Title:

Effects of processing parameters on ultrasound-assisted carotenoids and chlorophyll extraction from *Nannochloris sp.* and *Arthrospira platensis* dry biomass determined by factorial experiment design

Authors & affiliations:

 A. Vlaicu^{*,1,2}, P. Chipurici¹, A. Vintilă^{1,2}, I. Călinescu¹, M. Vînătoru¹, A. Trifan¹, A. Diacon¹
¹University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Bioresources and Polymer Science Department, 1-7 Gh. Polizu St., 011061, Bucharest, Romania
²National Research & Development Institute for Chemistry and Petrochemistry ICECHIM, 202 Splaiul Independentei St., 060021 Bucharest, Romania

Abstract:

Microalgae have attracted attention due to their potential for microalgae-based biorefineries given the presence of high-value compounds in their structure, such as carotenoids, chlorophylls, lipids, proteins, carbohydrates and others, with various applications in the food, nutraceutical and biofuel industries. Extraction techniques include Soxhlet/solvent extraction, microwave/ultrasound assisted extraction, supercritical fluid extraction, pulsed electric field extraction.

Carotenoids are isoprenoid pigments with antioxidant, photoprotection and provitamin A activity, with various commercial applications both in the food and pharmaceutical industries, the commercial value of animals and animal products has also been explored.

An experimental program (2^3) was carried out based on the software Design Expert 11. Three significant independent variables were chosen (temperature, ultrasonic amplitude, and solvent – solid ratio) and their effect on the extraction was determined.

Significant effects of extraction parameters on carotenoid concentration and carotenoids to chlorophyll ratio have been selected using Pareto charts (Figure 1).



Fig 1. Pareto charts of the standardized effects for US-assisted extraction from *Nannochloris sp.* (a) and (b) and *Arthrospira platensis* (c) and (d); influences on: carotenoids (a and c) and carotenoids/chlorophyll ratio (b and d).

Using the factorial experiment design, the response for each parameter (temperature, solvent to solid ratio, US amplitude) was estimated for two types of dry microalgal biomass, *Nannochloris sp.* and *Arthrospira platensis*. It was observed that the influence of US exposure was different for each species of microalgae, most likely as a result of the variety in terms of morphology and cell membrane structure between the various microalgal species.

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