Journal of Food Engineering 189 (2016) 123–134

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Effect of high-pressure compaction on supercritical CO_2 extraction of astaxanthin from *Haematococcus pluvialis*

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ARTICLE INFO

Article history:
Received 12 August 2015
Received in revised form
3 April 2016
Accepted 11 May 2016
Available online 13 May 2016

Keywords:
Astaxanthin
Broken-and-intact cell model
Haematococcus pluvialis
High-pressure compaction
Microstructure
Supercritical CO_2 extraction

ABSTRACT

Supercritical (sc) carbon dioxide (CO_2) has the potential of extracting high-value compounds such as astaxanthin from *Haematococcus pluvialis* microalgae. However, there is a significant risk of caking of *H. pluvialis* (a fine hygroscopic powder) during scCO_2 extraction that may lead to a decrease in extraction rate and/or yield. We propose pretreating *H. pluvialis* by High-Pressure Compaction (HPC) to increase the bulk density and prevent caking during extraction. The resulting microstructure may depend strongly on the conditioning of the material and compaction parameters. The objective of this work was to determine de effects HPC on the formation *H. pluvialis* compacts and scCO_2 extraction of astaxanthin from them. A goal of this study was to produce reproducible and defined microstructures by varying the die and compression parameters during uniaxial compression of *H. pluvialis* powder. We fabricated 42 types of compacts in a semiautomatic tableting machine by varying the compression pressure (15–400 MPa), die diameter (3, 5, and 7 mm), and depth fill (2, 5, and 8 mm). Statistical analysis identified three distinguishable clusters depending on the porosity (microstructural feature) and specific surface (macrostructural feature) of the compacts. Supercritical CO_2 extraction showed that clusters with low specific surface had lower extraction yield than clusters with higher specific surface. The effect of compact microstructure on kinetics of scCO_2 extraction was ascertained by computing a microstructural factor (*MF*) from the best-fitted inner mass transfer coefficient of Sovová's broken-and-intact cell model. Because *MF* depended on the shape of the compacts, this work explored their assimilation to infinity slabs or infinite cylinders. Resulting *MF* values were significantly larger in clusters with high porosity than clusters with low porosity. Finally, the fraction of broken cells in Sovová's model was higher in clusters subjected to higher compression pressures that may have favored cell disruption.

1. Introduction

Haematococcus pluvialis, a green microalgae, has been identified as the prime natural source of astaxanthin; a valuable ketocarotenoid and powerful biological antioxidant. Astaxanthin has ten times higher antioxidant activity than other carotenoids such as zeaxanthin, lutein, and β-carotene, and 500 times higher antioxidant activity than tocopherol (Higuera-Ciapara et al., 2006; Lorenz and Cysewski, 2000). The effects of astaxanthin consumption on human health has been described previously by several authors (Ambati et al., 2014; Dhankhar et al., 2012; Guerin et al., 2003; Hussein et al., 2006; Kidd, 2011; Yang et al., 2013; Yuan et al., 2011). The major market for synthetic astaxanthin (95% market share) is aquaculture, as a pigment for salmon and trout (Spolaore et al., 2006). Human supplementation, on the other hand, faces strict demands both in terms of quality (high purity) and antioxidant activity (Lorenz and Cysewski, 2000). Astaxanthin can be extracted from *H. pluvialis* using organic solvents, but they exhibit limited selectivity and favor astaxanthin degradation, and the extracts contain solvent residues potentially toxic (Crampon et al., 2011). This has motivated the use of supercritical (sc) carbon dioxide (CO_2) as an alternative to organic solvents because of its unique characteristics: it is nontoxic, non-flammable, cost-efficient, readily available, and easy to remove from the treated materials (Wang et al., 2012).

Successful scale-up for the industrial production of concentrated astaxanthin extracts depends on the fine-tuning of substrate

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http://dx.doi.org/10.1016/j.jfoodeng.2016.05.008
0260-8774/

pretreatment and extraction parameters (pressure, temperature, and superficial solvent velocity). Most studies on $scCO_2$ extraction of astaxanthin focused on quantifying the effect of extraction pressure, temperature, time, and/or co-solvents such as ethanol (Krichnavaruk et al., 2008; Machmudah et al., 2006; Pan et al., 2012; Reyes et al., 2014; Thana et al., 2008; Wang et al., 2012), but few authors additionally studied the effect of pretreatments such as milling or crushing (Nobre et al., 2006; Valderrama and Perrut, 2003). Most studies generally agree in the need of a cell disruption step to increase the availability of astaxanthin. However, disrupted *H. pluvialis* is usually a fine hygroscopic powder, which may cake inside the extraction vessel, thus causing CO_2 channeling and a decrease in extraction rate and/or yield (Pereira and Meireles, 2009; Reyes et al., 2015). Caking phenomena has been described in garlic (del Valle et al., 2012) and *Nannochloropsis oculatai* microalgae (Crampon et al., 2013).

To avoid caking phenomena during $scCO_2$ extraction of fine powders, the Extraction Laboratory of Biological Materials (LEMaB acronym from the Spanish name) proposes increasing the particle size by precompacting the powdery material using High-Pressure Compaction (HPC) processes such as tableting or pelletization. In the area of powder technology it is important to understand, quantify, and predict density variations within the compacts during HPC. For pharmaceutical tablets, the internal density distribution is relevant because it affects the local material properties, which in turn can influence the mechanical properties of the tablets and the bioavailability of the drug (Sinka, 2007). Similarly in food matrices, high shearing forces during HPC destruct cells walls and other mass transfer barriers within the solid (Uquiche et al., 2004). As a result, the solid matrix acquires a new microstructure, which in turn affects the mass transfer coefficient within the solid matrix (Aguilera and Stanley, 1999; Crossley and Aguilera, 2001). Hence, it is expected that the extraction rate and yield will depend strongly on the microstructure and size of the compact.

There have been significant efforts to fabricate predictable and reproducible microstructures in compacts (*e.g.* tablets, pellets) (Lannutti, 1997). The choice of powder composition (*e.g.*, moisture, granulometry) and process parameters (*e.g.*, compression pressure, die geometry, pressing schedule) determine the microstructure and final properties of compacts (Lannutti, 1997; Sinka, 2007). In an extraction process, Aguilera and Stanley (1999) proposed a Microstructural Factor (*MF*) that quantifies the effect of the microstructure on the diffusion of a solute within a solid matrix, which is defined as:

$$MF = \frac{D_e}{D_{12}}, \tag{1}$$

where D_e is the effective diffusivity and D_{12} the binary diffusion of the solute in $scCO_2$. For porous solids, they proposed the following relationship to estimate *MF* as a function of the solid particle porosity (ε_p) and tortuosity (τ_p):

$$MF = \frac{\varepsilon_p}{\tau_p}. \tag{2}$$

This factor has been successfully used to estimate the effective diffusivity as a function of the binary diffusion coefficient in mathematical models (del Valle et al., 2006; Uquiche et al., 2006). Uquiche et al. (2006) investigated the possibility of fabricating different microstructures by varying the moisture and granulometry of milled Jalapeño pepper flakes before pelletization. Their study showed mix results for moisture, however reducing particle size through milling reduced the porosity and increased the tortuosity of the pellets resulting in a smaller *MF*. The estimated D_e fitted reasonably well the experimental data, but further studies are required to understand this factor and to effectively reproduce these results.

The objective of this work was to determine de effects HPC on the formation of *H. pluvialis* compacts and the recovery of astaxanthin from the compacts by means of $scCO_2$ extraction. An important goal of this study was to produce reproducible and defined microstructures by varying the compression pressure and die geometry during uniaxial compression of *H. pluvialis* powder. *MF* was quantified by mathematical modeling of extraction curves.

2. Materials and methods

2.1. Samples

Disrupted, dried *H. pluvialis* (maximum of 1.5% w/w astaxanthin feed grade powder) was kindly provided by Atacama Bio Natural Products Inc. (Iquique, Chile). The powder was stored at −18 °C inside hermetically sealed desiccators containing silica gel to standardize its moisture and avoid stickiness during manipulation. This was also done with the compacts previous to $scCO_2$ extraction. The moisture of the powder and compacts was of 3.27 ± 0.19% in a dry basis.

2.2. High-pressure compaction of H. pluvialis

H. pluvialis powder was uniaxially compressed in a semi-automatic laboratory tablet press Natoli NP-RD10A (St. Charles, MO). A wide range of compacts were fabricated by changing the compression parameters such as diameter of the die (3, 5, and 7 mm), depth fill (2, 5, and 8 mm), and compression pressure (15, 25, 50, 100, 200, and 400 MPa). After setting the compression parameters the compaction process consisted in three steps: die filling, compaction until achieving the desire compression pressure, and ejection from the die. The usable compression pressure range was determined by the minimum allowed for detection (15–25 MPa) and the maximum permitted by the punch tooling elements (200–400 MPa) of the equipment. Each compact was labeled by its compression parameters. For example, 3.2.100 means a compact fabricated with a 3 mm die, 2 mm of depth fill, and 100 MPa of compression pressure.

2.3. Compacts characterization

The bulk density of the compacts in a packed bed (ρ_b) was determined in triplicate using the gravimetric procedure of Uquiche et al. (2004), whereas the true density (ρ_t) of the substrate was determined by N_2 pycnometry using a Quantachrome Ultrapyc 1200e (Boynton Beach, FL) device. The tablet press produces well defined compacts with small variations in dimensions; each compact consisted of a cylinder with 2 spherical caps. Thus, by measuring its length and diameter it was possible to calculate its volume, superficial area, and specific surface a_p (superficial area-to-volume ratio). Ten random compacts of each type were individually weighted and then photographed with a 13-Mpx camera; their length and diameter were precisely measured using software ImageJ 1.46 (NIH, USA). The compact's density (ρ_s) was calculated as the ratio between its mass and volume.

Porosity of the compact (ε_p) was measured from the calculated densities using Eq. (3):

$$\varepsilon_p = 1 - \frac{\rho_s}{\rho_t}, \tag{3}$$

then the bed porosity (ε_b) was determined as previously described by del Valle et al. (2006).