2nd FoodEnTwin Workshop
Experimental animal models for food and environment
February 3-4, 2020, Vienna, Austria

Book of Abstracts

Session 1: Mouse and rat models for food allergy

EoodEnTwin highlights — **Tanja Cirkovic Velickovic** — **University of Belgrade**

The objective of FoodEnTwin (http://horizon2020foodentwin.rs/) is to create a networking collaboration among the University of Belgrade – Faculty of Chemistry (UBFC) and its Center of Research Excellence for Molecular Food Sciences (CoE MFS) and four high renowned institutes from Sweden (Karolinska InstitutetI), Austria (Medical University of Vienna) and Belgium (KULeuven and Ghent University) providing a unique opportunity for UBFC and its partners to increase their scientific excellence and visibility, technology innovation capacity and enable frontier research at the crossroad of food, agriculture, chemistry, nutrition and environmental sciences by the infusion of –Omics technologies and experimental animal models. The project focuses on the key target actions of twinning of research activities through networking, training and a lecturing program resulting in a roadmap for a future collaboration, organization of four public Summers Schools, internal and external expert-driven Academia-Industry meetings, two workshops, and finally, bringing the European Food Chemistry conference (EuroFoodChem) in 2021 to the UBFC in Serbia to increase the UBFC, the Serbian and the European visibility in the fields of food sciences. The scientific topic addresses the major challenge of how environmental pollution affects the food we eat at the molecular level. The project will have a significant societal impact. Our dissemination approach will present our networking ideas to a broad public, from experts, the science community and industry stakeholder organization, to interested, non-professionals, making society more aware of the impact that environment has on food and the importance of new approaches in food, nutrition and environmental sciences. The aim of this 3-year project is to use cutting-edge -omics technologies (proteomics, transcriptomics, digestomics, allergomics, metalomics and lipidomics) and experimental animal models to address the challenges in food, nutrition and environmental sciences in a way that enables the creation of a pan-European research network through the twinning research activities in this project..To achieve the objectives of the FoodEnTwin project, the consortium partners have implemented a comprehensive set of measures within the project's key work packages (WPs): Short term staff exchanges; (WP1), Training workshops, and summer schools; (WP2), Dissemination and outreach. (WP3)

Rat models of food allergy — **Katrine Lindholm Bøgh** — **Danish Food Institute**

In this lecture, I will present an overview of the different parameters that you will need to consider when designing animal experiments. I will give examples of the impact of factors related i) to the proteins, such às dose-response relationship, protein preparation and processing, ii) to the host such as stain, gender and disease status, iii) to the environment such as diet, microbiome and housing condition, and iv) to the experimental design, such route of administration, use of adjuvant and end-point analyses. Allergenicity assessment of novel foods is a difficult task, and not animal models have been validation for such allergenicity assessment. This lecture will provide an example of how animal models can be used in the evaluation of the *de novo* sensitising as well as in cross-reaction capacity. Finally, as brief overview of how animal models can be used for preclinical assessment of new prevention and treatment strategies within food allergy.

Mouse models of food allergy — **Karine Adel-Patient** — **CEA**

In this lecture, we will have a view of mouse models of food allergy. which are not restricted to gastrointestinal sensitization, then mimicking the real life conditions. I'll present some of our own models and experiences, placing them in other published preclinical and clinical data.

performed two-dimensional gel electrophoresis along with in solution digestion with subsequent ZipTips purification as a preparatory step for mass spectrometry measurements.

Session 5: Oral Session

Thermal treatment effect on the antioxidant activity of ethanolic extracts of *Myrtus communis* L. — **Ahmed Snoussi** — **Ecole Supérieure des Industries Alimentaires de Tunis**

Heat treatments employed in the food industries, to improve the sensorial, nutritional and hygienic quality, might affect the phenolic content and the biological effects of formulated foods. Thus, the understanding of the chemistry and the biological outcome of this process on bioactive compounds is crucial for the development of food with beneficial effects on health. The objective of this research is to study the effect of heat processing at 70, 90 and 110°C for 120 min on the stability of the antioxidant activity of *Myrtus communis* L. ethanolic extracts. The obtained results showed that the degradation of phenolics compounds in myrtle extracts is influenced by the temperature. Heating at 110 °C led to the highest decrease in total phenols, flavonoids and proanthocyanidins amounts. The antioxidant activity of the extracts was tested by the DBBH and ABTS scavenging assays. Despite, the decrease of the phenolic compounds amounts of extracts, their antioxidant activities are not lost. Heating at 70 °C led to a decrease in the antioxidant activity of extracts. However, an increase was observed for extracts treated at 90 and 110°C. These results suggest that the products of degradation of polyphenols could have an antioxidant activity which sometimes superior to the native one.

Immunoproteomic study of raw and roasted peanut major allergen post-translational modifications (PTMs) — **Teodora Djukic** — **University of Belgrade**

Peanuts are widely used for the preparation of a variety of foods and are primarily consumed in roasted or boiled form. They have a high protein content of 24–29% from which these six are major peanut allergens: Ara h 1, Ara h 2, Ara h 3 and Ara h 6 because they cause an immune response in over 50 % of the patients allergic to peanut. Arah 1, 2, 6 are peanut storage proteins where Arah 2 is very similar to Arah 6, they share 58 % of homology in sequence. Both of these are heat stable and resistant to digestion. PTM profile may differ between raw and thermally treated peanut, which could affect its allergic potential depending on type, size and position of modifications. We focused our research on post – translational modifications (PTMs) of Arah 1, 2, and 6, due to roasting of the peanut and compared raw and roasted samples to see the difference in PTMs. The objective was to compare PTMs found using bioinformatic methods with results got using methods like 2D-SDS PAGE electrophoresis, Western Blot analysis and ELISA immunoassay. Protein extracts of raw and roasted peanuts were analyzed by Western Blot using antibodies on 7 PTMs. Same extracts were analyzed on 2D-SDS PAGE electrophoresis. After Western Blot analysis we focused on 4 modifications that showed the biggest difference between the two samples. We tested those 4 modifications using ELISA immunoassay with patient's sera who are allergic to peanut. What we concluded was that peanut allergens are indeed carriers of PTMs that differ in pattern and quantity between treated and non-treated. Roasted peanut extract carries more modifications on Arah 1, 2 and 6 than raw peanut especially Anti-Hydroxyproline (HyP), Anti-Carbamil Lysine (CarbK) and Methionine Sulfoxide (OxoM). Supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia GA 172024, and FoodEnTwin (Horizon 2020) GA 810752.

Higher degree of Mayar reaction induced by spray drying at high temperatures increases antioxidant activity of camel milk proteins. — **Ana Simovic** — **University of Belgrade**

Camel milk is highly nutritious food with many 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 examined the effect of freeze/spray drying treatment for camel milk powder production, on physiochemical and functional properties of camel milk proteins. 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. Structural and functional properties of camel milk proteins such as Maillard reaction products formation, antioxidant activity and protein solubility were assessed. 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. Higher inlet temperatures (230°C - 250°C) resulted in higher protein carbonyls formation and lower content of free amino groups as a result of Maillard reaction. 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. Our results showed that spray drying treatment promotes non-enzymatic glycation of camel milk proteins and exert significant effects on the techno-functional properties of CM powder such as nutritional value and shelf life. Thus, optimization of spray drying parametars is essential for production of high quality camel milk powders. This work was

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Microplastics determination in Korean clams **Maria Krishna de Guzman** **Ghent University Global Campus**

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 50 small (estimated age of ≤3 years) and 50 big clams (estimated age of >3 years) from the southwestern seas of South Korea was determined. Dissolution of organic matter was achieved by digestion in 10% w/w KOH. The isolated MP were visualized and counted using fluorescence microscopy with the aid of Nile red dye. Preliminary results from 24 small clams show that 95% of total MP detected are particles, followed by fragments (4%) and fibers (1%). In terms of size, 75% of the MP are < 250 □m, 16% are 250-1000 μm and the remaining 9% are ≥1mm. So far, the smallest particle detected from the clams is 10 μm. Given a total count of 523 MP from 24 small clams, the initial MP abundance is 22 microplastics per clam. In terms of weight, the MP concentration is 3.4 microplastics per gram wet weight. These current experiments will be continued to analyze all clam samples and chemical characterization will be conducted to identify the polymer type of the isolated MP. Additional studies will also be carried out to identify the effects that ingested MP has on human health.