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UNRAVELLING THE BIOACTIVITY POTENTIAL OF COMPLEX MATRICES: FOCUSING ON LIPIDS AND UNUSUAL AMINO ACIDS IN OILS

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The recent interest in the characterization of naturally occurring compounds is mainly driven by their potential health promoting bioactivities. Metabolites found in food and vegetables represent some of the mainly investigated analytes for the discovery of new bioactive compounds, which can later be exploited to valorize commercial products or find valuable natural sources of nutraceutical compounds.
Within this framework, extra virgin olive oil represents a typical product of the Mediterranean area since antiquity, known and appreciated also outside the boundaries of the Mediterranean Sea. Apart from the well-known content in polyphenols, several other metabolites characterize extra virgin olive oil and contribute to the bioactivity [1]. Among such compounds are polar lipids and trace metabolites, such as seleno-amino acids. As far as the polar lipids are concerned, extra virgin olive oil has a low content of phospholipids, compared to other vegetal oils, but they potentially provide interesting bioactivities, also in relation to different diseases and symptoms, such as inflammation, cholesterol absorption, coronary heart diseases, and cancer [2]. Moreover, these compounds could be used for authentication studies, being the phospholipid profile of olive oil peculiar [3]. Due to their low abundance, an enrichment method was devised to improve the phospholipid coverage, based on solid phase extraction on graphitized carbon black, liquid chromatography coupled to high resolution tandem mass spectrometry and bioinformatic analysis by Lipostar. A method was validated for target lipid standards, then applied to characterize by untargeted analysis extra virgin olive oils from different regions. As far as seleno-amino acids was concerned, they represent an important form of organic selenium, an essential micronutrient for humans [4]. Two methods were developed for direct enrichment of seleno-amino acids in oils. In the first method, a Chirobiotic TAG precolumn was employed to preconcentrate the analytes under Normal Phase (NP) conditions. Oil samples were diluted with dichloromethane and loaded on the precolumn using nitrogen as pressurizing gas. The use of NP allowed to efficiently eliminate oil traces and trap the analytes with high recovery. In the second method, a different sample preparation strategy was pursued, based on liquid-liquid seleno-amino acids extraction and -up by reversed phase/strong cation exchange OASIS MCX solid phase extraction. The second procedure allowed to lower both method detection and quantification limits below 1 ng g^{-1}. Both enrichment methods were coupled with an enantioselective separation of the target compounds (selenomethionine, selenocystine and selenocysteine) and triple-quadrupole single-reaction-monitoring mass-

spectrometry acquisition. Both methods were validated and finally applied to commercial oil samples and Italian extra virgin olive oils.

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