Optimization of Carotenoid and Antioxidant Compounds Extraction Combining Pressurized Liquid Extraction and Experimental Design from *Dunaliella salina* Microalga.

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Short title: PLE extraction of *Dunaliella salina*.

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Abstract.

In this work, extraction of antioxidant compounds from *Dunaliella salina* microalga is optimized combining pressurized liquid extraction (PLE) and experimental design. To carry out this optimization, a full factorial (3 levels) design was employed together with three different solvents (hexane, ethanol and water). Two main factors were considered, the extraction temperature (40, 100 and 160°C) and extraction time (5, 17.5 and 30 minutes). As response variables, the extraction yield (% dry weight/initial weight) and the antioxidant activity of the extracts (determined using the TEAC method) were used. The parameters of the model, for each response variable and each solvent, were estimated by multiple linear regression (MLR). Results showed that the extraction temperature was the factor having the strongest influence (positive) on the two response variables. The best yields were obtained with ethanol at the higher extraction temperature and time tested. Besides, although hexane extracts provided the best antioxidant activity, ethanol extracts were also very active. Taking into account these results, and considering that ethanol is a safe solvent (GRAS) for the food industry, it was selected as optimum for the PLE extraction of antioxidant compounds from *Dunaliella salina*. The chemical characterization of ethanol extracts was carried out using HPLC, and attempts have been made to correlate their chemical composition with the antioxidant activity measured. Results pointed out that the extracts contained, besides all-trans β-carotene and isomers, several different minor carotenoids that seemed to have a contribution on the antioxidant activity of the extracts. To our knowledge, this is the first work in which pressurized liquid extraction of antioxidant compounds from *Dunaliella salina* is optimized using an experimental design, correlating their composition with their antioxidant activity.

**Keywords:** PLE, *Dunaliella salina*, TEAC, antioxidant compounds, optimization.