From Agricultural By-Products to Bioactive Extracts: Advanced Green Extraction and Cutting-Edge MS-Based Vegetable Metabolomics

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Agricultural by-products represent a vast and largely underutilized reservoir of high-value bioactive compounds. In this study, the foliar biomass of PDO *Allium cepa*, typically discarded during post-harvest processing, was valorized within a circular-economy framework to obtain standardized extracts with potent antioxidant properties and potential applications in the food and pharmaceutical sectors.

Microwave-Assisted Extraction (MAE) conditions were first optimized using a Box–Behnken Design (BBD) combined with Response Surface Methodology (RSM) to maximize phenolic recovery and free-radical scavenging activity [a]. Given the remarkable chemical complexity of the resulting extracts, an online comprehensive two-dimensional liquid chromatography platform (RP-LC × RP-LC) was subsequently developed. Stationary-phase selectivity, mobile-phase gradient profiles, and flow rates were carefully optimized to maximize orthogonality and peak capacity.

Coupling the multidimensional separation system with High-Resolution Mass Spectrometry (HRMS) markedly reduced spectral overlap compared with conventional one-dimensional separations, enabling the tentative annotation of 147 secondary metabolites distributed across eight major chemical classes, including flavonoids, saponins, phenylpropanoids, isoprenoids, terpenes, dipeptides, fatty acids, and lipids. In addition, integration of an online pre-column 2,2-diphenyl-1-picrylhydrazyl (DPPH) reaction assay enabled direct functional bio-mapping of individual constituents, identifying flavonoids, isoprenoids, and phenylpropanoids as the main contributors to the antioxidant activity of the extracts [b].

Overall, this advanced analytical workflow provides a comprehensive chemical and functional characterization of PDO onion leaf by-products, supporting their valorization as a sustainable source of bioactive compounds and providing a robust analytical framework for the standardization of bioactive-rich natural extracts intended for nutraceutical and health-promoting formulations.

a) G. Aquino, M.G. Basilicata, C. Crescenzi, E.M. Sommella, E. Salviati, M. Manfra, G. Auriemma, P. Campiglia, G. Pepe, *Optimization of microwave-assisted extraction of polyphenols from onion (Allium*

cepa L.) leaves PDO variety using response surface methodology, Sci. Rep., 13 (2023), 14923, 10.1038/s41598-023-42303-x

b) G. Aquino, E.M. Sommella, E. Salviati, M. Manfra, G. Auriemma, P. Campiglia, G. Pepe, M.G. Basilicata, *Sustainable valorization of Allium cepa PDO leaves: Comprehensive profiling and antioxidant metabolite characterization via an advanced online LC×LC-HRMS approach*, J. Chromatogr. A, 1749 (2025), 465877, 10.1016/j.chroma.2025.465877

Short bio:

Giacomo Pepe is an Associate Professor at the Department of Pharmacy, University of Salerno. His research focuses on the chemical-functional characterization of plant matrices, agro-food upcycling, and DMPK/ADME studies of synthetic and natural molecules. He is a co-founder and board member of two university spin-offs: Sonda S.r.l., focused on multi-omics protocols and precision nutrition and medicine, and Aracne S.r.l., dedicated to innovative cosmeceutical and nutraceutical formulations. His work integrates circular economy principles, bioactive profiling, and pharmacokinetics to support preclinical drug discovery and health applications.