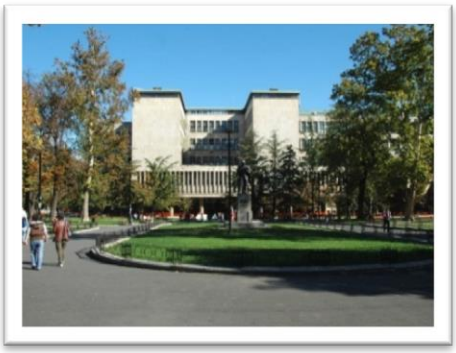
FoodEnTwin Symposium:

Novel analytical approaches in food and environmental sciences

Book of Abstracts



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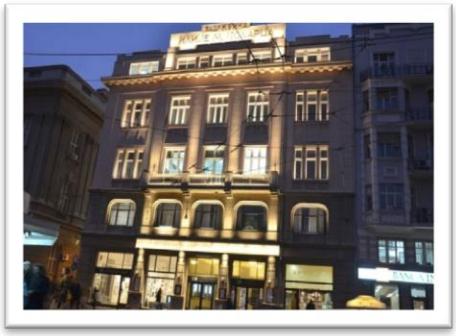
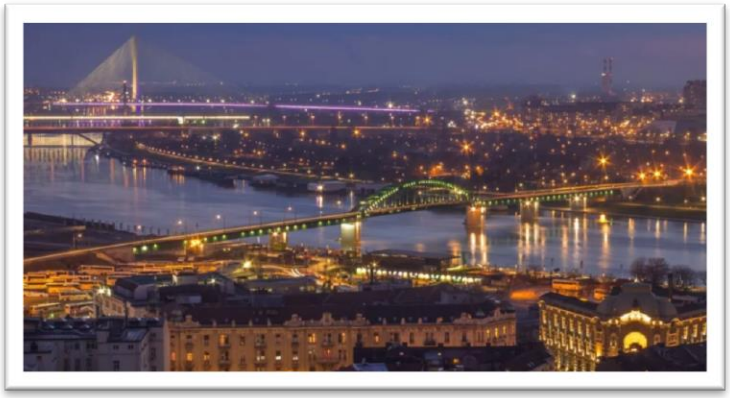
Belgrade, Serbia





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Session 2: Analytical methods development in food and environmental sciences

Invited lecture

METHODS DEVELOPMENT FOR PROTEIN MODIFICATIONS PROFILING

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Post-translational modifications (PTMs) occur in many forms, and broadly influence protein behavior. High-resolution tandem mass spectrometry coupled with engines for the identification of unspecified PTMs, is a first-choice method for their global mapping. The significance of untargeted, in-depth PTM profiling of various proteomes and its method development, just emerged, in contrast to proteomic studies dealing with few selected static and dynamic modifications. In 2018, we have finalized our first study of comprehensive analysis of multiple polluted and environmentally preserved Timothy grass pollen samples that included novel method of relative quantification of proteins expressions and of open, quantitative PTM profiling. It was among the first ones that has appeared in the proteomics field. In addition to this, relative quantitative PTMs profiling of the raw and roasted peanut allergens was completed in 2020, including validation of the method by specific antibodies. This peanut PTM study was inspiration for the idea that porcine trypsin used in proteomic experiments can serve as a probe to decipher differences in scissile bond hydrolysis caused by PTMs, with the steric and/or charge changes causing possible hindrance or facilitation to its activity. We further hypothesized that effects observed would be even more pronounced with human trypsin, since it is less efficient compared to the porcine counterpart. Finally, we developed dual manual method to reassess our data of the major peanut allergen Ara h 1 from the raw and roasted peanut, to confirm or rule out the first hypothesis (do PTMs positioned on lysine/arginine residues facilitate or hinder porcine trypsin digestion efficacy). We believe that this topic is relevant from the aspect of human gastrointestinal digestion of food allergens and PTMs introduced by food processing. For further advancement in this area, novel algorithms based on deep machine learning, capable of reporting cleavage and miscleavage events separately within unmodified and modified part of protein sequences are warranted.

Keywords: post-translational modifications, methods of profiling by mass spectrometry, validation, effects of modifications on digestion efficacy

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