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Characteristics of the extract of *Litopenaeus vannamei* shrimp obtained from the cephalothorax using pressurized CO_2

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ABSTRACT

The waste from shrimp processing contains various compounds such as proteins, lipids, chitin, carotenoids, minerals and aromatic compounds, whose percentages vary according to species, constituent parts, fishing site and season. Studies on the use of this byproduct by the food sector have been conducted, such as those regarding the flour of shrimp waste, preparation of flavored products, preparation of flavoring powder and even the recovery of the protein fraction of chitin and astaxanthin by enzymatic hydrolysis. In this study, we investigate the characteristics of an extract rich in carotenoids obtained with pressurized carbon dioxide and ethanol as a co-solvent from the cephalothorax of *Litopenaeus vannamei* shrimp. The operating conditions of the extraction involved two temperatures (40 and 50 °C) and two levels of solvent quality (CO_2 with and without ethanol). The pressure was gradually increased during each extract collection in 30-min starting at 150 bar and ending at 300 bar in increments of 50 bar. We observed that the productivity was higher in the presence of ethanol acting as a co-solvent at both temperature levels and was more pronounced at 50 °C; it was also observed that astaxanthin always appeared in greater amounts with respect to β-carotene for all experimental conditions evaluated in this study.

1. Introduction

Shrimp occupies a prominent place in the world fishery economy, both due its volume (about 20% of the world's market) and wide geographical distribution. In Brazil, starting in the seventies, there was a significant increase in shrimp production as a result of the beginning of shrimp farming. Initially, *Macrobrachium amazonicum* was the species used, being shortly followed by the native species *Farfantepenaeus brasiliensis* and *Macrobrachium japonicus* [1,2].

With the creation of the Brazilian Association of Shrimp Farmers (ABCC) in 1984 and the introduction of the exotic species *Litopenaeus vannamei*, shrimp farming in Brazil resumed its growth, as this species was well adapted to this activity.

In 2007, shrimp farming produced 65,000 tons of shrimp for processing according to the ABCC [3]. Considering the fact that byproducts account for roughly 50% of shrimp's total weight, it is believed that over 30,000 tons of heads and shells has been generated by the national production from shrimp farming [4].

Shrimp waste contains various compounds, such as protein, lipid, chitin, carotenoid, mineral and aromatic compounds, whose percentages vary by species, constituent parts, fishing site and season [5–8].

Thus, studies on the use of this byproduct by the food sector have been conducted, such as those regarding the flour of shrimp waste [9], preparation of flavored products [7], preparation of flavoring powder [10] and even the recovery of the protein fraction of chitin and astaxanthin by enzymatic hydrolysis [11]. Ogawa et al. [12] investigated the main pigments present in shrimp waste and concluded that astaxanthin is the most abundant carotenoid, given that each kilogram of shrimp cephalothorax provided 21.4 mg of astaxanthin.

This work investigates the acquisition of an extract rich in characteristic pigments and aromas from the cephalothorax of *L. vannamei* shrimp using pressurized carbon dioxide and ethanol as a co-solvent.

A major advantage of extraction using supercritical carbon dioxide is the processing of materials at low temperature, which is especially important when thermolabile compounds are present.

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Thus, it prevents the degradation of these compounds, which is particularly problematic because degraded compounds impair the quality of the final product and generate unwanted industrial waste that must be treated before disposal. Another advantage is the possibility of easy separation of the solvent after the extraction process simply by heating or reducing the pressure [13,14].

Meanwhile, depending on the particular compound to be extracted (molecular weight and number of polar bounds), they show very low or moderate solubilities in supercritical CO_2. Solubility of low volatile substance in unpolar supercritical gases decrease with increasing molecular mass and with increasing polarity and number of polar functional groups. Therefore, in several cases CO_2 added with a co-solvent has been proposed as the supercritical extraction medium. The most frequently proposed co-solvent is ethyl alcohol since its presence (in traces) in the final extracts does not compromise the use in nutraceutical or pharmaceutical applications [15].

The selection of the operating conditions depends on the specific compound or compound family to be extracted. Molecular weight and polarity have to be taken into account case by case. Multi-step operation is obtained varying pressure and/or temperature in each process step. This strategy can be used when it is required the extraction of several compound families from the same matrix and they show different solubility in supercritical CO_2. It takes advantage of the fact that supercritical CO_2 solvent power can be continuously varied with pressure and temperature [16].

2. Materials and methods

2.1.. Raw material

The raw material used in this study was the cephalothorax of sea shrimp (*L. vannamei*), waste generated from the production by the Nossa Senhora de Fátima Farm located in Curuçá, a city in the Pará state of Brazil. This material was initially pre-treated by drying, grinding and classifying it according to particle size.

About 2 kg of cephalothorax was ground in a home multiprocessor for 30 s. The resulting material was packed into glass containers covered with aluminum foil and stored in a vertical refrigerator (GELOPAR, Model GPTU-330L) at a temperature of 4 °C before drying. The material was distributed on an aluminum tray as a single layer and dried in an oven with forced air circulation (QUIMIS model Q-314M). The sample remained in the dryer for 12 h at 60 °C based on published studies [17,18]. After drying, the cephalothorax was ground again in the multiprocessor for 2 min. The resulting material was subjected to particle size classification using a Tyler series set of sieves (20, 38, 60 mesh).

By color analysis, it was observed that the fractions of smaller size had higher intensity for red, characteristic of carotenoids. Taking into account the yield with the color, the fraction of 60 mesh was chosen to conduct the study.

Table 1
Characteristics of cephalothorax and cephalothorax flour.

Parameters	Fresh[a] cephalothorax	Cephalothorax flour x[b]
Water concentration (%)	50.84 ± 0.2	8.75 ± 0.2
Crude protein (%)	26.25 ± 1.2	54.56 ± 2.8
Total lipids (%)	1.59 ± 0.0	6.54 ± 0.0
Ash (%)	17.90 ± 0.2	28.10 ± 0.2
Aw	0.79 ± 0.0	0.58 ± 0.0
Chloride (%)	18.03 ± 0.0	39.97 ± 0.0

Means and standard deviations. Analyses performed in triplicate.
[a] (b.u.).
[b] (b.s.).

2.2. Characterization of the raw material

The raw material was characterized according to the standard methods created by AOAC [19]: water concentration (932.12), total lipids (948.22), protein (940.25), ash (938.08) and chloride (937.09). The quantification of calcium, magnesium, sodium, potassium and phosphorus was carried out in an ICP-AES apparatus (VARIAN, model Liberty II), and water activity was determined using an AQUAlab instrument (DECAGON, Series 3TE).

2.3. Supercritical fluid extraction

The experiments performed to obtain shrimp extracts were conducted in a supercritical extraction apparatus with the configuration shown in Fig. 1.

Carbon dioxide (Gas Pará S.A., 99.9%, Belém, Pará) as pressurized to the required levels, circulated by a membrane compressor (HOFFER, Germany) through a fixed bed of solid material 2.7 cm in diameter and 14.5 cm in height and placed inside the extractor vessel (6.0 cm in diameter and 36.0 cm in height). The mixture

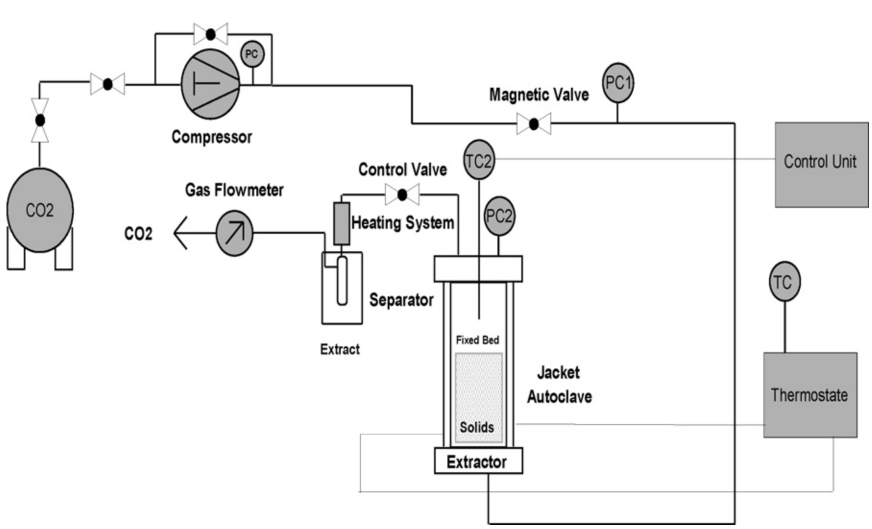

Fig. 1. Schematic flowsheet of supercritical extraction apparatus.