Empowering the antioxidant activity and lipid recovery from *Aurantiochytrium* sp. extracts with high pressure-assisted extraction

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Aurantiochytrium sp. is a marine microalgae composed of more than 50 % of its dry weight in lipids, within, ca. 63.5 % are polyunsaturated fatty acids, with docosahexaenoic acid (DHA) being predominant (with 42.6 %) [1], which makes this microalgae a potential alternative to the conventional sources of this polyunsaturated fatty acid, as well of triterpenic compounds, such as squalene (traditionally extracted from vegetal and animal-based sources), with high antioxidant activity.

An efficient extraction method needs to be designed to maximize the extraction yields and to avoid oxidation of polyunsaturated fatty acids by temperature (as well the destruction of other compounds) usually used in conventional extraction methods. High pressure-assisted extraction (HPE) is a non-thermal emergent extraction technique able to reduce the extraction time and solvent amount used, with higher extraction yields, improving also the selectivity and extraction efficiency [2].

The remaining biomass is still rich in squalene and other bioactive compounds, namely lipids. This way the lipid fraction of this remaining biomass was extracted with pressures between 300-500 MPa, during 15 min (**Figure 1A-C),** using 5 extraction solvent (ethanol, isopropyl alcohol, ethyl acetate and hexane), and after the first three sample-solvent ratio (1/10, 2/10 and 3/10) evaluation, another two (2.5/50 and 1/50) was assessed at maximum conditions of HPE (500 MPa).

HPE was increased the extraction yields (5-22%) in all solvents, compared to the extraction performed at atmospheric pressure (4-9%). The best extraction conditions allowed extracting 43 and 33 % of lipids on a dry weight basis, with a mass-solvent ratio of 1/50 and 2.5/50, respectively, using ethyl acetate as extraction solvent. Regarding the bioactive compounds of the extracted biomass, an increase of total phenolics content (≈ 50%) was observed in HPE extracts at 500 MPa on sample-solvent ratio of 1/50, compared to treated biomass at atmospheric pressure (0.58 mg GAE.g^{-1}). Also, the impact of high pressure-assisted extraction on radical DPPH scavenging activity of microalgae was clear, for instance, at mass-solvent ratio of 1/50 and after 15 min at 500 MPa of HPE, an augment of 70% was attained compared with control microalgae samples (without high-pressure treatment).

Overall, this study, proves the efficacy of HPE technology for extraction of bioactive compounds from the remaining biomass produced by Aurantiochytrium microalgae which can be applied, in the future, in food processing, as natural ingredient source with potential health benefits to consumers due the richness of bioactive compounds.

Figure 1: Representation of the high-pressure assisted extraction extraction. Showing the high pressure equipment (A) used, samples before (B) and after (C) the extraction process.

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