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FoodEnTwin
-omics technologies bridging food and environmental sciences

Универзитет у Београду - Хемијски факултет

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Content

Session 1: Omics and the environment

Invited lecture

Highly improved method for in-depth post-translational modification profiling: example of Timothy grass (Phleum pratense) pollen proteomes from polluted and preserved environments

Katarina Smiljanić[1], Ivana Prodić[2], Danijela Apostolović[3], Jelena Mutić[1,4], Marianne van Hage[3], Lidija Burazer[5], and Tanja Ćirković Veličković[1,4,6,7]

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Field-realistic exposure studies provide the most relevant assessment of the effects of the intensity and diversity of urban and industrial contamination on pollen structure and allergenicity. The significance of in-depth post-translational modification (PTM) studies of pollen proteomes, when compared with studies on other aspects of pollution and altered pollen allergenicity, has not yet been determined; hence, little progress has been made within this field.

Therefore, we created a comprehensive approach for the comparison of pollen from polluted and environmentally preserved areas. To examine the effects of long-term, in vivo pollen exposure to multiple source pollutants, *Phleum pratense* (Timothy grass) pollen samples were collected along a regional road in Kruševac, central Serbia. This road experienced moderate traffic and was located near a chemical plant that produces fertilizers. Pollen samples from this location were compared with pollen samples collected from a rural, environmentally preserved area over two consecutive pollination seasons. We combined the quantitative comparison of proteome expression profiles from in-solution and 2D gels with unrestrictive in-depth quantitative PTM profiling using high resolution tandem mass spectrometry and the PEAKS 8.5 Suite platform. This was followed by quantitative IgE enzyme-linked immunosorbent assays (ELISA) and one dimensional (1D) IgE immunoblots that were probed with the sera of grass pollen allergic patients and healthy control subjects from Serbia. In addition, elemental compositional analyses of Timothy grass pollen samples from both locations, and the surface grain structure and SPP releasing potential (including total protein and phenolic content), were assessed.

An increased phenolic content and release of sub-pollen particles was found in pollen samples from the polluted area, including a significantly higher content of mercury, cadmium, and manganese. Antioxidative defense-related enzymes were significantly upregulated and seven oxidative PTMs were significantly increased (methionine, histidine, lysine, and proline oxidation; tyrosine glycosylation, lysine 4-hydroxy-2-nonenal adduct, and lysine carbamylation) in pollen exposed to the chemical plant and road traffic pollution sources. Oxidative modifications affected several Timothy pollen allergens; Phl p 6, in particular, exhibited several different oxidative modifications. The expression of Phl p 6, 12, and 13 allergens were downregulated in polluted pollen, and IgE binding to pollen extract was substantially lower in the 18 patients studied, as measured by quantitative ELISA.

Quantitative, unrestricted, and detailed PTM searches using an enrichment-free approach was used for the first time to map extensive modifications in the pollen allergome, which was shown to reflect the increased environmental oxidative stress, primarily caused by increased content of heavy metals in pollen. With some modifications, this PTM profiling approach is suitable for exploring the oxidative stress effects in any proteomic source in a quantitative in-depth manner, thus enabling further data-driven research.

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Session 1: Omics and the environment

Invited lecture

Palynology - understanding the sources of ‘omics in the environment

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Any disease agent able to survive aerosolization and air transport is considered a potential cause of airborne disease. Aerobiology is the study of airborne organic particles passively transported in the atmosphere - their identity, behavior, movement, and survival. Although just a fraction of spectrum of bioaerosols suspended in the atmosphere, pollen is a good example of how biological molecules can affect health.

We are addressing here the origin, diversity and variability in quantity of airborne pollen and related allergens. The high diversity in airborne biota is challenging with respect to identification of taxa. Classical biological approaches, i.e., culture and microscopy, are not able to give complete information about spectra of airborne particles, which is particularly wide for fungi and bacteria. There is a lot of potential with for applying metagenomics and next-generation sequencing (NGS) in aerobiology.

By describing determinants of airborne pollen exposure in time and space we would like to emphasize importance of understanding atmospheric behavior of bioaerosols (including their genome, proteome and metabolome) when assessing the course and intensity of disease they are responsible for.

Session 2: Food and nutrition proteomics

Invited lecture

In vitro models of food digestion for risk assessment

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Food related non-communicable diseases are increasing in prevalence and understanding their associated risk factors is of increasing importance. The digestion of food is a complicated process and so it can often be useful to simulate various aspects of the process rather than undertake a human study. In recent years, the InfoGest network has driven the move towards the use of more physiologically relevant and consistent models of digestion. This talk will discuss the development of simple methods for a three phase static [1] model that can give a good indication of digestion end-points and a semi-dynamic model [2] that can be used to provide kinetic information. The static model consists of oral, gastric and intestinal phases there the conditions are fixed at the start of the phase and maintained throughout. The semi-dynamic simulation includes a gastric phase includes secretion of acid and enzymes as well as emptying into the intestinal phase.

I will also discuss some of the positive and negative aspects of using different static models for protein digestion in relation to allergy risk assessment. The current recommendation from EFSA is for the use of e pepsin resistance test, which is representative of the late gastric phase of digestion but may not be applicable to allergens that pose a risk for infants.

1. Minekus, M., et al., *A standardised static in vitro digestion method suitable for food - an international consensus*. Food & Function, 2014. **5**(6): p. 1113-1124.
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Session 2: Food and nutrition proteomics

Invited lecture

Omics of *Ixodes ricinus* ticks - involvement in mammalian meat allergy

Danijela Apostolović, Division of Allergy and Immunology, Department of Medicine Solna, Karolinska Institutet and University Hospital

During the last decade a red meat allergy was discovered, where patients have severe allergic reactions occurring several hours after red meat intake. The disease is caused by IgE antibodies directed against the carbohydrate epitope galactose-α-1,3-galactose (α-Gal) found in mammalian meat. Furthermore, a strong association between production of α-Gal specific IgE antibodies and tick bites have been highlighted. By using *omics* technologies together with red meat allergic patient material, we aimed to deeply investigate the link between *I. ricinus* ticks and red meat allergy.

IgE-binding activity of proteins from adult and larvae ticks as well as tick saliva was determined by western-blot and/or inhibition ELISA. Basophil activation test was used for assessment of allergenic activity. A comparative proteomics approach: 2D PAGE – western blot linked with mass spectrometry was used for identification of the α-Gal carrying proteins. Finally, allergen-specific T-cell responses from patients and healthy controls were scrutinized by flow cytometry and FluoroSpot assay.

Red meat allergic patients had IgE activity against *I. ricinus* proteins. IgE binding α-Gal-carrying proteins were recognized in tick saliva. All tested patients showed a higher allergenic activity to adult *I. ricinus* than to larvae. A comparative proteomics approach revealed vitellogenin and α-2-macroglobulin as IgE-binding α-Gal carriers. Tick proteins had a proliferative effect on red meat allergic patients' CD4+ T-cells compared to a response in healthy individuals. FluoroSpot analysis revealed dominant Th2 cytokine response in allergic group.

In conclusion, red meat allergic patients have, in addition to an anti-α-Gal IgE response, specific T_H2 derived cellular and humoral responses against *I. ricinus* tick proteins. *Omics* technologies revealed vitellogenins and α-2-macroglobulin as IgE binding α-Gal carrying proteins. The results support the strong relationship with tick bites for the development of mammalian meat allergy.

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Session 2: Food and nutrition proteomics

Invited lecture

Food and Nutrition Proteomics

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The international action Food and Nutrition proteomics were born in 2016 first under the behalf of the Human Proteome Organization and in 2018 was approved as an official initiative of the European Proteomics Association.

This action aims to bring together scientists involved in the field of food proteomics: from the study of food to food allergies and allergens to microbiology; microbiota applied for the improvement of food safety food quality, risk assessment, shelf life.

We eat three times a day and therefore, the prevention of non-communicable diseases is also achieved through food.

Proteomics plays a central role in the concept of one health where human is at the heart of the interactions with food, animals and the environment. The need, therefore, to have a network of food and nutrition proteomics in this area is essential to improve knowledge and consequently, to help the public health sector and to increase resilience. The proteome analysis is expected to play an essential role in solving major nutrition-associated problems in humans and animals, such as obesity, diabetes, cardiovascular disease, cancer, ageing, and intrauterine fetal retardation. The primary aim is to increase community and possibility to apply in different fields of research projects, especially for ESR; dissemination of proteomics in food and nutrition environments, creation of synergies with food industries, and also with regulatory agencies to set up new test based on proteomics.

Session 3: Novel in vitro and animal models for predicting the effects of environmental stress

Invited lecture

Animal models of allergic disease

Michelle Epstein, Medical University of Vienna, Vienna, Austria

There are many animal models, especially in rat and mouse that mimic allergic disease. Many models are available for allergic rhinitis, allergic asthma allergic conjunctivitis, atopic dermatitis and anaphylaxis models. Model development by experts in allergy, respiratory/pulmonary, dermatology, ENT and ophthalmology use approaches for further understanding underlying disease mechanisms and treatment modalities. This presentation will address basic aspects of experimental animal models predominantly for food allergy with knowledge on some of the key *in vivo* and *ex vivo* parameters measured, ethics for using experimental animals, and 3Rs.

Session 3: Novel in vitro and animal models for predicting the effects of environmental stress

Invited lecture

The search for protective antigens and selecting appropriate animal models: a curious case of *Chlamydia trachomatis*

Aleksandra Inić-Kanada, Institute of Specific Prophylaxis and Tropical Medicine; Center for Pathophysiology, Infectiology and Immunology; Medical University of Vienna

Infection with *Chlamydia trachomatis* (Ct) can be asymptomatic and result in trachoma and pelvic inflammatory disease, which can lead to infertility and ectopic pregnancy. Trachoma is a neglected tropical disease and the leading infectious cause of blindness. In 2016 the Global Trachoma Mapping Project was completed examining 2.6 million people in 29 countries and showing that 100 million people are at risk of blindness from trachoma. Since vaccination is expected to be the most cost-effective means of control, it appears as the solution of choice for controlling the sequelae of the ocular infection in low-income countries. Although intensive efforts to develop a trachoma vaccine including human trials, dating back to the 1960s, no vaccines for the disease are currently available mostly due to the Ct pathogen complexity and the lack of affordable animal models.

The quest for antigens has been revolutionized by the immunoproteomics, which has been used to identify immunoreactive proteins from many microorganisms. We have searched for immunogenic proteins of Ct ocular serovar B (CtB) in order to identify possibly novel intervention targets. Primary screening of antigen candidates was based on seroreactivity of serum samples from individuals with trachoma and matched controls from endemic countries against CtB proteome. Identification of major Ct antigens relevant in severe trachoma was performed by quantitative immunoproteomics of proteins solubilized from CtB elementary bodies. Prospective antigenic candidates of CtB were identified by high resolution, high accuracy nano-LC-ESI/Orbitrap tandem mass spectrometry. The relevance of the antigens was addressed in available animal models.

Although mice, guinea pigs, mini-pigs and non-human primates (NHP) are the common animal models for genital chlamydial vaccine research, mice are not suitable to assess the protective potential of a trachoma vaccine as they fail to develop ocular disease with Chlamydia. NHPs and guinea pigs develop ocular disease when infected with

Chlamydiae but bear many limitations. NHPs have substantial outbred genetic variation. Besides, the main reservation lies in NHPs procurement, coupled with ethical considerations and concomitantly higher costs associated with these experiments. Rodents show several differences to humans in terms of size, anatomy, physiology and immunology that do not always allow them to mimic the human course of infection and immune response. Pigs are susceptible to genital infection with Ct, and we investigated whether pigs are suitable models for the study of Chlamydia ocular pathogenesis and evaluation of the protective potential of vaccine candidates.

Session 4: Food lipidomics

Invited lecture

Lipid binding of allergens and their allergenic activity

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The main factors making a protein an allergen are still to be defined. However, it is evident that allergens usually are embedded in a mix of proteins, carbohydrates, small molecules and lipids. In the recent past increasing evidence has been provided on the interaction of lipids and the innate immune system contributing to the onset of an allergic immune response in predisposed individuals.

Lipids are sensed by dendritic cells (DCs) via toll like receptors, such as TLR2. As a consequence activated DCs will present lipids via CD1d molecules to other immune cells such as invariant natural killer T-lymphocytes (iNKTs). It has been shown that a number of allergens carry pollen- or microbial derived lipids and activate via DCs Th1, Th2 and Th17 mediated allergic inflammation.

Non-specific lipid transfer proteins (nsLTPs) are important food allergens and they are able to bind a range of different lipid molecules in their hydrophobic cavity. Recently, we investigated whether individual allergenic nsLTPs are able to interact with lipids in a similar way and if this ligand binding has an impact on the local protein structure and thus on the interaction with specific IgE antibodies. For Pru p 3, the major peach allergen, we could show that binding to certain lipids a local conformational change is detectable which facilitates the exposure of a dominant IgE epitope as compared to Pru p 3 without ligand. Binding to unsaturated fatty acids (using oleic acid as a representative) induces this conformational change as shown by IgE binding tests and basophil activation assays, while this effect is not detected when binding to saturated fatty acids (using stearic acid as a representative). In a subsequent study we focused on different nsLTPs: Mal d 3 from apple, Cor a 8 from hazelnut, and Hel a 3 from sunflower seeds. The main finding were the following: homologous allergens displayed different IgE binding capacity ranging from high to low allergenic activity; only oleic acid as a ligand could induce relevant structural changes and impact on IgE epitope as shown for Mal d 3 and Cor a 8, while Hel a 3 proved as a low allergenic protein. Further studies are needed to assess whether lipid binding will provide increased protein stability during digestion.

Session 4: Food lipidomics

Invited lecture

Lipid analysis to support research on the impact of environmental factors on the food lipidome

Bruno De Meulenaer, Ghent University, Department of Food Technology, Safety and Health, nutriFOODchem Group

Lipids consist of a diverse group of molecules which are, unlike other biomolecules, not characterized by a certain individual structure. They can be considered as hydrophobic or amphipathic small molecules originating from ketoacyl or isoprene building blocks. This results into a group of eight different categories of biomolecules, without considering the synthetic lipids such as particular emulsifiers. Moreover, lipids in foods are prone to a variety of chemical reactions of which oxidation can be considered as the most important. As result, analysing the lipidome of foods is a challenging task requiring a multitude of approaches.

On basis of a limited literature survey it can be concluded that the environment in which foods are produced impact the food lipidome. Using a variety of analytical approaches it was shown that feed composition of cows result in the presence of particular fatty acids in milk lipids; that heavy metals in soils have an impact on the fat content of legumes and their lipid composition; that insecticides impact the lipidome of beans.

Therefore an overview of the analytical approaches to characterise the food lipidome will be presented primarily using examples from our own research in the area of food lipids.

A first crucial step in the analysis of lipids consists of their quantitative extraction from the food matrix. This extraction can then be followed by a fractionation of different lipid classes. Various chromatographic approaches can be used such as thin layer chromatography, column chromatography and silver ion chromatography, typically all in a preparative context. Alternatively, saponification can be applied in order to isolate the, aqueous insoluble, unsaponifiable fraction, a minor fraction of the lipidome containing however a variety of interesting substances such as tocopherols, sterols and carotenoids amongst others.
The extracts and fractions thus obtained are then typically subjected to a more in depth chromatographic analysis, using both capillary gas and liquid chromatography.

Sometimes the extracts are analysed immediately using nuclear magnetic resonance spectroscopy.
For capillary gas chromatography, used in combination with flame ionization or mass spectrometric detection, direct liquid injection of the sample on a split/splitless injector or via a cold-on-column injector is performed depending upon the target analyte. Fatty acids and sterols are typically derivatised in order to increase their volatility and reduce their polarity. Headspace sampling, sometimes in combination with chemical derivatisation can be applied for target analysis of particular (oxidation) compounds.
For liquid chromatographic analysis, pending upon the analyte, fluorescence, evaporative light scattering or mass spectrometric detection are most appropriate. Typically reversed phase chromatography is used, albeit normal phase and gel permeation chromatography are used as well apart from other stationary phases. Mass spectrometric detection opened the door to detailed lipidomic analysis allowing the characterization of major classes of lipids present in a single chromatographic run or via direct infusion without chromatographic separation, i.e. shotgun lipidomics. These are combined with advanced data interpretation approaches.

Session 5: Food analytics

Invited lecture

Marine and freshwater "treasures" in the Arctic and sub-Arctic: biochemical status, adaptations and a quality of food

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The aquatic organisms of the Arctic and sub-Arctic have a great potential to synthesize biochemical molecules and components of unique structure that contribute to the effective adaptation to severe environment. Certain of these components are essential for human health and increase the adaptive capacity of humans living in the circumpolar region.

The study on mechanisms of adaptation to environment is topical for many general biological disciplines. In general, all the adaptive processes are based on biochemical adaptation that is the ability of living systems to adapt to changeable environment by modification of biochemical structure and metabolic reactions (Hochachka, Somero, 2002). The variety of adaptive mechanisms enables aquatic organisms to inhabit different ecological niches including those in such severe conditions that are typical for the northern latitudes. The study of mechanisms of adaptation to the complex environment (one of the most important factors are low temperatures and trophic) among aquatic organisms in the Arctic will afford to assess their ability of effective adaptation. The research of the structure, function and role of certain lipids, their fatty acids, activity of enzymes of energetic and carbohydrate metabolism, proteolysis in hydrobionts is important in terms of understanding of optimal functioning of all metabolic systems of organism under the conditions of changing environment. Among the studied organisms were invertebrates: zooplankton, mollusks (mainly *Mytilus edulis*); fishes: daubed shanny (*Leptoclinus maculatus*), three-spined stickleback (*Gasterosteus aculeatus*), and such commercially important fishes as White Sea herring (*Clupea palasii marisalbi*), Atlantic salmon (*Salmo salar*), and valuable aquaculture object as trout (*Oncorhynchus mykiss*). The modifications of the studied biochemical parameters (fatty acids of membrane and storage lipids, cathepsins etc.) were found and demonstrated differences in adaptive processes which had as general character among all studied organisms as were species specific.

The first data on proteome of parasites *Triaenophorus nodulosus* and *Schistocephalus solidus* (parasites of freshwater fishes) in ontogeny and relation in a system “parasite-host” will be given.

In addition, the evaluation of a quality of fishes assessed by the fatty acids with focus on the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA), – important and valuable compounds for human health, will be presented. The function of these fatty acids as beneficial health constituents of lipids from aquatic organisms, especially fish, has been reported and proofed in much research: brain and eyes systems development, preventing atherosclerosis, dementia, cognitive disorders.

Session 5: Food analytics

Invited lecture

Opportunities and Challenges of Multiomics Analysis

Sureyya Ozcan Kabasakal , Middle East Technical University, Ankara, Turkey

The multiomics approach by converging diverse omics aspects into a single platform is getting attention as a new future. The interrelation of different data layers has been shown to provide multidimensional insights into the biological processes. Through such a combinatorial approach it becomes much easier to determine the functional relationships between different data sets. However, it is a challenging approach due to differences in experimental design, analytical platform and data size. But the resulting interpretations and findings can help to explore the unseen relationships that have been hidden on a single platform. Multiomics data integration requires touse several statistical approaches. In this presentation, we will discuss the potential issues of integrating data sets as well as conventional and new statistical approaches including supervised and unsupervised statistical techniques.

Oral presentations

Simultaneous determination of macro and micro elements concentrations in the mussel *Mytilus galloprovincialis* as a food by rendgen fluorescent spectrometry

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The present study investigated the essential and non-essential elements in cultivated and wild mussels and presents the application of the ED-XRF method (Energy-Dispersive-X-Ray-Fluorescence Spectrometry) in determining the content of elements in the Mediterranean mussel (*Mytilus galloprovincialis*) sampled on the seven locations in the Boka Kotorska Bay, Montenegro, Adriatic Sea. This relatively new method was applied for simultaneous measurement of the content of many metals and enabled analyzing solid samples without dissolving them in acids, i.e. without destroying the mussel samples.

The selected locations were: Krasic, Kukuljina, Tivat, Arsenal, Opatovo, Sv. Stasija, Perast – Risan, Herceg Novi, located in the Bay where the influence of sea currents is much lower and the anthropogenic impact is much higher, compared to open coastline. Collected mussel samples from locations Krasic and Kukuljina were cultivated, while from the remaining locations were wild. The wild mussels were used as food from the locals, also.

The results showed that mussels tissue contained the next following elements: As, Ba, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Hg, Fe, I, K, Mn, Ni, P, Pb, Rb, S, Sb, Si, Sn, Sr, Th, Ti, V, Zn and Zr. In all mussels' samples, regardless of the location, the concentrations macronutrients were highest and declined in the following sequence: Cl > Si > S > K > P > Ca. The micronutrients concentrations of were 2 to 4 orders of magnitude lower than at the macronutrients, while concentrations of toxic elements were the lowest and declined in the following sequence: As > Sb > Pb > Sn > Cd > Hg.

On the basis of the obtained results for measured element concentrations, the correlation coefficient (r) was determined and their mutual influence with respect to bioaccumulation in mussels was monitored: there was a synergistic effect between certain metals, and an antagonistic effect among a great number of metals, giving a picture of the origin of these elements in the investigated mussels.

The content of more than 30 elements can be determined simultaneously in qualitative and quantitative terms quickly and without destroying the samples; a wide range of metal concentrations can be detected without preparing solutions and with automatic reading of results. Measurements of element contents in mussels give rise to the conclusion that the non – destructive ED-XRF method as a multi – element analysis can be applied in determining trace elements in food, as well as in many other biological samples.

Keywords: essential elements, toxic elements, mussel, correlations, ED-XRF

Oral presentations

Profiling of microplastics in Korean mussels: A preliminary study

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Microplastics (MP) pollution has reached a global scale. In the marine environment, it accounts for 92.4% of total plastic debris. Their high bioavailability to marine biota creates a serious health risk to consumers of seafood. In 2016, FAO identified South Korea as the top seafood consumer. With a consumption of 58.4 kg per person per year, South Koreans are more disposed to the negative health effects of MP. In this study, the MP content of mussels from the East and South seas of Korea was determined. Dissolution of organic matter was achieved by digestion in 10% w/w KOH. Preliminary results show that fibers (red, blue, black and green), films and granules are the frequently encountered type of MP. Majority of the particles detected are < 350 µm. In the case of fibers, the largest detected was 2000 µm. Through micro-FTIR analysis, the chemical composition of selected particles was determined to be polyethylene, polyvinylchloride, and acrylic/methacrylic (paint particle). These results suggest that the diet of the Korean population is contaminated with MP. Further studies should be conducted to determine the average annual intake of MP through shellfish consumption and its effects on health.

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Oral presentations

Improvement of Wheat bread quality using lentil flour

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Wheat bread, the worldwide most popular food, is considered a source of energy in many diets for its high amount of complex carbohydrates. However, it has low amounts of proteins, such as lysine and threonine, and fibres when compared to wholegrain bread. This difference is attributed to the use of refined white flour. Thus, enriching wheat with legumes flour, well known by their nutritional benefits, is an effective way to improve the nutritional quality of bread.

This work aimed to improve the bread quality by blending wheat flour with green lentil flour at 10, 15 and 20 (w/w; %). The study of the technological properties of dough and the characteristics of bread showed that lentil flour addition increased the dough development time, water activity and extensibility and decreased its tenacity, deformation energy and swelling index.

All Breads obtained from the different blends were acceptable when compared to the control (100% wheat flour). Whereas, an increase in dietary fibre, phenolic compounds and the overall antioxidant activity were observed. Furthermore, the amount of acrylamide, known for its toxicological effects on human health, in formulated bread was evaluated by HPLC-UV. The addition of 20% (w/w) reduced acrylamide amount up to 40%.

Keywords: Bread, wheat, lentils, nutritional value

Oral presentations

Toward Sustainable Small Scale Edible Insect Production through Life Cycle Assessment Methodology

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Many studies show that most of the food production systems are not sustainability friendly. In this regard, developing new alternative sustainable sources of food is highly important. In recent years, insect is recommended to be a new source for human feed and the amount of insect production units are increasing all over the world. So, many of these start ups and production units are producing insect in small scale units. In this regard, environmental impact is the key factor toward sustainable development of food production system. Thus, this study applied Life Cycle Assessment (LCA) to evaluate the environmental impacts of small scale edible insect production in South Korea (as an example of small scale edible insect production). Impact 2002+ was applied as the baseline impact assessment (IA) methodology and CML-IA baseline, EDIP 2003, EDP (2013), ILCD 2011 Midpoint, ReCiPe midpoint IA methodologies were also used for sensitivity analysis. The results highlighted that the edible insect production system has beneficial environmental impacts in some impact categories (ICs) including non-carcinogens, respiratory inorganics, respiratory organics, terrestrial ecotoxicity, terrestrial acid/nutria, land occupation, aquatic acidification, mineral extraction (8 impact categories out of 15). In other words, this food production system could mitigate environmental impacts of the aforementioned ICs. On the other hand, it has negative environmental impacts in some ICs namely global warming potential, carcinogens, ionizing radiation, aquatic ecotoxicity, aquatic eutrophication, ozone layer depletion, and non-renewable energy. For instance, the global warming potential of one kg dried insect and one kg protein production from insect were 5.08 and 2.54 kgCO_2eq, respectively. Based on the beneficial environmental impacts of edible insect production in some ICs, through management of some inputs consumption, edible insect could be considered as an environmental friendly food production system in human diets.

Keywords: Alternative food, Edible insect, Global warming, Sustainable food

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Poster presentations

P1

Interactions of ruthenium(II)-cymene complexes with cytochrome c

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The ruthenium-based antitumour compounds act more via protein targets involved in carcinogenesis, in contrast to platinum-based compounds. Also, after intravenous administration of antitumour complexes proteins are the first binding targets in circulation. Therefore, interactions of anticancer compounds with proteins are important for elucidation of their pharmacokinetic pathways. Four half-sandwich ruthenium(II)-cymene complexes (C1, C2, C3 and C4), developed earlier and with promising cytotoxic activity, are investigated for their interactions with cytochrome c (Cyt). Complexes were incubated with Cyt for 48 h at 37 °C and high-resolution LTQ-Orbitrap ESI MS was used to monitor the formed adducts. The changes in heme state and tertiary structure around the heme were monitored by CD and UV-VIS spectra in the presence of oxygen. The complexes containing two chloride ligands (C2 and C3) were more reactive toward Cyt than those with only one (C1 and C4). The complex with S,N-chelating ligand (C4) was less reactive than one with O,N-chelating ligand (C1). All complexes reduced heme iron of Cyt, but the extent of reduction was inverse to the order of their reactivity to Cyt (C1>C4>>C2>C3). CD spectra in Soret region indicated that Cyt reduction was accompanied with slight tertiary structure change, the rupture of ferro-Met-80 and occupation of this heme coordination site by His-33/His-26. Extent of heme reduction by compexes inverse with respect to their reactivity implies that initially noncovalent binding of complexes occures, causing heme reduction, followed by comlex coordination to protein. In the presence of less reactive complexes more intensive reduction of heme leaves less available histidine residues (main targets for Ru coordination), leading to less efficient formation of adducts.

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P2

Digestomics of raw and roasted hazelnut according to Infogest protocol and characterization of gastric-phase products

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Brief introduction: Stability to gastric digestion represents a very important parameter of food protein allergenicity. Usually digestion experiments are carried out on purified proteins or protein extracts; however, use of solid food is far closer to the *in vivo* situation, taking into account food protein interactions with other food components, such as polyphenols and lipids.
Objective: The aim of this study was to investigate and compare digestion stability and allergenicity of large and small peptides released after pepsin digestion of whole raw and roasted hazelnut kernels under standardized and physiologically relevant *in vitro* conditions.
Methodology: *In vitro* simulated oral and gastric phase digestion was carried out with ground raw and roasted hazelnut kernels. Digested proteins were extracted from the mixture and analyzed by SDS-PAGE, 2D-PAGE, and compared with Image Master 2D Platinum 7.0. Western blot probed with allergic patients' sera and specific antibodies for Cor a 8.
Main findings: Several important hazelnut seed storage digestion resistant proteins and peptides have been identified and characterized. Most abundant hazelnut allergens were resolved on a 2DE map, for instance acidic and basic chains of Cor a 9, and Cor a 11. Digestion-resistant peptides of Cor a 11 and Cor a 9 were able to bind IgE. Lipid transfer protein (Cor a 8) was highly resistant to gastric proteolysis. **Conclusion**: To conclude, roasted hazelnut is more prone to gastric digestion than raw, and cause milder IgE response in patients. Gastric phase digestion of raw and roasted hazelnut kernels results in partial extraction and digestion of Cor a 11 and Cor a 9 into digestion-resistant peptides with preserved IgE-binding epitopes. These results demonstrate substantial resistance of raw and roasted hazelnut allergens to gastric digestion since they remained mostly intact after 2 h of gastric (pepsin) digestion and retained their allergenicity.

Keywords: hazelnut allergens, digestion, food matrix

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P3

Investigation of raw and thermally treated peanut major allergen post-translational modifications (PTMs)

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Introduction. Peanut allergy affects a large portion of world population causing reactions ranging from mild to severe. Major peanut allergen IgE epitopes are well characterized but little is known about their post-translational modifications (PTM) and how they are affected by thermal treatment. PTM profile may differ between raw and thermally treated peanut, which could affect its allergic potential depending on type, size and position of modifications.

Objective. Our aim was to analyse and compare PTM profiles of 4 major peanut allergens - Ara h 1, Ara h 2, Ara h 3 and Ara h 6, as well as their amounts in raw and roasted samples using bottom-up proteomics methods.

Methodology. Full peanut protein extracts (both thermally treated and non-treated) were digested in gel and in solution, and analysed by a Top10 nLC-MS/MS method by LTQ Orbitrap XL (Thermo Fisher Scientific Inc., Germany). Within the extracts major allergens - Ara h 1, Ara h 2, Ara h 3 and Ara h 6 were identified, label free quantified (LFQ) and searched for PTMs by Peaks X software (Bioinformatics solutions Inc.I, Canada). Epitope sequences were acquired from the Immune Epitope Database (IEDB www.iedb.org).

Main findings. LFQ results show that there is no significant change in the amounts of any of the studied allergens between raw and roasted extracts. Out of the 4 allergens Ara h 6 is modified in the highest portion, with respect to the protein size: 15% and 12% of its positions are modified in raw and roasted sample, respectively. Total of 21 modifications were quantified between the two preparations, with oxidation (M), methylation (K,R) and dethiomethylation affecting the largest number of peptides.

Conclusions. Peanut allergen epitopes are indeed carriers of PTMs that differ in pattern and quantity between treated and non-treated extracts. The *in silico* discovered PTMs could affect protein digestibility and allergenicity. Further investigation is necessary in order to fully understand the impact protein modifications could have on their allergenic potential.

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P4

Physicochemical characterization of soluble proteins of whole camel milk powders produced by spray drying treatment at high temperatures

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Objective. Camel milk is highly nutritious food with numerous health benefits proposed. Demand for camel milk has increased worldwide. Production of camel milk powders facilitate its transport, prolonge shelf-life, and also offer an attractive additive for various food products. In this study we characterized proteins of soluble fraction of freeze/spray dried camel milk powders.

Material and Methods. Whole camel milk powders were prepared by spray drying treatment at six different inlet temperatures (190°C - 250°C) or by freeze drying. The soluble protein fractions upon the treatments were analysed by combination of electrophoretic and spectroscopic techniques. Functional properties, such as antioxidant activity and protein solubility were assessed.

Results. SDS-PAGE revealed non-uniform increase in Mw of major protein bands, while native electrophoresis revealed non-uniform decrease in pI values with increased inlet temperature of spray drying. That indicated attachement of lactose moieties to NH_2-group of proteins via non-enzymatic Maillard reaction. Spectrophotometric analysis showed formation of intermediate Maillard reaction products (increased absorbance at 294 nm) and no detectable late Maillard reaction products formation. Far-UV circular dichroism spectra showed no differences in secondary structures between freeze and spray dried samples. Antioxidant activity and protein solubility were increased with increase in inlet temperature.

Conclusions. Our results showed that spray drying treatment promoted non-enzymatic glycation of camel milk proteins. Glycation of food proteins affects their techno-functional properties, shelf-life and nutritional value. Thus, optimization of spray drying parametars is essential for production of high quality camel milk powders.

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P5

Polysaccharides-induced coacervation of camel milk proteins – A proteomic approach

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Background: Camel milk is often the most important camel product and source of proteins for people living in a harsh and arid Africa and Asia. Besides its nutritional properties camel milk shown to be valuable source of potent bioactive peptides released during gastrointestinal digestion. Anticancerous, antidiabetic, antihypertensive and antimicrobial effects of camel milk have been reported and might be linked to its unique protein composition. Casein micelles can be processed into many types of dairy products, but most of the attempts to make camel milk cheese shown major difficulties in process of milk coagulations. It has been reported that addition of polysaccharides can lead to destabilization and coagulation of milk micelles.

Methods: We tested effects of different concentrations of carboxymethyl-cellulose (CMC) on coacervation of milk proteins. We utilized one-dimensional (1D) and two-dimensional (2D) electrophoresis as a proteomic method of choice, to analyze polysaccharides-induced milk coacervation and the effect on individual camel milk proteins. Skimmed camel milk was mixed with the different % of CMC solutions and left to incubate in a shaker. After incubation, the milk samples were fractionated into the milk supernatant fraction (MSF) and the milk pellet fraction (MPF) by centrifugation. MSF samples were collected. Supernatants (MSF) were analyzed on a 1D and 2D electrophoresis to see how much protein was left in the MSF.

Conclusion: Our results suggested that carboxymethyl-cellulose is potent milk coagulant especially as the concentration of CMC increased. Here by we can say that most of casein fraction does go to MPF and that proteomic approach can be successfully used for this type of analysis.

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P6

Comparison of fatty acid profiles of Korean clams, scallops, mussels and cockles

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Background: Consumption of bivalve molluscs, such as oysters, mussels, clams, cockles and scallops, makes a significant part of the daily Korean diet. Beside high quality proteins with all the dietary-essential amino acids, vitamins, minerals and other bioactive nutrients, this food contains less than 5 percent of total fat, so it is considered a low-fat food. The proportions of saturated, monounsaturated and polyunsaturated fatty acids (FA) (SFA, MUFA and PUFA, respectively), as well as ratio of n-3 (ω-3) and n-6 (ω-6) PUFA in food are very important for the healthy diet. The main goal of this study was to compare the fatty acids profiles of four types of bivalves: clams, scallops, mussels and cockles.

Methods: Four species of clams of family Veneridae (*Ruditapes philippinarum* (Manila clam (RP)), *Cyclina sinensis* (CS), *Leukoma jedoensis* (LJ) and *Meretrix lusoria* (ML)), three species of scallops of family Pectinidae (*Mizuhopecten yessoensis* (MY), Ar*gopecten spp*. (AS) and *Chlamys farreri* (CF)), two species of mussels of family Mytilidae (*Mytilus californianus* (MC) and *Mytilus galloprovincialis* (MG)) and two species of cockles of family Arcidae (*Anadara broughtonii* (AB) and *Tegillarca granosa* (TG) were bought in two fish markets in Incheon, Korea, in order to determine FA composition using GC/EI-MS of fatty acid methyl esters (FAME). The FAME were identified by comparing their retention times with those of the FAME standards or by comparing their mass spectra with those stored in the NIST Mass Spectral Library.

Results: The SFA/MUFA/PUFA ratio and the ω-6/ω-3 PUFA ratio are the most significant markers of lipid composition in a healthy diet and should be 1.25:1.5:1 and <4, respectively. All analysed types of bivalves have the ω-6/ω-3 PUFA ratio between 0.11 and 0.38, which is below the recommended value. The recommended ratio of SFA/MUFA/PUFA has not been obtained for any of analysed types of bivalves.

Conclusion: From the aspect of human nutrition the scallops have the best ratio SFA/PUFA, while the clams and cockles have the best ratio of MUFA/PUFA 1.3, 1.2 and 1.35, respectively.

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P7

Comparative digestomics of Tropomyosin of vertebrates and invertebrates in real food matrix

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Shellfish, is a highly nutritive food resource in the world, but also among the eight allergic food groups accounting for approximately 90% of all immunoglobulin E food allergies worldwide [1]. This work focuses on the only well-recognized major allergen muscle protein tropomyosin(TM) that is responsible for cross reactivity between shellfish and other invertebrates [2]. By contrary, TM of vertebrates (chicken, pig, cow) is not a prominent allergen. The stability of food allergens to digestion is an important factor contributing to their allergenicity. Most *in vitro* digestibility studies are based on the protein extract rather than whole food matrix thus overlooking its effect on TM stability [3]. Our objective was to primarily test the pepsin digestibility of invertebrates and vertebrates (raw and thermally treated based on their real life consumption modes) mimicking the gastric digestion under standardized conditions. To closely observe and compare the vertebrates' and invertebrates' TM stability, we aimed to perform the specific antibody based western blot analysis with two primary antibodies; 1) Rabbit anti shrimp TM antibody (invertebrates), and 2) Rabbit anti human TM antibody (species reactivity to vertebrates).

Methods: Thermal treatment of selected samples to compare TM heat stability, Standardized static *in vitro* methods of simulated gastric digestion[4] for the evaluation and comparison of TM resistance to pepsin, Sodium Dodecyl Sulfate-Polyacryl amide Gel Electrophoresis (SDS-PAGE) of digesta supernatant under reducing and non-reducing conditions to quantify proteins and compare thermally treated invertebrates and vertebrates protein profiles focusing on TM, specific antibody based semi dry Western blot analysis.

Results and discussions: SDS-PAGE analysis of vertebrates and invertebrates samples showed a range of proteins in varied amounts between 10-250 kDa. Depending upon samples, varied numbers of prominent protein bands were observed including the distinct bands corresponding with the molecular weights of TM(37-39kDa). In agreement with publications, TM was, indeed, resistant against pepsin digestion as well as thermal treatment prominently in case of invertebrates than in vertebrates. This was confirmed upon Ab based Western blot analysis. Our results show that, upon thermal treatment but importantly with pepsin digestion, TM (allergen) is completely degraded in vertebrates in contrast to the invertebrates' TM (which is pepsin resistant and heat stable).

This result provides an insight on the differences in digestibility of allergenic versus non-allergenic TM in real food matrix and upon thermal treatments of solid food samples.

Keywords: shellfish allergy, thermal treatment, simulated gastric digestion, tropomyosin

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P8

Detection and characterization of novel allergens from *Anadara* seashells using a immunoproteomics approach

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Background: Seafood allergy is one of the most common in adults with and prevalence of 2.5% of the general population which have experienced adverse reactions to seafood. Diagnostics are limited to a few species and allergens for seafood with tropomyosin and actin as major allergens. Our aim was to identify new allergens from *Anadara* shells using immunoproteomics approach and to investigate level of cross-reactivity with shrimp.

Methods: Proteins from lyophilized *Tegillarca (Anadara) granosa* (Ag) and *Anadara broughtonii* (Ab) shells were extracted in 7M Urea, 2M thiourea containing 2%CHAPS and 0.5% NP40 and phosphate buffer saline solution (PBS). Proteins content was assessed by Bradford assay and SDS PAGE. Sixty-nine sera sensitized to shrimps and other seafood were used to determine IgEreactivity to shells protein extracts by quantitative IgE ELISA. IgE immunoblot was used to confirm IgE activity. Two-dimensional (2D) electrophoresis was used for comparison of shells proteome profiles where 2D immunoblot with serum pool from positive sera was used to investigate IgE-binding spots for identification of novel allergens.

Results: By using haotropic detergents better yield has been observed which was confirmed by SDS PAGE. Form 69 shrimps sensitized sera 23 were positive to our shells, and moderate correlation (rho=0.4, $p<0.001$) was found between IgE level to proteins from both types of shells and IgE levels to shrimps. In addition a high correlation between IgE levels to *Anadara granosa* and *Anadara broughtonii* was observed (rho=0.8, $p<0.0001$). This was confirmed by IgE immunoblot where the same proteins bands showed to possess IgE activity. 2D electrophoresis showed that most of the proteins are in acidic pI range with the most dominant spots in range of 37-50 kDa. Protein profile of two shells species was very similar. Only in *Ab* there were additional two spots at approximately 18 kDa. 2D IgE immunoblot reveal several IgE-binding spots in the acidic area around 45 kDa which could represent actin, and in area around 37 kDa representing tropomyosin. Additional spots were found at approximately 150, 75, 30 and 18 kDa.

Conclusions: By testing sera from shrimp sensitized population we found that 30% of population have IgE-crossreactivity with shells proteins, where tropomyosin and actin are the most dominant IgE-binding proteins. 2D immunoblot reveal more IgE binding spots which could be additional potential allergens for identification and characterization.

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P9

Sugar Profile of Some Autochthonous Apple Cultivars

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Besides volatile compounds and polyphenols, sugar profile is one of the guide marks for fruit quality and consumer acceptance. Chemical characterisation of cultivated plants, such as apple (*Malus domestica* Borkh.), can be useful in breeding programs, innovation in agricultural and processing practice and better understanding of plants adaptation to diverse environmental conditions. Autochthonous cultivars are usually characterised by good adaptability to the local environment and represent a valuable source of genetic variability.

The aim of this study was to determine and compare sugar profiles of mesocarp from 18 autochthonous apple cultivars. Sugar content was determined by High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Determination of 15 sugars and 4 alditols was performed. The dominant sugars in the mesocarp were glucose, fructose and sucrose, which ranged from 57-154 g/kg fresh weight (FW), 150-228 g/kg FW and 32-166 g/kg FW, respectively. The most abundant alditol (sugar alcohol) in the studied samples was sorbitol, with range 29-40 g/kg FW. The similarities and dissimilarities of the sugar profiles established for different apple varieties were discussed.

P10

Characterization of rape honey by stable carbon isotope ratio analysis

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Analysis of stable isotopes by Isotope Ratio Mass Spectrometry coupled to Elemental Analyzer (EA-IRMS) has been developed for the authenticity proof of different foodstuff (wine, juice fruit, honey, etc.). Since 1998, comparison between the isotope ratios of carbon (δ13C) determined in honey and the relative extracted proteins has been used as the official method to detect the fraudulent addition of C4 plant-derived sugars to honey (AOAC 998.12 method). Stable carbon isotope ratio values have not only been used for the detection of adulterated honey, but also for the botanical and geographical characterisation of honey. It has been accepted that honey characteristics depend primarily upon the botanical origin of nectar. The floral source of the nectar predominantly affects the chemical composition of honey in terms of its protein, carbohydrate enzyme, mineral, and organic acid contents.

Some articles dealing with the impact of botanical origin on carbon stable isotope ratio of honey revelaed that due to the narrow range of δ13C values of different origins of honey and relatively dispersed measurements, this parameter is not practicable for honey classification. Additionally, samples from different European areas but the same botanical types are showing relatively large differences depending on the climate area. In this study, EA-IRMS method was used for characterization of botanical origin of rape honey, as very rare type of honey which was not evaluate by its carbon stable isotope ratio previosly. Eleven honey samples collected from several regions in Serbia were analysed. Mean δ13C values of honey was –27.36‰ and the values were in the range of –25.36‰ to –28.30‰, while for protein isolated from honey mean value was –27.13‰ and the values were in the range of –26.22‰ to –28.66‰. Compared to the range of δ13C values for other types of honey, such as acacia, linden and other monofloral honey most commonly used in this region, it could be noticed that rape honey has higher δ13C values. These results could imply the botanical variety as one of the most important factor affecting the isotopic characteristics of honey. Physicochemical parameters of honey samples are also determinated, and met the relevant EU standards.

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P11

Evaluation of physicochemical parameters, total phenolic content, and radical scavenging activity of rare unifloral false indigo (*Amorpha fruticosa* L.) honey

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Honey is a popular natural sweet substance produced by honeybees, from the nectars of plant flowers and/or honeydew. Due to its nutritional, antimicrobial, and antioxidative properties honey has been used for centuries as both a food and a medicine. Chemical composition and, hence, health-promoting properties of honey depend of various factors whereby its floral origin is the most important one. In this work we have investigated 15 samples of unifloral false indigo honey. False indigo (*Amorpha fruticosa* L.) is an aggressive invasive shrub whose flowers with rich nectar production have an important melliferous potential.

The aim of the current study is to evaluate physicochemical properties[1] (moisture, electrical conductivity (EC), optical rotation, acidity, pH values), total phenolic content (TPC) and radical scavenging activity (RSA) of **false indigo honey samples**.

The study revealed that regarding the basis physicochemical parameters studied honey samples met the relevant EU standards[2]. **Samples were characterised with TPC values ranging between 271.04 and 847.81 mg gallic acid/kg, while the results of RSA ranged from 16.03% to 29.93%. The results obtained for physicochemical characteristics of analysed honey** samples **indicate a good quality level**, adequate processing, and good maturity. High phenolic content and radical scavening activity showed that **false indigo honey could be rich source of bioactive compounds.**

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P12

Optimization of the coffee samples preparations for the determination of polycyclic aromatic hydrocarbons by gas chromatography - mass spectrometry

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Polycyclic aromatic hydrocarbons (PAH) are pollutants of great concern due to their toxic, mutagenic and carcinogenic properties. European Commission classified 16 compounds as priority pollutants: acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fuoranthene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenzo [a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene, and pyrene. They are formed by incomplete combustion of coal, oil and gas, or other organic substances from tobacco or grilled meat and can easily enter environment. Through food chain, they can reach human organism and reveal toxic effects. PAH analysis in food samples is of great importance, but due to PAHs low concentrations and low selectivity of organic solvents is challenge. Extraction techniques used for sample preparation for PAH analysis are time consuming and often involve usage of large volume of organic solvents. The aim of this study was to optimize QuEChERS (Quick Easy Cheap Effective Rugged Safe) extraction technique for analysis of 16 priority PAHs from coffee samples using GC - MS. Three different solvent systems (acetonitrile/water (2:1, v/v), hexane/acetone (2:1, v/v) and hexane) were used for PAHs extraction. Obtained extracts were subjected to clean up using thermally threated clinoptilolite. Method was validated in terms of linearity, recovery, precision, limit of detection (LOD) and limit of quantification (LOQ). The best recovery values were obtained by QuEChERS technique using acetonitrile/water (2:1, v/v) as extraction solvent. Recovery values varied between 51.18% for benzo[g,h,i]perylene to 115.59% for phenathrene. Out of 16 analyzed compounds only 3 of them (benzo[g,h,i]perylene, benzo[b]fluoranthene and benzo[k]fluoranthene) had recovery values lower than 70%, so this method could be used for analysis of 13 out of 16 priority PAHs. Method LOD and LOQ for analyzed PAHs were in the range of 0.12–8.88 and 0.4–29.6 μg kg^{-1}, respectively. Proposed QuEChERS technique is accurate and precise, but also cheaper and simpler than other methods used for PAH extraction from coffee samples. Optimized QuEChERS technique was applied on real coffee samples consumed in Serbia. The content of total PAHs varied from 169.58 – 427.98 μg kg^{-1}.

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P13

ICP-MS assessment of essential and toxic metal/elements levels in wild edible mushroom species *Butyriboletus regius* and *Butyriboletus fechtneri*

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In recent times, mushrooms have assumed great importance in human diet. Many wild mushrooms have both nutritional and medicinal value. Mushrooms are protein rich food, but also contain high amounts of essential elements such as potassium, calcium, zinc, cooper and iron. The determination of metal concentration in the fruiting bodies of mushrooms is essential in dietary intake studies. In the present study authors reported determination of the essential trace elements (Zn, Cu, Fe and Mn) and other trace/toxic elements (As, Ni, Cd, Hg, Bi) in two species of wild rare edible mushrooms from family *Boletaceae, genus Butyriboletus (Butyriboletus regius* and *Butyriboletus fechtneri*). Determination of the trace elements (that include essential and toxic elements) is of great importance for public health due to the fact that mushroom are widely consumed in many countries.

After microwave digestion essential trace elements (Zn, Cu, Fe and Mn) and toxic elements (As, Ni, Cd, Hg, Bi,) *were determined* by inductively coupled plasma - mass spectrometer (ICP-MS). All metal concentrations were determined on a dry weight basis (d.w.). The results showed that the essential trace elements have higher content than other elements with predominant amount of Zn in both species (92.61 mg kg^{-1}d.w.for *B. fechtneri* and 78.71 mg kg^{-1} d.w. for *B. regius*) and Fe (56.97 mg kg^{-1}d.w. for *B. regius* and 24.40 mg kg^{-1} d.w. for *B. fechtneri*). Amount of investigated toxic elements content of As, Cd, Hg varied from 0.33 mg kg^{-1}d.w. of As in *B. fechtneri* to 2.54 mg kg^{-1}d.w. of Hg in *B. regius*.

Present results prove that examined wild edible mushroom species could be used in well - balanced diets due to their high contents of functional minerals. Low contents of toxic metals show that collection areas are not polluted, therefore booth collected edible mushroom species can be unreservedly consumed without any health risk.

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P14

Accumulation of Cadmium in Selected Species of Mushrooms from Southeastern Serbia

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We are witnesses of the golden age of mycology, the rapid rise of the science of mushrooms that began in the twenties of the last century. The reason for the great interest in this type of food in many countries is their excellent taste and high nutritional value. Mushrooms are the sources of biologically active compounds, which contribute to their antioxidant, antitumor and antimicrobial properties. They contain large amounts of minerals necessary for human health, essential amino and fatty acids, fibers and vitamins, polysaccharides while content of fat is very low.

Monitoring of the occurrence of heavy metals in edible mushrooms is of considerable importance since they might constitute a possible toxicological hazard. It is known that wild-edible mushrooms can accumulate some toxic heavy metals, such as mercury, lead and cadmium. The accomplished research has shown that cadmium does not have any biological role in the human body, but the screening of its content in mushrooms can be used as bioindicators of environmental pollution. **There are a lot of reports about the ability to take up and accumulate cadmium of wild growing mushrooms.** The aim of this study was to evaluate the contamination of five wild-edible species of mushrooms (*Russula virescens, Clitocybe odora, Amanita caesarea, Cantharellus cibarius* and *Leccinellum pseudoscabrum*) collected from non-contaminated zones by cadmium. The samples were prepared by microwave digestion and the measurement of cadmium content was carried using an inductively coupled plasma with mass spectrometer (ICP-MS). Cadmium concentrations were determined on a dry weight basis (d.w.).

The ability to accumulate metals is characteristic of each species and various mushrooms growing on the same substrate, absorb different concentrations of the same elements. So that, the highest Cd level was observed in *A. caesarea* (6.29 mg/kg d.w.) and the lowest value, which was around six times lesser, was found in *C. cibarius* (0.98 mg/kg d.w.). *C. odora* and *L. pseudoscabrum* showed similar values for cadmium (2.47 – 2.94 mg/kg d.w.), respectively.
Cadmium concentrations were compared to data in the literature and to levels set by legislation. It was concluded that consumption of examined mushrooms is not a toxicological risk concerning cadmium content.

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P15

Antimigratory potential of *Coprinus comatus* mushroom extract on colorectal cancer cells

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Background: *Coprinus comatus* is culinary-medicinal mushroom used in folk medicine since ancient times in treating large number of diseases, including cancer. The present study evaluates antitumor effects of methanol extract from fruit-bodies of *C. comatus* on migratory/invasive potential of two colorectal cancer cell lines (HCT-116 and SW-480).

Methods: *Transwell* method was applied to investigate migratory potential. Expression and localization of migratory protein markers: E-cadherin, N-cadherin, vimentin, nuclear and cytoplasmic beta catenin and Frizzled 7 receptor, was analyzed by immunofluorescence. Determination of MMP-9 concentration was carried out spectrophotometrically. Mushroom extract was applied in two sublethal doses (10 and 50 μg/mL) and all parameters were tracked after 24 h.

Results: Higher basal migratory potential and promigratory/invasive marker MMP-9 was observed in HCT-116 cells when compared to SW-480 cells. Extract inhibited migratory potential of HCT-116 cells, especially in higher applied concentration. Meanwhile, promigratory potential of extract was observed on SW-480 cells. Treatment reduced MMP-9 in HCT-116 cells, while SW-480 cells responded poorly, compared to control cells. Extract was able to increase E-cadherin in HCT-116 cells and relocate it into cell-cell junctions lowering the number of protrusions, while in SW-480 cells lowered expression of this protein was noted. Extract induced localization of cytoplasmic ß-catenin cytoplasmic into cell-cell junctions with aggregation of cells and firmer intercellular connections, which correlates with the antimigratory effects of this treatment. Significantly lower ß-catenin was observed in SW-480 cells after treatment. Nuclear ß-catenin was significantly reduced in HCT-116 cells in comparison to untreated control cells, while higher concentration limited presence of nuclear ß-catenin in nucleus. In SW-480 cells nuclear ß-catenin was reduced by treatment in lower concentration and mainly located in cell cytoplasm. Both applied doses induced higher Frizzled-7 protein expression with its cell membrane localization in both cell lines. Dose-dependent reduce of N-cadherin expression was noted in HCT-116 cells after treatment, while no effect was observed in SW-480 cells. Vimentin protein expression was significantly reduced in treated HCT-116 cells, in comparison to control group, while in SW-480 cells extract induced higher expression of this protein.

Conclusion: Extract exhibited cell-specific responses, with remarkable suppression of cell migration/invasion of HCT-116 cell line. Therefore, its recommendation as nutritional supplements with pronounced antitumor effect in invasive forms of colorectal carcinoma.

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P16

Antitumor potential of *Hygrophorus eburneus* mushroom extract on colorectal cancer cells

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Background: *Hygrophorus eburneus* is edible mushroom with insufficiently explored medicinal properties on cancer. We evaluated for the first time antitumor effects of acetone extract from fruit-bodies of *H. eburneus* on cytotoxic (proapoptotic) and anti-migratory potential of two cancer cell lines: HCT-116 (colorectal) and MDA-MB-231 (breast).

Methods: Cytotoxic potential was determinated with MTT test in concentration range from 1 to 500 μg/mL. Type of cell death was analyzed by Acridine orange/ethidium bromide stainning in two high concentrations (100 and 250 μg/mL). Results of these methods were measured after 24 and 72 h of treatment. Anti-migratory effect was evaluated using Wound healling assay in two sublethal doses (10 and 100 μg/mL) for 24 h.

Results: Treatment reduced HCT-116 cell viability (IC50 = 178.54 μg/mL after 72 h) and had no significant effect on viability of MDA-MB-231 cells. Extract induced cell death mainly by apoptosis (50.29%), with high percent of necrotic HCT-116 cells (20.12%) after 24 h. Number of cells in late apoptosis and necrosis was increased after 72 h, and morphological changes typical for apoptosis were observed. *H. eburneus* induced notable percent of MDA-MB-231 cells in early apoptosis after 24 h, while these effects were reduced after 72 h. Basal migration of MDA-MB-231 cell was more prominent in comparison to HCT-116 cells. No effect of extract was observed on migration of MDA-MB-231 cells, while significant inhibition of HCT-116 cells migration was observed in higher (100 μg/mL) concentration after 12 h when compared to the migration of control cells and cells treated with 10 μg/mL, after same time of exposure. After 24 h, migration was inhibited for about 30% when compared to control after same time of exposure. Generally, non-toxic (sublethal) doses of tested extract exerted cell selective effect with obvious anti-migratory potential on colorectal carcinoma cell line (HCT-116).

Conclusion: Tested *H. eburneus* mushroom extract expressed cell selectivity, with notable effects observed on HCT-116 cells when compared to MDA-MB-231 cell line. Effect on reduced cell viability of HCT-116 cells is result of strong proapoptotic potential of this extract. Also, extract significantly inhibited migratory potential of colorectal cancer (HCT-116) cell line. Breast cancer cell line (MDA-MB-231) was less sensitive to treatments and no significant effect on cytotoxicity or migration of these cells was noted.

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P17

Improving food quality in working dogs feeding

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Dogs are usually feed with food present on the market in one country. Usually its quality is good enough for house keeping dogs but working dogs has some special requirements concerning the incresed everyday physical activities. Aim of our study was to examine fatty acids profiles in everyday used food in working dogs and to suggest some improvments which could be of great importance for them.

In kennel number 1, dogs were fed with commercial granular foods that normally satisfy the normal needs of dogs in nutrients. In kennel number 2, dogs were fed with Premuim granulated foods of high quality to widespread and most-sold foods in our country. The analysis of improved Food by fish ingredients (Farmina, Italy) was our suggestion for their further feeding.

In this study, approved by the Ethics Commission of the Faculty of Veterinary Medicine are included dogs from two straps (1 and 2), the Belgian Shepherd (Malinoa) breed. In both straps we took 10 dogs, n-10 (5 females and 5 males), age categories of 3 to 7 years, body weight 30.2±2.2 kg. As for the condition of the dogs they had their activities in the morning and in the evening for 60 minutes (walking, running).

Results showed that alpha-linoleic acid, ALA, (18:3, n3) was significantly ($p<0,001$) decreased in Food 1 and 2 compared to enriched food by fish. Eicosapentanoic (EPA) (20:5, n-3) and docosahexanoic acid (DHA) (22:6, n-3) were also significantly ($p<0,001$), decreased compared to enriched food by fish. There were changes also in other fatty acids especially in overall n-3 and n-6 in those three examined food.

In conclusion our suggestion is that especially in working dogs feeding, fish should be in composition considering all benefit effects of ALA, EPA and DHA as a part of n-3 fatty acid family such as cardiovascular protection, decreased inflammation, long-term physical improvement, antioxidant activity, dyslipidaemias regulation.

P18

The presence of α-Gal epitopes on the protein surface reduces transcytosis through a Caco-2 monolayer

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Background: Transepithelial transport of proteins is the first step in the cascade of events during an immune response to food allergens. Red meat allergy is characterized by an IgE response against the carbohydrate galactose-α-1,3-galactose (α-Gal) and severe allergic reactions several hours after red meat consumption. The aim of this study was to reveal if the presence of α-Gal epitopes on the protein surface influenced transcytosis through a Caco-2 monolayer (an in vitro cell-based system that faithfully mimics gut transportation of food allergens).

Methods: Bovine serum albumin (BSA) and BSA conjugated α-Gal (BSA-α-Gal) were labeled with Alexa488 flotescent dye. Caco-2 cells were seeded on polycarbonate membrane inserts and cultured for 21 days. Cell monolayers with a transepithelial electrical resistance (TEER) above 300 Ω cm2 were used and the transport of 40 μg or 400 μg of Alexa488-labeled proteins was evaluated. Fluorescence of passed protein was measured at various time points (1, 2, and 4 hours) and calculated from standard curves of the corresponding protein using a spectrofluorimeter. For intracellular detection of proteins inside the cells, after 4h of transcytosis, SDS-PAGE of Caco-2 cell lysates was performed and subsequent Western blot immunodetection of α-Gal epitope was conducted.

Results: Our preliminary results showed a constant increase in transported protein over time. The amount of transported BSA-α-Gal when 400 μg was applied on the monolayer was in the range from 69.7 ± 18.8 ng after 1 h of transcytosis up to 298.6 ± 74.8 ng of protein after 4 h of transcytosis. When BSA was administrated in the same amount, levels were in the range of 136.4 ± 38.7 ng up to 564.8 ± 113.5 ng for 1 and 4 h of transcytosis, respectively. This was an approximately 2 times higher amount in comparison with transcytosis for BSA-α-Gal. No statistically significant difference in the rate of transcytosis between 40 μg and 400 μg of applied proteins was found. After 4 h of transcytosis, no change in TEER values was noted, suggesting that the monolayers stayed intact. After SDS-PAGE, strong protein bands corresponding BSA and BSA-α-Gal

were detected after 4h of transcytosis. Western blot analysis confirmed that the α-Gal epitope was present in Caco-2 cell lysates after 4h of transcytosis of BSA-α-Gal.

Conclusions: We showed that transcytosis of proteins was a well-defined process dependent on the bioavailability of the protein and not on the applied concentration. The prolonged transepithelial transport of BSA-α-Gal may contribute to the explanation why red meat allergic patients experience delayed symptoms after mammalian meat consumption.

Keywords: α-Gal, Caco-2 monolayer, red meat allergy, transcytosis, epithelial barrier

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P19

Determination of toxic elements (mercury, cadmium, lead and arsenic) in shellfish samples

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Bivalve molluscs, which include mussels, oysters and clams, have high nutritional value. On the other hand, seafood may also contain harmful contaminants and other undesirable substances such as mercury and persistent halogenated compounds. Four species of bivalve molluscs Ruditapes philippinarum (Manila clam, MC), Yesso scallop (YS), Tegillarca granosa (TG) and Anadara broughtonii (AB) were bought in Incheon, Korea, in order to determine content of As, Cd, Hg, and Pb and consequently, to estimate the health hazards associated to dietary intake. The samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion. All species showed As content higher than the maximum tolerable limit specified by EFSA. Estimated daily intake of Hg, Cd and Pb from consumption of 300 g was very low and hence poses no toxicological risk.

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P20

Chemometric characterization of sellfish according to their element composition

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The main aim of current study was classification of four biologically different sellfish species such as bivalve molluscs Ruditapes philippinarum (Manila clam, MC), Yesso scallop (YS), Tegillarca granosa (TG) and Anadara broughtonii (AB) bought in the Incheon, South Korea. Content of essencial ellements such as Co, Cr, Cu, Mn, Ni, Se, Zn, and Fe were determined by using inductively coupled plasma mass spectrometry (ICP-MS) after closed-vessel microwave digestion. Chemometrics techniques showed classification of sellfish smaples based on biological species and identified elements most important for classification.

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P21

Study of element contents in bivalve molluscs from Korea

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Bivalve molluscs, which include mussels, oysters and clams, have high nutritional value. They are regarded as a good source for proteins, lipids, carbohydrates and minerals [1]. On the other hand, seafood may also contain harmful contaminants and other undesirable substances such as mercury and persistent halogenated compounds, which has resulted in a number of risk-benefit assessments during the last decade [2].

Four species of bivalve molluscs Ruditapes philippinarum (Manila clam, MC), Yesso scallop (YS), Tegillarca granosa (TG) and Anadara broughtonii (AB) were bought in two fish markets in Incheon, Korea, in order to determine content of As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Zn, and Fe and consequently, to estimate the health hazards associated to dietary intake. The samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) after closed-vessel microwave digestion. The analytical accuracy of the method was evaluated by using the SRM (TORT-2, lobster hepatopancreas). Application of principal component analysis (PCA) and hierarchical cluster analysis (HCA) showed a tendency to form three groups between samples belonging to different genus of samples.

European food safety authority (EFSA) has established recommended daily intake (RDI) values for Cu, Fe, Mn and Zn of 3, 14, 5 and 10 mg/day, respectively. We calculated the RDI for daily consumption in milligrams per 300 g of sample. Our results showed that these species could serve as a good dietary source of essential elements, especially Fe, Mn and Zn. However, all species showed As content higher than the maximum tolerable limit specified by EFSA. Seafood is the major contributor to As in the diet though As in seafood mostly occurs as organic As species [3]. In addition, content of Mn in Yesso scallop is few times higher than in other species.

Table 1. Results found in bivalve molluscs species (mg/300 g, w.m.)

Elements	MC	AB	TG	YS
As	0.78 ± 0.09	0.42 ± 0.23	0.45 ± 0.15	0.31 ± 0.08
Cd	0.03 ± 0.01	0.15 ± 0.14	0.26 ± 0.16	0.34 ± 0.27
Co	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
Cr	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Cu	0.23 ± 0.04	0.32 ± 0.19	0.36 ± 0.18	0.31 ± 0.27
Hg	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.01
Mn	0.40 ± 0.15	1.08 ± 0.70	1.12 ± 0.52	17.8 ± 17.5
Ni	0.17 ± 0.05	0.02 ± 0.01	0.04 ± 0.01	0.10 ± 0.11
Pb	0.01 ± 0.02	0.03 ± 0.02	0.04 ± 0.04	0.02 ± 0.01
Se	0.16 ± 0.03	0.10 ± 0.04	0.16 ± 0.04	0.12 ± 0.03
Zn	2.51 ± 0.29	3.53 ± 0.92	3.74 ± 0.79	9.15 ± 6.19
Fe	10.5 ± 6.6	16.7 ± 8.7	20.6 ± 6.9	4.4 ± 2.6

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