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Extraction of oil and carotenoids from pelletized microalgae using supercritical carbon dioxide

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ABSTRACT

Nannochloropsis gaditana is a microalga characterized by its high content of lipids and as an important source of carotenoids, which are recognized to be potent natural antioxidants. A central composite design using response surface methodology was used to study the effects of temperature (36–64 °C) and CO_2 density (914–956 kg/m^3, in a pressure range between 31.7 and 54.3 MPa) on the recovery of oil and carotenoids from pelletized *N. gaditana* by supercritical CO_2. Extraction yields of oil and carotenoids varied between 110.1 and 152.9 g/kg dry substrate and between 393.0 and 773.7 mg/kg.d.s., respectively. The recovery of oil and carotenoids increased with the temperature and CO_2 density, reaching the highest recovery at 64 °C and 956 kg/m^3 (59.3 MPa). Temperature had a greater effect on response variables than CO_2 density. Antioxidant activity (DPPH assay and bleaching β-carotene assay) and anti-inflammatory activity (lipoxygenase inhibition) were measured in selected supercritical extract, and important antioxidant properties were demonstrated.

1. Introduction

Nannochloropsis gaditana is a microalga belonging to the class *Eustigmatophycea* whose cell size varies between 2 and 4 μm [1]. The cell wall contains cellulose material that is protected by a lipid outer layer called "algean", mainly composed of long-chain aliphatic hydrocarbons which provide resistance to cell rupture [2]. *N. gaditana* is characterized by its high capacity to accumulate lipids, between 65 and 70% (dry basis) [3]. *N. gaditana* is also an important source of pigments with a high commercial value such as carotenoids, including astaxanthin, β-carotene, canthaxanthin, neoxanthin violaxanthin and zeaxanthin [4]. Carotenoids are important in use as a functional additive given their antioxidant activity and nutritional value as provitamin A, and as a food colorant. These natural compounds may have nutraceutical applications and be used in the development of functional products.

Oil and carotenoids were extracted from microalgae by supercritical extraction with carbon dioxide (CO_2) as the solvent. CO_2 is the most commonly used supercritical solvent because it has a relatively low critical temperature and pressure (31.1 °C and 7.39 MPa). Obtaining solvent-free extracts at low temperature with a high carotenoids content is favored by the use of supercritical CO_2 extraction, since carotenoids are heat sensitive compounds and the process avoids heat-degradation of active compounds. Furthermore, it provides higher selectivity, shorter extraction times and does not use toxic organic solvents [5]. Supercritical extraction for solid substrates is limited to batch operation because of the requirement for high-pressure conditions. One way of overcoming this limitation is by compacting the substrate, e.g. by pelletization, thus increasing the load of the extraction vessel and the "volumetric yield" of the process [6]. Extraction from pelletized substrate has been carried out for Jalapeño pepper [6], hops [7], mushrooms [8] and red pepper [9]. Furthermore, pelletizing prevents filter clogging by fine particles.

Temperature and pressure are the principal factors affecting the behavior of supercritical extraction, because these factors determine the solvent power of the supercritical fluid. Response surface methodology (RSM) is an effective statistical method for optimizing the extraction process while reducing the number of experimental trials required; furthermore, it allows evaluation of the effects of the factors and their interactions on one or more response variables. An important aspect of RSM is the experimental design in which the factors and their experimental levels are set. The object of experimental design is to select the points at which the response

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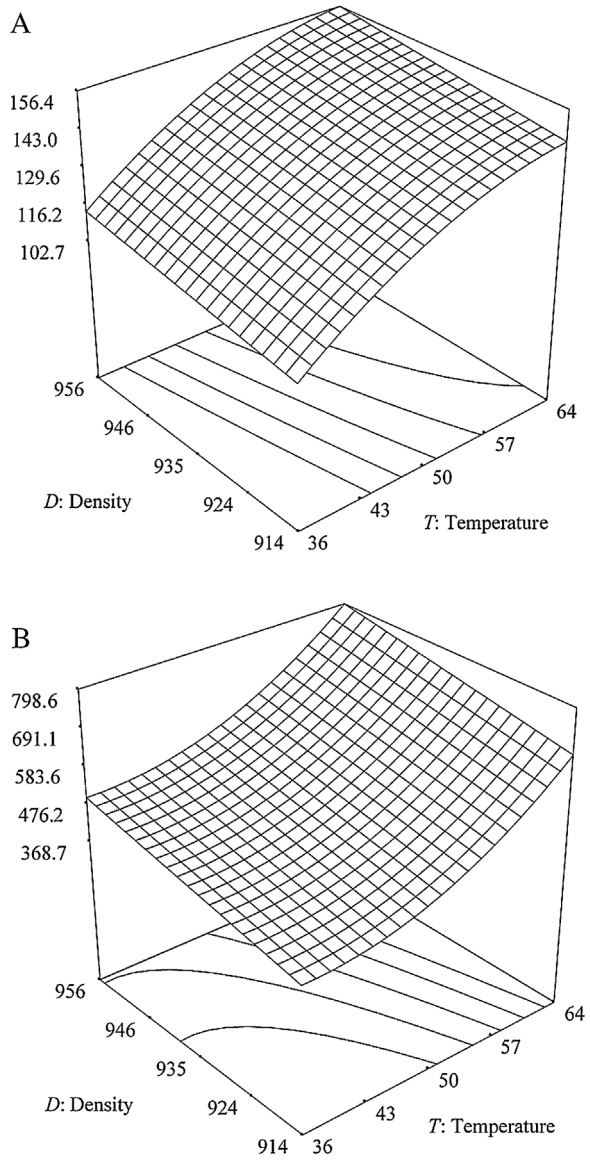

Fig. 1. Surface plot as a function of temperature (T, °C) and density (D, kg CO_2/m^3) of supercritical extraction for: (A) oil yield (Y_{oil}, g/kg d.s.) and (B) carotenoids yield (Y_{car}, mg/kg d.s.).

should be evaluated [10]. These designs are called response surface design and have been applied, for instance, to optimize supercritical CO_2 extraction of carotenoids and chlorophylls from *N. gaditana* [11], from *N. gaditana*, *Synechococcus* sp. and *Dunaliella salina* [12], antioxidant activity of extracts from *Spirulina platensis* [13], astaxanthin from *Haematococcus pluvialis* [14,15], carotenoids from *Scenedesmus almeriensis* [16] and oil from *Scenedesmus* sp. [17].

Macías-Sánchez et al. [11] reported that the extraction of carotenoids from lyophilized *N. gaditana* increased with the temperature (40–60 °C) and pressure (10–50 MPa), the highest extraction yield (343 mg/kg dry substrate) being obtained at 60 °C and 40 MPa (~891 kg CO_2/m^3). Macías-Sánchez et al. [12] studied the extraction of carotenoids from lyophilized *N. gaditana*, using ethanol as a cosolvent. Carotenoid extraction increased with the temperature (40–60 °C) over 30 MPa, and with the pressure (20–50 MPa) at 50 or 60 °C. The highest carotenoid extraction yield (2.893 g/kg d.s.) was obtained at 60 °C and 50 MPa (~934 kg/m^3). In these studies the positive effect of pressure on extraction yield was explained by the increased CO_2 density, which improves the solubility of the solutes in the supercritical phase. Supercritical extraction from other *Nannochloropsis* species has been reported, such as *N.* sp. [18,19], *N. salina* [20] and *N. oculata* [21–23]. Nobre et al. [19] studied the effect of temperature (40–60 °C) and pressure (12.5–30 MPa) on oil extraction from lyophilized *N.* sp. The highest oil extraction yield (330 g/kg d.s.) was obtained at 40 °C and 30 MPa (~911 kg CO_2/m^3). Andrich et al. [18] studied the effect of pressure (40–70 MPa) and temperature (40–55 °C) on oil extraction from lyophilized *N.* sp. Oil extraction increased markedly with pressure and to a lesser extent with temperature. The highest extraction yield (250 g/kg d.s.) was obtained at 70 MPa and 55 °C (~1009 kg CO_2/m^3). The authors used the Chrastil equation to show the dependence of the extraction efficiency on CO_2 density. The Chrastil equation has been used for predicting the solubility of oils in CO_2, showing the direct dependence of the solubility on CO_2 density at constant temperature. CO_2 density directly affects extraction efficiency, since as density increases the distance between the molecules decreases; this leads to greater solute-solvent interaction which improves the solubility of the solute in the supercritical phase [24].

Our objective was to evaluate the effect of the temperature and CO_2 density on the supercritical extraction of oil and carotenoids from pelletized *N. gaditana* using CO_2 as solvent. Response surface design was used to evaluate the effect of extraction conditions on oil and carotenoids recovery from microalgae. There are no previous reports of supercritical extraction from pelletized microalgae.

2. Materials and methods

2.1. Substrate

Saline microalgae *N. gaditana* were supplied by Universidad de Antofagasta (Antofagasta, Chile). This strain was cultured under outdoor production in pilot-scale panel reactors [25]. The microalgae with a moisture content of 30% (wet basis) were pelletized by extrusion in a laboratory single-screw extruder Haake Poly Drive 0–120 Nm (Thermo Electron, Karlsruhe GmbH, Germany), with barrel length to diameter ratio 25:1, internal barrel diameter 19 mm, screw compression ratio 3:1 and a die nozzle with 3 mm diameter. The extruder was feed mechanically. The barrel had three sections with independently controlled electric heaters set at 60, 80, 60 °C. The screw was turned at 45 rpm and the extrusion die was kept at 60 °C. Compressed air was circulated around the barrel to maintain precise control of the temperature of the barrel and die assemblies. The pellets (extruded substrate) were dried to a moisture content of 6.6 g/100 g d.s. in a convection oven (model UF110, Memmert, Schwabach, Germany) set at 60 °C. The pellets were carefully milled with mortar and pestle until a particle size of less than US sieve screen number 18 (1 mm openings) was obtained. A load of 100 g of substrate in each batch was sieved for 20 min through seven US Sieves, size (ASTM E11:95) numbers 18, 20, 25, 35, 50, 60, 140, using a Ro-Tap testing sieve shaker (model RX-29-10, W.S. Tyler, Mentor, OH). The mass retained in each sieve was weighed in an analytical balance (model XB220A, Precisa Gravimetrics AG, Dietikon, Switzerland). The average particle diameter ($d_p = 0.615 \pm 0.021$ mm) was determined using Eq. (1) of the standard method S319.3 [26].

$$d_p = log^{-1}\left[\frac{\sum_{i=1}^{n} w_i log\bar{d}_i}{\sum_{i=1}^{n} w_i}\right] \quad (1)$$

$\bar{d}_i$ (mm) is geometric mean diameter of particles on the ith sieve, or $(d_i \times d_{i+1})^{1/2}$; where d_i is the nominal sieve aperture size of the ith sieve and d_{i+1} is nominal sieve aperture size of the next larger than ith sieve. w_i is the mass of particles with average diameter of $\bar{d}_i$.