

Supercritical fluid extraction of carotenoids and chlorophyll *a* from *Nannochloropsis gaditana*

M.D. Macías-Sánchez^{a,*}, C. Mantell^a, M. Rodríguez^a, E. Martínez de la Ossa^a,
L.M. Lubián^b, O. Montero^b

^a Science Faculty, Department of Chemical Engineering, Food Technology, and Environmental Technologies, P.O. Box 40,
University of Cadiz, 11510 Puerto Real, Cádiz, Spain

^b Institute for Marine Sciences of Andalusia (CSIC), Avda. República Saharaui 2, 11510 Puerto Real, Cádiz, Spain

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Abstract

Traditional methods for the extraction of carotenoids and chlorophylls from microalgae frequently require more than one extraction step with organic solvents, which are forbidden in the processing of food additives. In addition, further process steps are necessary for the separation of carotenoids from chlorophylls. Consequently, faster processing methods that are compatible with food production are extremely important.

The aim of this study was to ascertain the influence of pressure and temperature on the supercritical fluid extraction of carotene and chlorophyll from a freeze-dried powder of the marine microalgae *Nannochloropsis gaditana*. The operating conditions were as follows: pressures of 100, 200, 300, 400 and 500 bar and temperatures of 40, 50 and 60 °C. The extracts were analysed by measuring the absorbance at 665 and 480 nm. Empirical correlations were also developed.

The results demonstrate that it is necessary to work at a pressure of 400 bar and a temperature of 60 °C to obtain a significant yield in the extraction of the pigments. The best Carot/Chlor ratio was obtained at 200 bar and 60 °C. It was also found that excellent selectivity can be obtained under these operating conditions and this could enable the separation and purification of these kinds of extracted pigments.

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1. Introduction

Microalgae are the subject of numerous research projects at an international level and this interest is due to their potential as a source of pigments (Ip, Wong, & Chen, 2003; Lorenz & Cysewski, 2000). A range of different pigments are present in microalgae but two in particular should be highlighted; the chlorophylls and the carotenoids.

The main interest in the use of carotenoids is based on the advantage that they are not affected—unlike many other dyes—by the presence of ascorbic acid or heating and freezing cycles. Furthermore, carotenoids are extremely strong dyes and already give the desired

properties in food even at levels of parts per million. Carotenoids are increasingly used in food technology, mainly due to consumer pressure and more demanding regulations regarding the use of artificial dyes (Gordon & Bouernfeind, 1982).

With regard to the interest in chlorophylls in food technology, studies are mainly aimed at avoiding the degradation of the material during processing and storage so that it is present in a natural way in the food (Schwartz & Lorenzo, 1990). In addition, the use of chlorophylls is authorized in the dyeing of foodstuffs such as cold drinks and ice creams amongst others (Directive 94/36/CE, 1994; Madrid & Madrid, 1990).

Nannochloropsis gaditana is a microalga that belongs to the group of brown algae in the class Eustigmato-phycea. This alga is used in aquaculture for the cultivation of fish, either directly or via rotifers, as it is a microalga with very stable behaviour during the

* Corresponding author. Fax: +34-95-601-6411.

E-mail address: dolores.macias@uca.es (M.D. Macías-Sánchez).

cultivation process. The alga has adapted to the climatological conditions of the Bay of Cádiz and possesses a good nutritional profile (Lubián & Cañabate, 1987, 2001).

Studies on the morphology, ultrastructure and growth physiology of this system have been described in the literature (Lubián, 1982).

Nannochloropsis gaditana stands out as an important source of pigments of great commercial value. The major pigments present are chlorophyll *a*, beta-carotene, violaxanthin and vaucherixanthin.

Conventional methods based on the solvent extraction of these substances from natural matrices are time-consuming since they require multiple extraction steps and need large amounts of organic solvents, which are often expensive and potentially harmful.

Extraction with carbon dioxide under supercritical conditions constitutes an emerging technology in terms of environmental impact. The advantages in using carbon dioxide include its lack of toxicity, chemical inertness, low cost and ready availability (Hawthorne, 1990). Furthermore, the use of carbon dioxide is also beneficial in adding quality to the products obtained since this technique does not give rise to excessive heating, which usually has a negative effect on the thermolabile compounds.

Investigations carried out previously have demonstrated the feasibility of extracting pigments with supercritical carbon dioxide and examples include carotenoids, starting from carrots (Bath, Zhou, Kute, & Rosenthal, 1995), cabbages (Albino, Penteadó, Lanças, & Vilegas, 1999) and microalgae (Mendes et al., 1995). These processes allow good extraction yields to be obtained. On the other hand, studies concerning the extraction of chlorophylls are scarce.

In the work described here, starting from experimental data, a factorial multilevel experimental design was carried out in order to analyse the effect of temperature and the operating pressure on the extraction yield of carotenoids and chlorophyll *a* when supercritical carbon dioxide is used as the solvent. Subsequently, the program STATGRAPHICS Plus 4.0 (1994–1999, Statistical Graphics Corp.) was used to develop empirical equations that are able to predict the yields obtained in the extraction processes for carotenoids and chlorophyll *a*. Finally, the yield of the supercritical carbon dioxide extraction process was compared with the conventional method using methanol as a liquid solvent.

2. Experimental

2.1. Raw material

The raw material employed in the experiments was the microalga *Nannochloropsis gaditana* and this was

obtained from the Marine Microalgae Culture Collection of Instituto de Ciencias Marinas de Andalucía (CSIC, Spain). The biomass was freeze-dried after being cultivated in sea water enriched with *f/2* medium (Guillard & Ryther, 1962) at 20 °C and aerated with atmospheric air. The illumination conditions were 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from daylight fluorescence lamps. Once the sample had been obtained it was stored under vacuum in darkness until the extraction procedure was carried out.

2.2. Chemicals

Extractions were carried out with high purity carbon dioxide (99.995%) purchased from Carbueros Metálicos (Spain).

Methanol (HPLC grade) from Panreac was used as the extraction solvent. The solvent was flushed with a stream of nitrogen from Air Liquid (France). Ethanol (instrumental analysis grade) was used as the collection solvent for the extracts.

2.3. Supercritical fluid extraction

The experimental development was carried out in a micro-scale supercritical extraction apparatus obtained from Isco (Nebraska) (model SFX 220). The equipment consisted of an extractor, an SFX 200 controller, a restrictor and a syringe pump (Fig. 1).

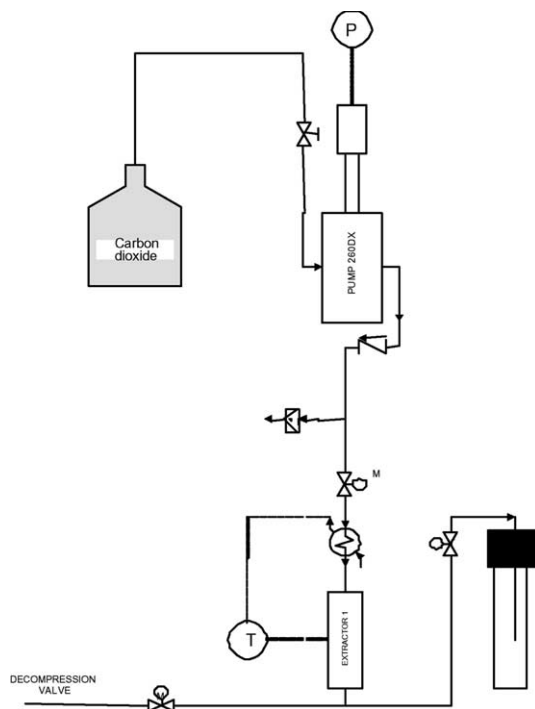


Fig. 1. Diagram showing the flow of the extraction process with supercritical carbon dioxide.

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