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Functional food oil coloured by pigments extracted from microalgae with supercritical CO_2

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Abstract

A functional food oil, rich in fatty acids and antioxidants, coloured with pigments (carotenoids) extracted with supercritical CO_2 from the microalga *Chlorella vulgaris*, was produced, having in view its use in food industry (namely for derived seafood). The supercritical fluid extraction (SFE) was carried out in order to study the effect of several modifiers (oil mixed with the microalga and ethanol with the supercritical CO_2), the degree of crushing of the microalga and the supercritical fluid flow rate, at a pressure of 300 bar and temperature of 40 °C. Moreover, the microalga pigments were also extracted with acetone and with vegetable oil at room and high temperature. The recovery of carotenoids was 100% with oil at room temperature for 17 h, 70% with oil at 100 °C for 30 min, 69% with supercritical CO_2 at 40 °C and 300 bar. In SFE the degree of crushing strongly influenced the extraction recovery and higher pigment recoveries were obtained with well crushed biomass.

The stability of soybean oil containing the extracted pigments was also evaluated (light protected) over six weeks, in terms of total carotenoid content and peroxide value. Carotenoids exhibited good preservation over the time, practically without loss. Peroxide values were stable for all extraction systems, showing only a slight increase over time.

Keywords: Pigments; Carotenoids; Microalgae; *Chlorella vulgaris*; Supercritical fluid extraction

1. Introduction

Modern food industry leads to an increase of cheaper, healthier and more convenient products. On the other hand, the increase of world dependence on fish as a food source, due to population increase and meat related problems and the decrease of natural catches, leads to a growing interest in aquaculture products and profitable protein isolates from unused species. To increase the commercial value of new seafood- and/or aquaculture-derived products, consumer-attractive product innovation, with a high nutritious value, must be developed, e.g., surimi, hamburgers, sausages, *pates*, nuggets and seafood analogues (such as shrimps and crabs).

Colour, widely used in the food industry, is an important factor for appeal, a major criterion of identification, indicator of quality and freshness and is a determinant of consumer acceptability, image, market size and value. Seafood-derived products must be coloured to ensure that they are "nature-identical". In the past, synthetic pigments were used indiscriminately to manipulate food colour, but the current trend is to replace artificial (synthetic) by natural colorants (Britton, 1999). Newly manufactured foods, using

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natural ingredients, with functional activity (e.g., as nutritional and antioxidant agents), are now more attractive.

Microalgal biomass is a natural purveyor of almost unlimited biologically active compounds, such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins and sterols. Some microalgae, such as the carotenogenic *Chlorella vulgaris*, with colouring properties (Gouveia et al., 1996a) have already been tested with success as a pigment source for farmed products (Gouveia et al., 1996b, Gouveia, Gomes, & Empis, 1996c, Gouveia, Gouveia, Rema, Pereira, & Empis, 2002, Gouveia,, 2003) with functional activity (e.g. as antioxidants) (Gouveia, Raymundo, Batista, Sousa, & Empis, 2006).

Supercritical fluid extraction, a ''natural and green'' way for product extraction, has received increased attention as an important alternative to conventional separation methods, because it is simpler, faster, efficient and avoids the consumption of large amounts of organic solvents, which are often expensive and potentially harmful. Supercritical CO_2 presents some unique characteristics as a solvent, since it is not toxic. So, it can be a good candidate for carotenoid extraction from the microalga *C. vulgaris*, as was successfully carried out by Mendes et al. (1994, 1995a, 2003).

The aim of this work was the production of a functional soybean oil rich in polyunsaturated fatty acids and antioxidants, coloured from microalgae *C. vulgaris* pigments extracted with supercritical CO_2. To compare this kind of extraction with the more conventional ones, the pigments were also obtained from the microalga through acetone and oil extractions. Furthermore, the stability of soybean oil with the added pigments was evaluated over 6 weeks, in terms of total carotenoid content and peroxide index value.

2. Materials and methods

2.1. Microalgae

The microalga used in this study was *C. vulgaris* Beijerink INETI 58C, cultivated in a Sorokin and Krauss growth medium (Vonshak, 1986) in *air-lift* reactors and polyethylene bags with air injection, at 25 °C, continuously illuminated. Carotenogenesis was induced by nutrient starvation and sodium chloride addition, at high luminosity, according to Gouveia et al. (1996a). Harvesting was done, without prior flocculation, by simply stopping agitation, concentrating by centrifugation and freeze-drying. The carotenoid content of microalgae was 0.43% (ash free) (determined by extraction with acetone – see below).

2.2. Extraction

Microalga biomass was manually crushed – slightly (degree 1), manually crushed mixed with dry ice – moderately (degree 2) and crushed with a disk vibratory mill (NV-TEMA, Labor-Scheibenschwingmuhle, type T100, 0.75 kw, 1000 V/min) – completely (degree 3).

The carotenoids of the microalga were extracted:

(a) with acetone (Gouveia et al., 1996a). The microalga was submitted to extraction with small portions of acetone, with glass beads (425–600 μm) alternately in an ice bath and in a vortex, to complete extraction, which was detected by the absence of colour in the solvent. The acetone extracts were combined and the carotenoid quantification was carried out by spectrophotometry (Hitachi U-2000). Spectra were run, between 380 and 700 nm, and the concentration of carotenoids was determined using the Lambert–Beer law at the maximum absorbance. The value used for the specific optical extinction coefficient ($E^{1\%}_{1\ cm}$) was 2150.
For the stability studies (see below), the acetone was evaporated under vacuum and the carotenoids dissolved in commercial soybean oil.
(b) with soybean oil at room temperature. These extractions were repeated several times (over 17 h) with stirring until a colourless residue (and clean oil) was obtained. The carotenoid content of the extract was determined spectrophotometrically. To determine the specific optical extinction coefficient ($E^{1\%}_{1\ cm}$) of the carotenoids in oil, several solutions of known concentration were evaporated, the residue dissolved in oil and the maximum absorbance was measured. The value found for $E^{1\%}_{1\ cm}$ was 1912.
(c) with soybean oil at 100 °C for 30 min. The carotenoid content of the extract was determined spectrophotometrically, as described above.
(d) with supercritical fluid extraction.

The flow type apparatus used in these studies, which was described in detail in a previous paper (Mendes et al., 1995b), allows working at pressures and temperatures up to 40 MPa and 80 °C, respectively.

The liquid CO_2 (99.998% purity) from a cylinder was compressed to the working pressure at the temperature of a water bath. The pressure was controlled by a back-pressure regulator, and the fluid, before reaching the extractor, passed through a coil immersed in a water bath at a temperature above the supercritical one.

After passing through the alga bed, the supercritical fluid was expanded to atmospheric pressure through a three-way valve and the solutes were collected in cooled glass U-tubes filled with glass wool. Gas flow rate was monitored with a rotameter and the total volume of gas was measured with a wet test meter.

At the end of the runs, the extracted carotenoids were collected, washing the inside of the three-way valve and the expansion tubing with the soybean oil. The glass wool inside the U-tube was also washed with acetone.

Fractions of 5–20 l of expanded gas were collected over time.

To assess the amount of extracted carotenoids, UV–Vis spectra were run, between 380 and 700 nm, and the concen-