomized controlled trials (RCTs) and observational studies in children that investigated the relationship between iodine intake/or status and developmental outcomes: intelligency, mental and psychomotor performance. For status we included only previously established the best status markers, i.e. urinary iodine, serum/plasma thyroidstimulating hormone, serum/plasma thyreoglobulin and dried blood spot thyreoglobulin.
Design: Search included Ovid MEDLINE, EMBASE (Ovid), Cochrane Library and bibliographies (to February 2010**).** Studies were assessed for inclusion and validity, with independent duplication. This search was originally planned as a meta analysis but published data often provided z-scores or without necessary data on variance and non-comparable cognitive test.
Results: Thirteen studies (5 RCTs, 4 cross sectional studies and 4 nested case control studies) were included in the systematic review. The observational studies in severely ID indicated a strong relation between iodine intake or status and mental impairment. The results of RCTs studies were inconsistent, probably due to high or moderate risk of bias in most of the included studies. The number of included studies in moderate ID was generally small and methodologically weak.
Conclusion: There was some evidence that iodine supplementation improved general cognitive function in ID children, but this requires confirmation with well-powered, blinded, independently funded RCTs in different age groups of children, measuring relevant long-term developmental outcomes across all levels of baseline iodine status.

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Oral 6.3. **Cross-linked peanut proteins exhibit lower allergenic risk in a mice model of peanut allergy**

Radosavljević, Jelena[1]; Mihajlović, Luka[1]; Willemsen, Karina[2]; Selinheimo, Emilia[3]; Buchert, Johanna[3]; Pieters, Raymond[2]; Pennings, Maarten[4]; Ćirkovic Veličković, Tanja[1]; Smit, Joost[2,5]

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Background: Recently, more effort has been put into investigation of food processing which can yield food with reduced allergenic risk. Cross-linking enzymes have potential in production of food with lower IgE-binding potential. However, no study has been performed on allergenic risk of food treated by cross-linking enzymes.
Here we used enzymatic cross-linking to obtain mixtures of high-molecular weight cross-linked proteins, with potentially altered allergenic properties due to changed susceptibility to digestion and/or route of uptake in the gut.
Methods: Peanut extract (PE) was cross-linked using the following enzymes: mushroom tyrosinase from *Agaricus bisporus*, tyrosinase from *Trichoderma reesei*, microbial transglutaminase and laccase from fungus *Trametes hirsuta*. In order to investigate the allergenic capacity of different cross-linked proteins C3H/HeOuJ mice were sensitized by intragastric dosing to PE or cross-linked PE in the presence of the mucosal adjuvant CT on 3 consecutive days, followed by weekly dosing (4 weeks). At day 28, mice received a challenge with peanut protein. The capacity of cross-linked material to induce oral tolerance was investigated by intragastric exposure of C3H/HeOuJ mice to PE or cross-linked PE on 3 consecutive days, followed by two weekly intraperitoneal injections of PE /alum. From all mice spleen cell cultures were stimulated with PE and medium and in the cell-culture supernatants IL-13 and IFN-γ were determined by ELISA. IgE, IgG1 and IgG2a levels in the blood were obtained by ELISA. Digestibility in *in vitro* gastric and intestinal fluids was assayed with cross-linked material and peanut extract as control. Also, Western blot probed with anti-Ara h1, anti-Ara h 2, anti-Ara h 3 and anti-Ara h 6 antibodies was performed in order to provide information about preserved structure of allergenic peanut proteins in cross-linked material upon digestion in simulated gastric and intestinal fluid.
Results: The crosslinked proteins from tyrosinase (from *T. reesei*) or laccase treatment showed significant lower antibody levels in the blood and levels of both IL-13 and IFN-γ in cell-culture supernatants in comparison with native peanut extract No effect of crosslinked proteins compared to native peanut proteins were observed in induction of oral tolerance. Digestion of cross-linked material revealed prolonged survival of structures of higher molecular weight, especially in laccase induced crosslinked proteins. Structures obtained upon digestion of cross-linked material contained Ara h 1, Ara h 2, Ara h 3 and Ara h 6 proteins. This feature could explain results obtained in *in vivo* studies of cross-linked peanut proteins sensitizing potential.
Conclusion: Cross-linking of peanut proteins with laccase and tyrosinase led to a reduced allergenic responses in a mouse food allergy model, implying a lower allergenic risk of cross linked proteins. Therefore, crosslinked proteins with improved immunological properties could be exploited for use in foods or immunotherapy for food allergy.

Oral 6.4. **The Effect of Iron Intake on Cognitive Functions and Psychomotor Development in Children and Adolescents**

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Background: Iron deficiency (ID) is a systemic condition, which has many non-hematological consequences: it impairs physical endurance, infant and children growth and development, and depresses immune function. A lack of sufficient iron intake may significantly delay the development of the central nervous system in infants and children as a result of alterations in morphology, neurochemistry, and bioenergetics that could affect mental and/or psychomotor performances later in life.
Objective: To systematically evaluate the effects of iron intake by children and adolescents on psychomotor development and cognitive function. Thus we conducted a comprehensive literature search which included MEDLINE, EMBASE (both on Ovid), Cochrane Library and bibliographies (to February 2010**).** A general search strategy was devised including terms for [study designs in humans] AND [intake or status] AND [iron]. All studies were assessed for inclusion and validity, with independent duplication. This search was originally planned as a systematic review with meta-analysis but cognitive tests used in most studies were not comparable.
Results: Ten studies (RCTs) met the inclusion criteria and were included in the systematic review. The results of RCTs studies were inconsistent, probably due to high or moderate risk of bias related to proper randomization, funding etc. in most of the included studies (8 out of 10). There was some evidence that iron supplementation improved general cognitive function in ID children, but the number of included studies was generally small and methodologically weak.
Conclusion: The evidence on the effect of iron intake on cognition in children and adolescents was not fully convincing, maybe due to confounding environmental factors or possible irreversibile effects of iron deficiency on the developing brain in infancy and early childhood, and this requires further confirmation with high quality RCTs.

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Poster session

P.1. **The optimization of enzymatic synthesis of ascorbyl oleate in organic solvents**

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Antioxidants are widely used as additives in food and cosmetics. At present, synthetic antioxidants such as BHT and BHA are being used to prevent oxidation of lipids in these products, but their application is questionable because of their potential toxicity. Natural antioxidants which could be used as adequate substitute in this purpose are fatty acid esters of vitamin C, since they are lipophilic (in distinction to vitamin C), their free radical scavenging capacity is high, their usage does not pass on any additional odor, flavor, and color to the product, and their degradation forms are not toxic (unlike synthetic antioxidants). Nowadays, efforts are being made in order to develop industrial process for enzymatic synthesis of vitamin C fatty acid esters.

In this study, ascorbyl oleate was synthesized by using immobilized lipase from *Candida antarctica* as catalyst and vitamin C and oleic acid as substrates. Organic solvents: acetone and *t*-butanol were used as a reaction medium. HPLC analyses of reaction mixtures confirmed that reaction occured in both solvents and that it was selective, since by-products were not detected. Changes in product yield were studied for the following parameters: temperature, enzyme concentration, water content, and substrate molar ratio. Since increase of temperature from 45°C to 55°C had crutial effect on reaction rate, further examinations were caried out at 55°C. The results showed that yeald in acetone can be rised by using oleic acid in excess (substrate molar ratio 1:5) and by adding 0.5 g/l of molecular sieves after 4 hours in reaction medium with starting water content of 0.05 % (v/v). Optimal enzyme concentration was 0.5 % (w/v).

Key words: antioxidant, vitamin C, oleic acid, ester, lipase

P.2 **Screening tests in using essential oils as natural preservatives in bakery industry**

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This paper aims to present the results of *in vitro* antifungal activity of seven essential oils (EOs) tested against several toxigenic fungi that commonly cause spoilage of bakery products.

Seven EOs represented by cinnamon leaf oil (*Cinnamomum zeylanicum*), garlic oil *(Allium sativum),* onion oil *(Allium cepa),* white thyme oil (*Thymus vulgaris),* oregano oil (*Thymus capitatus),* basil oil *(Ocimum basilicum),* clove bud oil (*Eugenia caryophyllata)* were purchased from Sigma – Aldrich, Germany.

The EOs were first solubilized in DMSO then diluted in sterile distilled water to obtain work concentrations of 800, 400, 200, 100 and 50 ppm.

Toxigenic strains (*Fusarium graminearum, Fusarium culmorum, Aspergillus flavus and Aspergillus oryzae*) were used in the study. Spore suspension (picked using 10 ml of NaCl: DMSO solution) obtained from a 7 days culture on PDA at 25^0C was used to test the antifungal activity of the EOs.

Antimicrobial assay of EOs was conducted using agar disc diffusion method. Sterile filter discs (6 mm diameter) impregnated with 10 µl of each concentration of the EOs were placed on the PDA medium plate previously inoculated with 100 µl volume of spore suspension of each selected fungi. The plates were incubated at 25^0C for 5 days. At the end of the incubation period the diameter of inhibition of fungal grow was measured using a milimeter ruler. Results showed that *A. flavus and A. oryzae* were more sensitive to the inhibitory action of EOs presenting a greater inhibition diameter at any tested concentration. Oregano oil presented the highest inhibitory activity against the named moulds, especially against *A. oryzae* at a minimum inhibitory concentration (MIC) of 400 ppm. Garlic and onion oils had the biggest inhibitory effect against *F. graminearum,* shown by the large growth inhibition halos (>10 mm). The MIC in this case was 100 ppm. Cinnamon oil presented the biggest inhibition diameter at a MIC of 400 ppm against *F. culmorum*. Comparative analysis of the diameter of fungal growth inhibition showed that EOs were more efficient at a concentration of 400 ppm, especially onion, garlic and oregano essential oils.

Based on these results, it may be suggested that EOs can be use as natural antifungal additives and could be an alternative to chemical preservatives used in bakery industry to prolong shelf life of products. Future research activities are based on the use of EOs in active packaging of bread, to prevent fungal growth and to increase shelf life.

Experiments were performed in the Contract 51-005/2007 financed by Romanian Ministry of Education and Research - Partnerships in Priority Area.

P.3. **One-step method for isolation and purification of native β-lactoglobulin from bovine milk with separation of A and B β-lactoglobulin isoforms**

Marija Stojadinović[a], Lidija Burazer[b], Dilek Ercili-Cura[c], Ana Sancho[d] , Johanna Buchert[c], E. N. Clare Mills[d] , Tanja Ćirković Veličković[a] and Dragana Stanić-Vucinić [a]

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The major whey protein, β-lactoglobulin (BLG) has been widely studied for its functional properties. Until now many methods for isolation of this protein have been described. The scope of this paper was to develop an easy, inexpensive, one-step method for isolation and purification of BLG while preserving its native structure. BLG was purified from defatted whey obtained from raw cow's milk by anion-exchange chromatography. Protein purity and identity was determined using reversed-phase HPLC and mass spectrometry. Total BLG yield was 80% with purity from 97-99%. The structure and native conformation of the isolated BLG and its potential to bind anti-BLG rabbit antibodies were compared to the standard commercial BLG by circular dichroism (CD) spectrometry and by inhibition ELISA respectively. Their susceptibility to various cross-linking enzymes was also studied. Far-UV CD spectra indicated that the isolated and the standard BLG molecules had similar secondary structures, while near-UV CD spectra showed that the standard BLG had slightly disrupted tertiary structure compared to the isolated BLG. The isolated protein bound anti-BLG rabbit antibodies with higher affinity (IC_{50}=0.163 µg/mL) then the standard BLG (IC_{50}= 0.414 µg/mL). Their susceptibility to cross-linking enzymes pointed out that the purified BLG is more compact than the standard BLG.

P.4. **Optimization of peanut protein crosslinking by oxidase and transglutaminase enzymes and the effects on IgE binding**

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Background: Enzymatic crosslinking has a growing role in today's food, pharmaceutical and nutriceutical industry. Peanut (*Arachis hypogea*) protein extract was crosslinked by several enzymes including laccase and tyrosinases from fungal sources and a microbial translgutaminase. in order to obtain high molecular mass crosslinks with modified allergological properties.
Material and methods: The extraction process was optimized to obtain sufficient phenolic compound concentration in the protein extract. This to simplify the procedure and allow extensive crosslinking by oxidases and transgulataminases - enzymes with potential use in food processing. Crosslinked proteins were analyzed by SDS-PAGE on 7% and 12% gels as well as on composite 0.5% agarose gels. IgE binding was determined by immunoblot, ELISA inhibition and basophil activation experiments in four allergic patients. Incorporation of peanut allergens, Ara h 1, Ara h 2, Ara h 3 and Ara h 6 was determined by immunoblot using specific antibodies produced in mice and rabbits.
Results: The molecular weight of the crosslinked proteins varied depending on the enzymes used, with the largest crosslinks (obtained by laccase and tyrosinase from *A. bisporus*) having molecular weight of around 5MDa. Immunoblots showed that certain peanut allergens (i.e. Ara h 1, Ara h 6) are less prone to crosslinking, with reaction efficiency depending on the enzyme. Due to the shifts in molecular masses it is not possible to directly compare IgE binding in immunoblot. Therfore ELISA inhibition was used, showing slightly increased IC50 values for crosslinked proteins made by laccase, transglutaminase and *A. bisporus* tyrosinase, indicating a decrease in IgE binding in these samples. In some of the tested patietns, the crosslinked proteins exhibited reduced basophil activation in the concentration range of 0.5-10µg/mL, with results showing similar or lower activation than untreated peanut proteins at other examined concentrations.
Conclusion: The obtained crosslinked proteins have molecular masses on the order of MDa, with moderate reduction of IgE binding during the crosslinking process. Therefore, enzymatic cross-linking has potential for use in immunotherapy of food allergies and creation of hypoallergenic and safer food products.

P.5. **Synergic effects of modified atmosphere packaging (MAP) and antifungal rye sourdoughs on the shelf life of bread**

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The study aims testing and establishing different optimization techniques of bread making process and its subsequent stages, to extend the shelf life of the selected product.

The experimental activities were pursued to determine the influence of modified atmosphere packaging (MAP) and the addition of calcium propionate on the shelf life of sourdough breads. Two antifungal sourdoughs were prepared based on rye flour, water and three selected *Lactobacillus plantarum* strains: LP C71, LP C78 and LP C81, using two mixture ratios, respectively 3:1:2 (variant A) and 2:3:1 (variant B). After 24 hours of incubation, at 32^0C, the sourdoughs were added to dough preparation, using a 2% sourdough to wheat flour ratio, via the direct technological process of bread making. During dough mixing, 0.1% and 0.2% calcium propionate concentrations were used, with respect to wheat flour quantity. The end products, wheat breads based on rye sourdough, were packed using three MAP gas mixtures: 50% CO_2:50% N_2; 60% CO_2:40% N_2; 80% CO_2:20% N_2. Periodically, the packed breads were analysed in terms of microbiological and physicochemical analysis in order to determine the optimal variant that could be used in bakery industry, with the aim of shelf life extending.

Good results in terms of an increased shelf-life were obtained for the breads produced using sourdoughs instead of control – with no sourdough. In all investigated options, the best results were obtained using variant A of *Lb. plantarum*. Lowest fungal contamination was observed for MAP packaged bread with 60% CO_2 and 80% CO_2, the shelf life being extending up to 11 days compared to 3 days for the control bread, with no significant differences between these two gas mixtures.

Taking into account the production costs, the MAP packaging using 60% CO_2:40% N_2 gas mixture can be considered more efficient for bakery industry.

P.6. **Production of β-limited dextrins by soybean α-amylase and their use in zymography**

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Apart from being a major constituent of foodstuffs, starch is very important as a raw material for the production of sweeteners, thickening and binding agents, and adhesives. Starch utilisation requires degradation to maltodextrins, maltose, and glucose which could be done by a palette of amylolytic enzymes of varying specificity. Appropriate combinations of these enzymes can lead to complete depolymerisation of starch to high-maltose or high-glucose syrups. β-Amylase is used for production of high maltose syrup from starch.

β-Amylase is the major starch-hydrolysing enzyme in soybean, which is an exo-amylase that hydrolyses the β -1,4-glucosidic linkage of a non-reducing ends of starch molecules, giving maltose units. Maltose has a wide range of applications in the food and pharmaceutical industries, since its properties are represented by mild sweetness, good thermal stability, low viscosity in solution, and lack of color formation.

In this work β-amylase from soybean was purified with purification yield of 35% while specific activity was increased 8-fold at the same time. Purified enzyme had molecular weight of around 50kDa, pI value between 5.2 and 6.2 and pH optimum of 5.0. Purified enzyme was employed for the production of maltose and β-limited dextrins from raw wheat starch. After 40 hours of starch hydrolysis in batch reactor at 37°C, hydrolysis was completed and maltose and dextrins were separated. Dextrins were used for preparation of native gels for zymogram detection of β -amylase. After optimization of this method α- and β -amylases from different sources were compared and distinguished. In addition, purification of α-amylases from triticale was successfully monitored.

P.7. **Potato waste as a source of polyphenol oxidase for removal of aqueous phenol and phenol derivatives**

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Phenols containing halogens, which tend to deactivate the aromatic nuclei, constitute a significant category of highly toxic and difficult-to-degrade pollutants from a wide variety of industries. Inherent toxicity of chlorophenols or of the intermediates produced during their degradation compromises the ability of microorganisms to completely mineralize the chlorophenols present in the wastewater. Therefore, biological treatment techniques, if used alone, have a serious limitation in treating non-biodegradable/toxic chemicals. Another alternative is to use enzymes, such as polyphenol oxidases and peroxidases which can act on specific recalcitrant pollutants by precipitation or transforming to other products and permitting a better final treatment of the waste. There is always a search for cheaper support and enzyme for preparing an immobilized enzyme preparation for such application. Polyphenol oxidase (PPO) from potato is exceptionally cheap enzyme because it can be purified from the food industry potato waste. It has been shown that PPO activity was the greatest at the tuber exterior, including the skin and cortex tissue 1 to 2 mm beneath the skin.

The main purpose of this work was to obtain an inexpensive immobilized enzyme for removal of phenols. Partially purified potato polyphenol oxidase (PPO) was immobilized onto different commercial and laboratory produced carriers. The three of the obtained biocatalysts, with the highest PPO activities, namely Eupergit C250L-PPO; Celite-PPO and CelluloseM-PPO, were tested in the batch reactor for phenol, 4-chlorophenol and 4-bromophenol removal. In the case of 2.5 mM substrates with Eupergit C250L-PPO, around 45% removal of 4-bromophenol was achieved, while 4-chlorophenol and phenol were removed 35% and 20%, respectively. The reusability of Eupergit C250L-PPO for the removal of 4-chlorophenol has been tested. After eight times of repeated tests the efficiency of 4-chlorophenol removal by Eupergit C250L-PPO immobilizate decreased to 55%.

P.8. **Development and simple preparation method for determination ochratoxin A in cereals and green coffee by ultra-high performance liquid chromatography/tandem mass spectrometry**

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Mycotoxin ochratoxin A (OTA) has been classified as a possible human carcinogen by the International Agency of Research on Cancer as Group 2 carcinogen. Cereals are the major contributor for ingestion of OTA but it is also found in a variety of food products ranging from coffee to nuts, wine, beer, dried fruits and spices. The EC regulations 1881/2006 and 576/2006 lay down limits for OTA presence in food and feed as follows: 5 μg/kg for cereals intended for food, 0.25 mg/kg for cereals intended for feed. The limit for green coffee has not been defined yet. In 2010, the EU Reference Laboratory for Mycotoxins (EU-RLM) at Institute of Reference Materials and Measurement (IRMM), (Geel, Belgium) has organized a proficiency test (PT) for the network of appointed National Reference Laboratories (NRLs) to determine OTA in food and feed test samples. The only Serbian laboratory involved in this PT scheme was the Laboratory for Mass Spectrometry of Centre of Excellence in Food Safety and Emerging Risks established at the Faculty of Technology, University of Novi Sad. This paper describes a simple preparation method developed in this laboratory for the determination of OTA in cereals and green coffee by ultra-high performance liquid chromatography with heated electrospray ionization triple quadrupole mass spectrometry (U-HPLC/HESI-MS/MS, ACCELA-TSQ VANTAGE, Thermo Fisher Scientific, US). The material used in this study was provided by the EU-RLM. The OTA was determined with the application of the positive ion mode in chromatographic run of 8 min each samples. The solvent mixture acetonitrile-water (84:16, v/v) has been used as the best solution for the extraction of the analyte from cereals and green coffee samples. Raw extracts were injected without any clean-up step. The method recovery, repeatability, linearity, limit of detection (LOD), and limit of quantification (LOQ) were determined. Calibration with matrix matched standard solutions were performed. Calibration curves for OTA in cereals and green coffee were linear within the working range from 8 μg/kg to 1600 μg/kg. Squared correlation coefficients (R^2) were in the range of 0.9990-0.9998 for 6 point calibration curves. The LODs (as signal to noise ratio S/N=3:1) for the established method were 2.5 μg/kg and 0.2 μg/kg for cereals and green coffee, respectively, while the corresponding LOQs (as S/N=10:1) were 8.3 μg/kg and 0.7 μg/kg. Recoveries of the analyte relative to the assigned values of the materials were 87% and 90% for cereals and green coffee, respectively.

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P.9. **Cereal-Based Products Rich In Prebiotics And Bioactive Compounds**

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Adequate nutrition is an important condition for quality of life, health and welfare. Cereals have an important role in the daily diet. The optimum intake of dietary fiber from food based on cereals can contribute to a balanced diet. The purpose of this research was to develop new cereal-based products rich in prebiotics and other bioactive compounds (omega-3, lutein and vitamins: B6, B12 and folic acid). The influence of different amounts of inulin (0-10 %) as a soluble fiber added in wheat flour was analyzed using farinographic measurements. Baked rolls were obtained from the mixes of wheat flour with inulin, adding lutein, omega-3 and a vitaminic complex in powder form. The rolls were tested concerning sensorial properties and physical-chemical indicators. The energetically values of the obtained products are around 230 kcal/100g end-product and they have an increased content in bioactive compounds.

P.10. **Supercritical CO_2 extraction as an alternative to organic solvents in the production of soybean oil**

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People nowadays generally expect the food that they buy to be of a high quality, minimally processed, „natural“, additive-free and high in nutritional value. The unique effects of pressure appear to be able to meet these requirements. Subjecting foods to pressures is now starting to be done by some of the world's leading food manufacturers. Organic solvents (mainly hexane) have been the preferred extraction solvents for soybean oil for a long time. Offlate, there is a growing interest in alternative processes that can minimize the environmental impact, decrease the toxic residues, more efficiently use the sub-products and also produce higher quality foods. Supercritical fluid technology is a viable alternative to current extraction methods. Supercritical CO_2 is an ideal solvent because it is nontoxic, nonexplosive, inflammable, cheap, readily available and easily removed from extracted products. The oil extracted from soybeans with supercritical CO_2 is of much higher quality than the hexane-extracted oil. It does not contain any phospholipids, thus eliminating the degumming step. The great advantage of the extraction of soybean oil with CO_2 compared to the conventional extraction is that the refinement stages are simplified significantly and the solvent distillation stage is completely removed (the two most costly steps in terms of energy consumption). In the terms of industry supercritical CO_2 extraction still hasn’t replaced hexane extraction. It may be not too long to wait before supercritical fluid extraction becomes routinely used for the production of soybean oil, and according to that fact it is very important to understand the effects of different process parameters on the yield of soybean oil as well as on the oil quality. The knowledge of these influences is not only useful for the optimization and economic evaluation of the process, but also for the ability to predict the extraction process, which is useful for scale-up as well as for the design and the optimization of an industrial plant.

In this work the scale-up between laboratory-scale high pressure extraction plant and pilot plant was successfully performed according to the mass transfer mechanism involved on the extraction and geometric proposal. Soybeans processed into oil, leaving a by-product with large amount of phenolic compounds known as isoflavones. The content of total and individual isofavones in soybean meal after supercritical fluid extraction was determined by high performance liquid chromatography. The total isoflavone content in soybean seed meal was 21.8 mg/g of extract. The most abundant isoflavone was genistin.

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P.11. **Influence of the microenvironment of thiol groups in low molecular mass thiols and protein on the reaction with methylglyoxal**

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The reaction between cysteine thiol group and α-oxoaldehydes (glyoxal, methylglyoxal (MG)) occurs during food production and also it takes place in some pathological states in which α-oxoaldehydes generated in high extent. The products of reactions between α-dicarbonyls, (byproducts of alcoholic and malolactic fermentations) and cysteine (Cys) have characteristic odors that significantly influence wine quality and character. On the other hand, reactive α-oxoaldehydes that are produced in higher quantities in diabetes, uremia, oxidative stress, aging and inflammation, react with the thiol groups of proteins. This causes protein modification, formation of advanced glycated end-products (AGEs) and cross-linking. Low molecular mass thiols can be used as competitive targets for MG, preventing the reactions mentioned above. Therefore, this paper investigated how the microenvironment of the thiol group in low molecular mass thiols (cysteine, N-acetylcysteine (NAcCys), carboxymethylcysteine (CMC) and glutathione (GSH)) and human serum albumin (HSA) affected the thiol reaction with MG. The SH group reaction course was monitored by ^{1}H-NMR spectroscopy and spectrophotometric quantification. Changes in the HSA molecules were monitored by SDS-PAGE. The microenvironment of the SH group had a major effect on its reactivity and on the product yield. The reactivity of SH groups decreased in the order Cys > GSH > NAcCys. CMC did not react. The percentages of the reacted SH groups in the equilibrium state were almost equal, regardless of the ratio of thiol compound/MG (1:1, 1:2, 1:5): 38.1 ± 0.9%; 38.2 ± 0.7% and 39.0 ± 0.8% for Cys; 26.5 ± 0.6%; 26.6 ± 2.6% and 27.4 ± 2.5% for GSH; 10.8 ± 0.9%; and 11.2 ± 0.7% and 12.2 ± 0.9% for NAcCys, respectively. Our results explain why substances containing α-amino-β-mercapto-ethane are successful scavengers of MG. In equilibrium, HSA SH reacted in high percentages both with an insufficient amount and with an excess of MG (55% and 65%, respectively). An analysis of the hydrophobicity of the microenvironment of the SH group on the HSA surface showed that it could contribute to high levels of SH modification, leading to an increase in the scavenging activity of the albumin thiol.

P.12. **Biogenic Amines in Fish**

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Fish is very perishable food item, submissive to intense post mortem changes if not manipulated in proper way. Biogenic amines are non-volatile organic bases of low molecular weight that can be synthesized by microbial, animal and plant metabolic processes. Low concentrations of these substances are naturally present in most seafoods but growth and activity of bacteria is required to produce the high concentrations of biogenic amines that can be responsible for food-borne intoxications or used as indices of fish spoilage. Basically, if the high protein foods, like fish, with a specific amino acid content, is exposed to conditions suitable for growth and development of microorganisms, it is expected that there will be formation of biogenic amines, which are joined by biogenic amines in fish already present. The final content of various amines depends on several factors including the nature of the product, storage conditions, especially temperature, and the presence of microorganisms. High concentrations of biogenic amines in fish indicates low quality and inadequate processing, therefore understanding the formation and the role of biogenic amines is needed to enhance the production steps and conservation processes in fish and seafood production. This is important because high concentrations of histamine, tramine or β-phenylethylamine can result in food-borne human intoxications. Despite the modernization of equipment and refrigeration systems for fish handling, histamine and other biogenic amines poisoning rate has not decreased during the last two decades. Furthermore, concentrations of biogenic amines, including cadaverine, histamine, putrescine, spermine, spermidine and tyramine, have been suggested as single or multiple compound quality indices for seafood or feed to fish. However, groups of bacteria produce markedly different profiles of biogenic amines. Analytical methods that determine a profile of biogenic amines therefore can help identify the type of bacteria responsible for their formation and in that way contribute to a better understanding and management of the problem.

P.13. **Healthy invert sugar in food industry**

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Equimolar mixture of fructose and glucose, known as invert sugar, was known in the beginning of XIX century. Invert sugar is sometimes referred to as artificial honey since its composition and properties are nearly same. Invert sugar is 30% sweeter than sucrose, so the amount of sugar required for a particular degree of sweetness is also reduced. Main consumers of invert sugar are the baking, beverages, canning, confectionery and dairy industries. Invert sugar is prepared by the hydrolysis of sucrose. The conventional method of manufacturing invert sugar involves acid hydrolysis of sucrose. The acid hydrolyzed product also contains impurities introduced by uncontrollable parameters during inversion. Enzymatic invert sugar is an absolutely health-friendly sweetener. The enzyme invertase is baker yeast's enzyme, which catalyses the hydrolysis of sucrose. The price of enzymatic produced invert sugar is important for industry use. Enzyme immobilization offers technical and economical advantages, such as cost-reduction of biocatalysts (as they can be reused many times), easy separation from reaction mixtures and the possibility of using higher enzyme activity per volume in the reactor, compared to soluble enzyme preparations. The price of invert sugar can be reduced by using of immobilized cell wall invertase. In this work is presented economic and health benefits of enzyme produced invert sugar immobilized cell wall invertase. Two types of reactors for production of invert sugar were used. Both of them were operated under approximately the same conditions, at 45°C and 70% (w/w) sucrose. Packed bed reactor shown better productivity. Obtained invert sugar is colorless, pH neutral, low conductivity and without unhealthy byproducts. Invert sugar produced by immobilized CWI is an easy storage product. It was stable more than 6 months storage at room temperature, without crystallization of product and also without microbial contamination. Obtained invert sugar was compared with commercial (acid hydrolyzed) samples from some Serbian food industry.

P.14. **Antioxidant activity of hydroxybenzyl alcohol (HBAs) glycosides**

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Autoxidation in food and biological systems is responsible for a multitude of adverse effects and implications in human health as well as in food stability and preservation. Antioxidants play a major role in preventing or delaying autoxidation and have attracted much attention as food stabilizers, dietary supplements and natural health products. Both synthetic and natural antioxidants are widely used in food products. Also, increasing attention has been paid to the antioxidants identical to the natural antioxidants found in foods, but synthesized in the industry. They are called nature-identical antioxidants. Contrary to natural antioxidants, they are pure substances, relatively cheap, easily available, and of reproducible properties, including antioxidant activity. Therefore, they combine advantages of the synthetic and the natural antioxidants. Phenolic glycosides, which widely exist in fruits, nuts, grains and vegetables are reported to have multiple biological effects, including antioxidant activity, antitumor, antimutagenic and antibacterial properties. In this work, glycosides of *o*, *m* and *p* hydroxybenzyl alcohols (HBAs) were synthesized via environmentally benign and energy economic process, using α-glucosidase from bakers yeast as a catalyst. Antioxidant activity of glycosides and corresponding alcohols was tested using DPPH scavenging test and assay of lipid peroxidation. It was shown that glucosides of HBAs exhibit antioxidant activity as it was expected due to presence of phenolic hydroxyl group. Among 3 isomers of HBAs glucosides, 2-HBA glucoside and 4-HBA glucoside are found to have better radical scavenging and antioxidant properties than 3-HBA glucoside. However HBAs are more efficient antioxidants than corresponding glucosides, which is probably result of free primary hydroxyl group presence, another structural feature that may increase antioxidant capacity. Primary hydroxyl group is glucosylated in a case of HBAs glucosides and cannot contribute in radical scavenging process as in a case of HBAs. However, slow hydrolysis of the glucosides in food, possibly enhanced by a suitable enzyme added to the application, could results in a delayed release of HBAs, which are promising antioxidants.

P.15. **Development of the Sheep Cheese with Addition of Probiotic Bacteria**

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Functional food is a food which enhances physiological performance or prevents /treats diseases and disorders, beyond its nutritional properties. Nutriceuticals are integrants of functional food and can be classified by its origin, action or chemical nature. Probiotics are living microorganisms that can improve the microbiologic intestinal balance of the host. They are added to foods in order to include them in human diet. Some of their benefits are normalisation of intestinal microflora and lactose hydrolysis. To categorize the food as a functional, probiotic bacteria must be viable not only during the food production and commercialization, but also during the passage of the food through the digestive apparatus. The beneficial health effects are registered at about 10^7 CFU/g or ml of probiotic bacteria count at the moment of food consumption. Dairy foods are commonly selected as probiotic bacteria carriers because they have an environment adapted for lactic acid bacteria. Among other dairy products, cheese has advantage of having high pH and higher fat content. Also, it's more closed texture may protect bacteria more efficiently than a fluid environment. References about sheep cheeses with probiotic bacteria are very scare. Taking this into account, development of a sheep cheese with addition of probiotic bacteria seemed as a logical choice.

The aim of this study was to incorporate probiotic bacteria into sheep cheeses produced with a technology developed in institute for researching in dairy products. During the period of ripening (45 days), physicochemical and microbiological determinations were made. Cheeses without probiotic bacteria were used as a control. Probiotic bacteria counts were made in order to verify in the population and were inside the limits established for functional food. The cheeses with good sensory characteristics were obtained. Cheeses at the end of ripening have satisfy all the criteria to obtain the name of the probiotic.

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P.16. **Complex compounds of *3d* ions (Fe(III), Cu(II), Ni(II), Co(II)) with 2-deoxy-D-glucose**

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Saccharides are widespread in nature and their role in biological activities is recognized. Dietary supplementation with 2-deoxy-D-glucose improves cardiovascular and neuroendocrine stress adaptation in rats and glucose metabolism by decreased concentrations of blood glucose and insulin under non-stress conditions [1]. Treatment with 2-deoxy-D-glucose mimics the beneficial effect of dietary restriction *in vivo* and protects cultured dopaminergic cells against oxidative, preserved mitochondrial function and metabolic insults relevant to the pathogenesis of Parkinson's disease [2]. The interactions between metal ions that are essential or found in human body with saccharides represent an area o interest for various potential applications such as: biotechnology and pharmacology.

This paper present the synthesis of four complex compounds of some *3 d* ions (Fe(III), Cu(II), Ni(II), Co(II)) with 2-deoxy-D-glucose (L). These compounds were characterized on the basis of elemental chemical analysis, electronic and infrared spectra and formulated as binuclear species with the following formulas: $Na[M_2(L)_2Cl_3(H_2O)_3]$, where M is Cu(II), Ni(II), Co(II) and $Na_2[Fe_2(L)_2\,(OH)_2(H_2O)_2\,Cl_2]$. The aqueous solution stability was investigated by UV-Vis spectroscopy. The electrochemical properties of the complexes were studied using a type of potentiodynamic electrochemical measurement, cyclic voltametry, under various conditions (pH, complex concentration, scan rate). The biological activities of the as-synthesized complexes were studied on *Pseudomonas Aeroginosa*, *Aspergillus Niger*, *Fusarium Oxisporum* and *Candida Scotti*.

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P.17. **Analytical analysis of traditional foods: Filling the gap in Serbian FCDB information**

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Background/aim: Adding new traditional food analitical values is an ongoing requirement for development and updating of food composition database (FCDB). The list of commonly consumed Serbian traditional foods was identified: gibanica (filo pastry with cheese fill), prebranac (first cooked, than baked beans), ajvar (cooked pepper and aubergine spread), fresh cheese, kajmak (creamy dairy product), and vanilice (cookies) .The aims of our study were; 1) to obtain analytical values of representative traditional foods following EuroFIR criteria, 2) to fulfill the gap in traditional food composition knowledge and 3) to get high quality analytical data for inclusion in Serbian FCDB.
Methods: Fresh cheese, kajmak and ajvar were bought in three different stores and 100g of each were pooled and 200g was taken for further analysis. Gibanica, vanilice and prebranac were homemade using the most common receipt.
Nutritional analysis was carried out by two accredited laboratories and included determination of water, ash, protein, fat, vitamin A, vitamin E and minerals (zinc, copper, manganese, iron). The samples were collected, prepared and distributed to the laboratories according to instructions given by EuroFIR Traditional food work package.
Results: Analytical determination showed the following nutritional features:
Moisture /%/100g: 70.96 (fresh cheese), 35.82 (kajmak), 79.74 (ajvar), 51.39 (gibanica), 67.24 (prebranac), 13.29 (vanilice).
Ash /%/100g: 3.54 (fresh cheese), 1.81(kajmak), 2.48 (ajvar), 2.25 (gibanica), 1.86 (prebranac), 0.51 (vanilice). Proteins /%/100g: 16.20 (fresh cheese), 3.35 (kajmak), 1.85 (ajvar), 11.28 (gibanica), 5.77 (prebranac), 7.16 (vanilice). Fat /%/100g: 2.86 (fresh cheese), 60.53 (kajmak), 2.80 (ajvar), 10.77 (gibanica), 1.70 (prebranac), 25.05 (vanilice).
Vitamin A /µg/100g: 54.0 (vanilice), 120.0 (kajmak), 70.0 (fresh cheese).
Vitamin E /µg/100g: 230.0 (fresh cheese).
Minerales /mg/kg: Zn-12.32, Cu-0.28, Mn-0.20, Fe-1.16 (fresh cheese), Zn-2.55, Cu-0.19, Mn-0.06, Fe-0.38, (kajmak), Zn-3.44, Cu-0.96, Mn-1.42, Fe-5.40 (ajvar), Zn-8.56, Cu-0.65, Mn-1.53, Fe-8.46 (gibanica), Zn-7.76, Cu-1.81, Mn-3.60, Fe-14.16 (prebranac), Zn-4.46, Cu-0.69, Mn-3.54, Fe-8.52 (vanilice).
Conclusion: Six traditional foods were selected for analyses on account of their importance and frequent consumption in Serbian cuisine. By using standardised procedures for sample collections, preparation and conducting analyses in accredited laboratories high quality data were produced and included in national FCDB.

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P.18. **Evaluation Of Wheat Flour Quality**

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Bread and bakery products are basic products for human alimentation. As the main ingredient in these products production, the wheat flour should fulfill quality requirements before entering any panification production line [1, 2]. Besides physical parameters, such as humidity, falling index, deformation index, and wet gluten, generally used to assess the bakery value, the heavy metals content should be also known for food safety reasons. Lead and cadmium were analyzed by atomic absorption spectroscopy with graphite furnace atomization [3] and ion chromatography with post−column derivatization [4] and results were compared in terms of sample preparation, linearity, and associated uncertainty. Providing comparable results, ion chromatography based on *UV-VIS* detection may become an alternative method for heavy metal quantification in several solid and liquid food matrices.

Three types of flour were evaluated in the current study and the physical parameters signaled good panification values. Humidity varied between 14 and 15.6 %, all falling index values were above 250 s, while the wet gluten exceeded 23 % in all cases. The heavy metals contents of maximum 8.7 ppb Cd^{2+} and 0.2 ppm Pb^{2+} were recorded; all bellow the officially admitted concentrations, so the samples evaluated are considered safe from the security point of view.

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P.19. **Losses of bioactive polyacetylenes during minimal processing of carrots**

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Vegetables of the *Apiaceae* plant family such as carrots, parsnip, celery and parsley, contain in minor quantities, a group of bioactive aliphatic C17-polyacetylenes (falcarinol, falcarindiol, falcarindiol-3- acetate). Recent studies have highlighted important biological functions in vitro and in vivo (animal studies) although the beneficial effect in human nutrition attributable to an increased in polyacetylenes diet are yet to be confirmed. Carrots not only contain relatively high polyacetylene content but also form a significant part of many countries dietary habits. Carrots are also present in the composition of some ready-to-eat foods such as chilled freshly prepared salads, as part of the increasingly popular minimally processed foods. Whereas the effect of conventional processing (boiling, vacuum processing) on the levels of polyacetylenes has been studied, the effect of minimal mechanical operations such as “peeling”, “mechanical cutting” and “chlorinate washing” are unknown. For this work, fresh carrots (cv. *Nantes*) were processed in a fresh produce processing plant (Wonderfoods Ltd, Dublin, Ireland) using industrial equipment.

The results showed that the initial total polyacetylene levels of unprocessed carrots (500 mg/kg dry carrot) were significantly decreased the end of the minimal pro\cessing. This decrease was more evident when carrots were cut in disks (5mm thickness), cubes (0.5x0.5x0.5cm) on batons (4x0.5x0.5cm) and less where shredded. There was no significant difference in losses for all three polyaceytelenes although falcarinol, the most bioactive of the polyacetylene family, was retained better than the rest. Storage studies on the fate of polyacetylenes were limited to 7d which is a maximum expected shelf life on a minimally processed product. During this period there was a slight decrease in falcarinol content especially in carrots cut in disks, cubes and batons. However this trend was not significant. For the other two polyacetylenes, results showed little differences between day 1 and day 7. Overall, the levels of polyacetylenes are fairly stable during chill storage.

In conclusion, this work showed that polyacetylenes in minimally processed carrots are affected particularly during the initial mechanical operations (mainly during peeling as carrot skin is rich in polyacetylenes) and remain rather stable during short term chill storage. Even if these operations are essential, there is scope for optimisation of conditions during peeling (the depth of peeling, the time in contact with chlorinated water during washing or size of carrot cut) to minimise the losses of polyacetylenes. These optimisations - that could come in the form of recommendations for processors - should however take into account the microbiological safety, the organoleptic quality as well as the equal retention of other bioactives present in carrots such as vitamins and minerals.

P.20. **Proteomics analysis and protein structure identification of *Escherichia coli***

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In order to separate *Escherichia coli* proteins we have developed a two-dimensional electrophoresis system. For the iso-electric focusing, immobilised pH gradient, dehydrated polyacrylamide gels cast on solid films was used as a supporting matrix. Protein complexes are an intrinsic aspect of life in the membrane. Knowing which proteins are assembled in these complexes is therefore essential to understanding protein function(s). Unfortunately, recent high throughput protein interaction studies have failed to deliver any significant information on proteins embedded in the membrane, and many membrane protein complexes are poor defined. In this study, we have optimized the SDS-PAGE technique for the study of protein complexes of Escherichia coli correlated with 2D-PAGE. In combination with second dimension SDS-PAGE and further on mass spectrometry, we should be able to identify distinct protein complexes. Our reference two-dimensional SDS-polyacrylamide gels will facilitate future studies of the assembly and composition of *E. coli* membrane protein complexes during different growth conditions and in different mutant backgrounds.

Keywords: *E. coli*, SDS-PAGE, membrane proteins, 2D-PAGE

P.21. **Antioxidant activities of essential oils and ethanol extracts from myrtle (*Myrtus communis* L.) leaves, berries and floral buds**

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This study examined the chemical composition and antioxidant activity of essential oils and ethanol extracts of *Myrtus communis* L. leaves, berries and floral buds.

Berries and floral buds essential oils were characterized by high proportions of monoterpenes hydrocarbons due to α-pinene with 34.3% for berries and 48.9% for floral buds. Leaves essential oil was rich in oxygenated monoterpenes with a large contribution of 1,8-cineole (61.0%). Significant differences were found in the amount of total phenols, flavonoids and condensed tannins among the studied organs. Myrtle berries were the richest part on total phenols while floral buds were the richest in flavonoids.

Antioxidant activities of the essential oil and the ethanolic extract from different myrtle parts were assessed by means of β-carotene-linoleic acid bleaching method and DPPH radical scavenging test. Results showed that ethanol extracts of all parts of *Myrtus communis* L. exhibited higher antioxidant activity than essential oils. Floral buds showed the highest activity of the tested parts.

Keywords: *Myrtus communis* L., organs, essential oil, extract, polyphenols, antioxidant activity.

P.22. **Production of amylases by *Aspergillus niger* in solid state fermentation**

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Amylases are used in food industry for starch saccharification to obtained dextrins syrups with dextrose equivalent (DE) from 3 to 30. Cultivating *Aspergillus niger* (ATCC 10864) on natural substrate using solid state fermentation (SSF), amylase complex (AC) which consist of α-amylases and glucoamylases were obtained. Using AC for starch saccharification will result in obtaining dextrin syrup with wide range of DE in one step. For AC production the granulated grain of triticale (*Triticosecale*) was used as substrate in SSF. Optimization of SSF to obtained AC with maximum number of amylase isoforms is shown in this work.

Production of amylases during fermentation was examined by spectrophotometric assays and by zymography detection. Several isoforms were detected. Influence of quantity of triticale grain on amylase production in SSF was examined. Three different quantities (16 g, 32 g and 48 g) were used. Using of 48 g triticale grain for *A. niger* SSF was produced superlatively amylase. Fermentation was carried out for 5 days.

Amylase isoforms were analyzed using zymogram technique for simultaneous detection of α-amylase and glucoamylase. During first 36 h of fermentation the production of α-amylase was emphasized, while production of glucoamylase was intensive after 36 h to 120 h. Ratios of glucoamylase and α-amylase during SSF were different when different quantities of triticale grain was used. Glucoamylase and α-amylase were produced in the same ratio after 96 h of fermentation. Amylase extract obtained from *A. niger* using 48 g of triticale grain on SFF, after 96 h can be used for starch saccharification to get useful dextrin syrup with DE range below 30.

P.23. **SDS-PAGE analysis of whey proteins from different heat-treated commercial bovine milk**

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This aim of this study was to investigate the impact of heat treatment on whey proteins of bovine milk. Different heat-treated (pasteurized and UHT) commercial available milk samples, by several producers were purchased from Cluj-Napoca local markets. SDS-PAGE (sodium dodecyl sulphate – polyacrylamide gel electrophoresis) and densitometric analysis was used as preliminary study to compare whey proteins from raw milk (control) and heat-treated milks. In comparison with raw milk, whey proteins from commercial milk samples have undergone major changes due to different heat-treatments used. Their level decreased with the temperature of heat-treatment.

Keywords: bovine milk, heat-treatment, SDS-PAGE, whey proteins

P.24. **MPO mediated oxidation of dizinon as a pre-step in determination of organophosphates in food samples by AChE based bioanalytical method**

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The aim of our work was to find a rapid, effective and specific method for oxidation of diazinon, as the model compound for organophosphates (OPs), in order to enhance the sensitivity of AChE based bioanalytical method for OPs detection in food samples. It was accomplished by converting diazinon from its thio form into the oxo form, wich is a more potent AChE inhibitor. Diazinon was oxidized into diazoxon using enzyme myeloperoxidase (MPO). The comparison of degree of AChE inhibition by diazinon at $1x10^{-5}$M - $1x10^{-7}$ M before and after the 10 min oxidation by 100 nM MPO was made. A calibration curve for diazinon was constructed. While the lower detection limit of diazinon using native and immobilized enzyme (10% AChE inhibition) before oxidation was ca. $1x10^{-5}$ M, the detection limit after oxidation was below $1x10^{-7}$ M. As conclusion, MPO mediated oxidation of OPs may be regarded as an excellent method for improving sensitivity of bioanalytical method for determination of diazinon and other OPs.

P.25. **The Influence of Extraction Method on Protein Profile of Soybeans**

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Dominant fraction of soybean proteins represents storage proteins, namely glycinin (11S protein) and conglycinin (7S protein). Extraction conditions influence not only the amount of extracted protein but also its form since it is known that different pH values of extraction media induce various changes such as: reversible dissociation of trimeric β conglycinin into subunits, transition of hexmeric glycinin into trimeric form and re-association of dissociated products forming complexes containing different combinations of glycin and conglycinin subunits.

The aim of this work was to find the most suitable method for protein extraction from both young tissues of developing soybean plant as well as from mature bean. The main difference between mature seed and young tissue is high oil content in seed. Traces of lipids are co-extracted thus interfering resolution of proteins during 2D electrophoresis.

For that purpose, we compared several soybean protein extraction methods which are conducted prior to 2D electrophoresis analysis (phenol extraction, extraction with urea solubilization buffer, acetone extraction and direct extraction in IEF buffer) with commonly used methods for extraction of soybean protein (Tris-buffer or Tris-urea extraction).

Although phenol extraction (pH 8) is usually used for young tissue it is also applicable when working with seeds since it gives high number of intense spots on the gel. However, this method of extraction is characterized with huge vertical stricking (especially in pH region 4-6) probably due to aggregation of dissociated subunits of glycinin and conglycinin into high molecular aggregates.

Extraction with urea solubilization buffer (pH 8.8) produces less number of much intense spots with less vertical stricking. Probable explanation for this is reduced solubility of proteins in this buffer where urea masks their charges and suppresses hydrophobic interactions hence inhibiting complexes formation. Acetone method of extraction gives worst results due to poor solubility of produced pellet in the appropriate IEF buffer. Direct extraction in IEF buffer (pH 8.8) gives similar results as urea solubillization buffer methods with the difference that direct IEF buffer solubilization gives fewer spots as a consequence of lower urea concentration and thus poorer separation of subunits.

The advantage of Tris-buffer (pH 8) extraction method is that it enables a good separation of basic subunits and allows examination of less abundant proteins (e.g. 2S fraction) but it gives a smaller number of well defined spots. Possible explanation for these results could be that although solubility of proteins in this buffer is the highest there are a lot of non-protein compounds that interfere with isoelectric focusing. Tris-urea (pH 8) method of extraction gives better results than Tris-buffer extraction method in that sense that gives higher number of less intense spots.

On the basis of current results, extraction methods allowing best separation of soybean proteins is Tris-urea extraction modified by using longer extraction duration and higher ionic strength of the buffer.

P.26. **Digestibility of β-lactoglobulin following cross-linking by *Trametes versicolor* laccase and apple polyphenols**

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β-lactoglobulin (BLG) is an important nutrient of the dairy products and an important allergen in cow's milk allergy. The aim of this study was to investigate a potential of laccase to cross-link the BLG in the presence of an apple phenolic extract (APE) and to characterize the obtained products for their digestibility by pepsin and pancreatin. The composition of apple phenolycs used for cross-linking was determined by LC-ESE-MS. Apple phenolic extract contains significant amounts of quercetin glycosides, catechins and chlorogenic acid. The laccase cross-linked BLG in the presence of apple phenolics. The polymerization renders a protein insoluble in the reaction mixture. SDS PAGE analysis of cross-linking reaction mixture revealed a heterogeneous mixture of high molecular masses (cross-linked BLG), with a portion of remained monomeric BLG. Enzymatic processing of BLG by laccase and apple polyphenols as mediators can decrease bi-phasal pepsin-pancreatin digestibility of monomeric and cross-linked protein, thus decreasing its nutritional value. Also, reduced BLG digestibility can decrease its allergenic potential. Apple polyphenols can find usage in creation of new, more functional food products, designed to prevent obesity and hypersensitivity related disorders.

P.28. **Study of the traditional fruit wine using ^{1}H-NMR spectroscopy**

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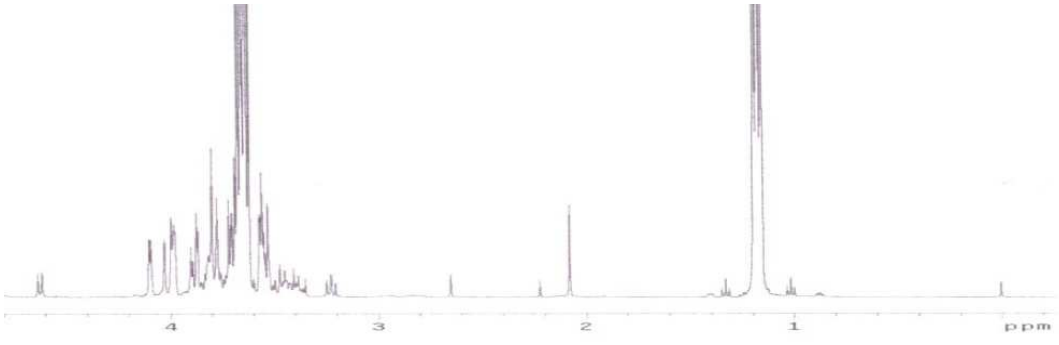
Wines obtained from fruit are a known initiator of the body's well-being, due to the high content of biologically active compounds. The main parameters that influence these wines quality are: alcoholic concentration, total mass of solid compounds, total acidity and total content of polyphenols.[i, ii]

NMR spectroscopy is a very useful tool for quantitative and qualitative determinations.[iii,iv]



Section ^{1}H-NMR spectrum of a blueberry wine

In this paper were analyzed several wines obtained through fermenting of the following fruits: strawberries, blueberries, raspberries and blackberries.

The samples were analyzed using NMR spectroscopy; the total polyphenols were determined using the Folin-Ciocaltau method.

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