The impact of 4D metabolomics and wide-scope screening methodologies for the investigation of Greek bee products' bioactive content

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In recent years, a growing scientific interest in the analysis of bee products has been developed, due to their high nutritional and financial value. Propolis and royal jelly have gained particular attention because of their promising beneficial properties. Propolis is a wax-like mixture, mainly consisted of resin and secretions from the salivary glands of the bees (*A. mellifera*). It also contains smaller amounts of other compounds, such as aromatic and essential oils, pollens, and phenolic compounds [1]. Royal jelly is a gelatinous and creamy secretion produced by the glands in the hypopharynx of young honeybee workers, called nurses. It represents the food source for all honeybee larvae during the first three days following birth, and the exclusive food of the queen bee for her entire life [2]. Due to their positive health impact, numerous pharmaceutical companies have promoted products such as food supplements, and cosmetics, using propolis and royal jelly as the main ingredients. Thus, further investigation of their bioactive components is of utmost importance.

For the determination of bee products' bioactive compounds, a novel analytical methodology has been developed, using Ultra High-Pressure Liquid Chromatography (UHPLC) coupled with Trapped Ion Mobility Spectrometry – Quadrupole Time of Flight Mass Spectrometry (TIMS-QTOF-MS). A Vacuum Insulated Probe Heated Electrospray Ionization (VIP-HESI) was used as the ion source offering several key analytical benefits, including increased sensitivity, and robustness, and decreased thermal degradation, resulting in zero loss or fragmentation of sensitive compounds [3]. Moreover, the incorporation of TIMS in High-Resolution Mass Spectrometry (HRMS) workflows provides an extra dimension of identification, introducing the Collision Cross-Section value (CCS value). This 4D metabolomics approach integrating qualifier ion, Retention Time (RT), fragmentation ions, and CCS values increases the identification confidence of the analytes, resulting in the separation of isomeric and co-eluted analytes [4]. Target and suspect screening workflows were followed, to allow the detection of several polyphenols. To the best of our knowledge, it is the first time that this powerful cutting-edge methodology is used to determine phenolic compounds in bee products.

Thirty-six propolis and twenty-two royal jelly samples from different regions across Greece were collected and analyzed achieving a comprehensive characterization of their phenolic content. In royal jelly samples, target screening allowed the identification and quantification of 35 bioactive compounds while in propolis 48 analytes were determined. In addition, following a thorough literature review, information on bioactive compounds previously mentioned in bee products was gathered, and a suspect database of 190 compounds was created. Suspect screening results revealed the presence of more than 110 compounds in propolis and 70 compounds in royal jelly. Based on the results, quinic acid was among the dominant compounds in royal jelly, while galangin and pinocembrin were dominant in propolis. The findings of this work are important for analytical and food chemistry. The capabilities of the technique UHPLC-VIP-HESI-TIMS-QTOF-MS were explored, while the identification of more than 100 analytes resulted in the widest chemical characterization of royal jelly and propolis based on their bioactive composition that has never been conducted.

References:

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