BOOK OF ABSTRACTS

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Detailed Program

22th November (CET)	
14:30-14:45	Opening Ceremony
Plenary session 1	
Virtual Room 1 – Chairs: **Tanja Cirković Veličković** and **Michael Murkovic**	
14:45-15:20	**Richard Stadler** - Real and not-so-real problems of chemical food safety
Virtual Room 1 – Chairs: **Tanja Cirković Veličković** and **Michael Murkovic**	
15:20-15:35	OC01 – David Moreno González – A worldwide study of pesticide residues in fruit-based soft drinks using liquid chromatography/tandem mass spectrometry
15:35-15:40	F01 – Maria da Luz Galante Maia – Synthetic musks in shrimp and seawater samples from the NW Portuguese coast
15:40-15:45	F02 – Cátia Sofia Faria Martins – Extension of shelf-life of lager beer can be a solution to prevent beer wastage resulting from its reduced consumption during the SARS-Cov-2 pandemic?
15:45-15:50	F03 – Lukas Bodenbender – Development of a prototype GC-(ion trap)MS/MS-IMS-system
Virtual Room 2 – Chairs: **Slavica Ražić** and **Maja Natić**	
15:20-15:35	OC02 – Margita Utczás – Analysis of WADA prohibited substances in ecdysterone-containing dietary supplements
15:35-15:40	F04 – Bram Miserez – Food fingerprinting techniques for the authentication of oregano
15:40-15:45	F05 – Philipp Weller – Non-targeted VOC profiling by GC-IMS and machine learning - principles and applications
15:45-15:50	F06 – Mónica Honrado – DNA-based methods as a powerful tool for the entomological authentication of honey
15:50-16:40	***Poster session*** – Wonder platform
Plenary session 2	
Virtual Room 1 – Chairs: **Joana Amaral** and **Manuel Coimbra**	
16:40-17:15	**Michele Suman** – Untargeted analysis with GC-Orbitrap as powerful tool for the authentication of spices and herbs: focus on oregano
Virtual Room 1 – Chairs: **Joana Amaral** and **Manuel Coimbra**	
17:15-17:30	OC03 – Leslie Valeria Simon – Deep-learning assisted data augmentation of spectral data for the authentication and quality analysis of food products
17:30-17:45	OC04 – Charlotte Capitain – Optimized headspace gas chromatography-ion mobility spectrometry (HS-GC- IMS) and non-negative matrix factorization (NNMF) for non-targeted VOC profiling of fermented dairy

DNA-based methods as a powerful tool for the entomological authentication of honey

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Honey is a food widely consumed worldwide and much appreciated for its nutritional, organoleptic and health properties. However, it is also considered one of the food products most prone to be adulterated in the EU. Up until now, honey authenticity addressed mainly the issue of sugars addition and botanical origin. Still, increased attention has recently been paid to honey entomological origin as it also relates to its geographical origin since honeybees carrying mitochondrial DNA (mtDNA) of distinct ancestries can be found across Europe. While in Portugal mtDNA of the autochthonous subspecies *Apis mellifera iberiensis* belongs to the African (A) lineage, in the northeastern part of Iberia African mitotypes are replaced by mitotypes of western European (M-lineage) ancestry. The native distribution of the M-lineage *A. m. mellifera* expands from the Pyrenees to Scandinavia and from the British Isles to the Ural Mountains while the C-lineage *A. m. ligustica* and *A. m. carnica* subspecies are naturally found in the Apennine and Balkan peninsulas, respectively [1]. Also, certain honeys holding the protected designation of origin (PDO) label should be produced by autochthonous *A. mellifera* subspecies, as mentioned in their EU geographical indications register. Because honey's entomological origin also relates with geographical origin, the development of entomological authentication tools can contribute to detect geographical origin mislabelling, which is a fraud difficult to identify. Within the project Autent+, new tools are being developed to discriminate the honey produced by the native A-lineage *A. m. iberiensis* from others of different lineages. To that end, two methodologies were developed using different technologies, namely: (i) a real-time polymerase chain reaction (RT-PCR) coupled to high-resolution melting (HRM) analysis and (ii) a DNA-metabarcoding approach using next generation sequencing (NGS). The mitogenomes of 121 individuals, representing *A. m. iberiensis* (lineages A and M), *A. m. mellifera* (lineage M), *A. m. carnica* (lineage C) and *A. m. ligustica* (lineage C), were used to select the most promising regions for primers design. A total of 35 honeys, including samples of known entomological origin provided by beekeepers from Portugal, Spain and Italy, and honeys purchased in supermarkets, were submitted to DNA extraction using an in-house optimized pre-treatment step to eliminate interferents and the NucleoSpin Plant II kit (Macherey-Nagel, Germany). The extracts were analysed using both HRM and NGS methods. For RT-PCR, the optimized conditions allowed establishing an absolute limit of detection (LOD) of 0.1 pg of honeybee DNA, a reaction efficiency of 93.4% and a R^2 of 0.998. For NGS, DNA extracts were first amplified using the newly designed primers attached to suitable adapters. Then, the products were amplified in a second PCR with a set of appropriate indexes and sequenced on the Illumina MiSeq platform. The obtained sequences were analyzed using a bioinformatics pipeline tailored for assigning sequencing reads to the different mitochondrial lineages and corresponding *Apis mellifera* subspecies.

The developed HRM analysis allowed the successful differentiation of honeybees from lineages A, M and C in three different clusters with high percentage of confidence (>99%) and when applied to honey analysis, the authenticated samples provided by beekeepers were correctly assigned proving the efficacy of the proposed method. However, some commercial samples were not clustered, suggesting the presence of a mixture of honeys produced by honeybees of different ancestries. NGS confirmed the HRM results, allowing to further identify the honeybee subspecies and estimate their percentage for the samples having a mixture of honeys. In particular, some honeys from Spain showed the presence of DNA mixtures from lineages A and M, which is consistent with the distribution of *A. m. iberiensis* in the Iberian Peninsula. For a sample produced in Faial, Azores, a mixture of DNA from lineages A and C was identified by NGS, which is also consistent with the subspecies used in beekeeping in that island. Overall, RT-PCR amplification with the fluorescent dye EvaGreen followed by HRM analysis proved to be a simple, fast and cost-effective approach, although it does not allow for the identification of honeybee lineages in case of honey mixtures. In contrast, this can be achieved by NGS that also allows for high-throughput analysis despite being a more laborious approach, requiring the availability of expensive equipment.

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References:

[1] F. Ruttner, in Biogeography and Taxonomy of Honeybees. Heidelberg, Berlin, New York: Springer Verlag, 1988.