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CHARACTERIZATION OF SPIRULINA MICROALGAE POLAR LIPIDS PROFILE COMPARED TO ENRICHED EXTRACT AND COMPREHENSIVE IDENTIFICATION WITH LIPOSTAR

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Microalgae species are characterized by different bioactive components, such as lipids, proteins, carbohydrates and pigments. Nowadays, polar lipids classes represent an important analytical target due to their bioactivity and biological functions. Until now, microalgae lipid profiling was mainly focused on free fatty acids and triacylglycerols, whereas information on the occurrence of glyco-, sulpho- and phospholipids is rather scarce [1]. In the present work, an optimized extraction procedure was employed in order to maximize lipids recovery using the *Arthrospira platensis*, also named spirulina microalgae. A solid-liquid extraction procedure was exploited, based on the use of a mixture CH_3OH:$CHCl_3$:H_2O. The hydroalcoholic phase was analyzed by Ultra High Performance Liquid Chromatography (UHPLC) coupled to high resolution tandem mass spectrometry (MS) followed by a bioinformatics procedure conducted by Lipostar, a comprehensive platform-neutral cheminformatics tool for lipidomics. A chromatographic evaluation, based on the type and concentration of mobile phase additives, gradients and pH of mobile phase, was carried out to separate the largest number of specific lipid classes with emphasis on glyco-, sulpho- and phospho- lipids under MS-compatible conditions. The chromatographic parameters were calculated both in negative mode, for sulfolipids and phospholipids, and in positive mode, for glycolipids, to obtain the best separation on a standard mixture. Afterwards, in collaboration with software creators and developers, Lipostar was implemented to improve the identification of phosphoglycerolipids and the identification of glycosylmonoradyl- and glycosyldiradylglycerols classes. Finally, the polar lipid extract of spirulina microalgae has allowed the identification of 205 lipids [2]. Afterwards, cause to the relevant abundance of sulfolipids in this matrix, an enrichment procedure based on graphitized carbon black (GCB) to enhance the specificity of the analysis about this specific lipid classes were performed. To this scope, after a solid-liquid extraction, sample was treated with GCB and analyzed with the proper UHPLC and MS conditions. A comparison between the GCB enrichment protocol and a no enrichment approach was also carried out to establish the enrichment efficiency in term of recovery and number of identifications. With a reliable lipid structure assignment conducted by Lipostar, the identification of 199 sulfolipids and only 60 sulfolipids in the no enriched sample was identified. This approach allowed us to characterize sulfolipids profile, identifying the highest number ever reported for the *Arthrospira platensis* species. Finally, a method validation in terms of precision, accuracy, recovery and limit of quantitation and

detection and a semi-quantitative analysis was carried out to characterize its sulfolipids profile and to estimate the concentration levels.

References

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