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Proximate composition and extraction of carotenoids and lipids from Brazilian redspotted shrimp waste (*Farfantepenaeus paulensis*)

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ABSTRACT

The chemical composition of redspotted shrimp (*Penaeus paulensis*) waste was investigated. The shrimp waste (freeze-dried heads, shells and tails) was found to have high protein (49% d.w.) and ash (27% d.w.) contents, but a low lipid content (4.9% d.w.) although the latter was higher than those found in other kinds of shrimp captured in Brazil. The fatty acid compositions showed that the lipids had a high content of unsaturated fatty acids, mainly EPA (C20:5; n-3) and DHA (C22:6; n-3). In order to establish an efficient and environmentally friendly recovery process for the astaxanthin (principal carotenoid and antioxidant present in the waste), the following processes were examined: traditional solvent extraction (TSE), super-critical fluid extraction (SC-CO_2) and super-critical fluid extraction with co-solvent (SC-CO_2 + ethanol). The temperature and pressure conditions for all the SC-CO_2 extractions were 50 °C and 30.0 MPa. The results showed that the mixture of 60% (v/v) *n*-hexane:isopropyl alcohol gave the highest (53 mg/kg waste) carotenoid extraction yield as compared to acetone, SC-CO_2 and SC-CO_2 + ethanol. The SC-CO_2 showed the lowest extraction yield of astaxanthin, but the addition of the entrainer (10% w/w) produced an important effect, increasing the astaxanthin extraction to values of 57.9%, similar to extraction with acetone (63.3%).

1. Introduction

World production of shrimp for the year 2008 was 6.519 million tons, showing a growth rate of 79.6% between 1998 and 2008. Of this total, 52.1% came from aquaculture, a captive production process that has risen on average 245% in the last decade and 3.6% since 2007. By the end of the year 2008, Brazil had established itself as the sixth largest producer of shrimp in the continent, with 100,540 tons, shrimp being the largest marine export from the country. With respect to aquaculture, Brazil has emerged as third in America, after Mexico and Ecuador (FAO, 2010). The waste generated during the industrial processing of shrimp is about 40–50% of its total weight depending on the species, this by-product consisting of the head, shell and tail (Heu et al., 2003; Sachindra et al., 2005a; Ogawa et al., 2007). Due to the increased world production and consumption of shrimp, the seafood industry is focused on an appropriate destination and/or reuse for this waste, since its improper disposal, without any attempt to use it causes a serious environmental problem. These waste products are economically recoverable, because they have high-quality protein, chitin, minerals, carotenoids, such as astaxanthin, and lipids that are high in ω-3 fatty acids. Thus there is great interest in developing alternative uses to generate value-added products from the waste (Shahidi et al., 1992; Ibrahim et al., 1999; Nargis et al., 2006; Assunção and Pena, 2007; Rødde et al., 2008). Astaxanthin (ASX) is a pigment belonging to the xanthophyll family, and is the major carotenoid in red aquatic animals such crustaceans, salmonids and other farmed fish feeds (Saito and Regier, 1971; Meyers and Bligh, 1981; Sachindra et al., 2005b). It is mainly used as a dyeing agent in the diets of aquaculture salmon and other species, but is also used in the cosmetic and pharmaceutical industries (Higuera-Ciapara et al., 2006). It has been reported that ASX has up to 10 times the antioxidant activity of other carotenoids such as zeaxanthin, lutein, canthaxanthin and β-carotene; and 100 times more that of α-tocopherol (Miki, 1991). In general, carotenoids and lipids are soluble in non-polar solvents and these types of solvent have traditionally been used to extract them (Britton, 1985; Mercadante, 2008; Sachindra et al., 2006). Thus, several organic solvents have been permitted for use in food industries like acetone, ethyl acetate, hexane, isopropanol, methanol, methyl ethyl ketone and ethanol; and other normally used solvents like dichloromethane, dimethyl sulfoxide and chloroform are not allowed, because their toxicity (U.S. FDA, 2010). With regard to the extraction of carotenoids present in the shrimp waste, it has been reported that the use of polar solvents such as acetone (Saito and Regier,

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Nomenclature

ASX	astaxanthin	$SC\text{-}CO_2$	super-critical carbon dioxide
TLC	thin-layer chromatography	TSE	traditional solvent extraction
EPA	eicosapentaenoic acid	v/v	volume to volume
DHA	docosahexaenoic acid	w/w	weight to weight
d.w.	dry weight	X_0	global yield extraction
Rf	retention factor		

1971; Mandeville et al., 1991; Britton, 1985) and a mixture of polar and non-polar solvents (Sachindra et al., 2006) improve yields considerably relative to other types of solvents. However, the disadvantage is that this method of extraction generally decreases the stability of carotenoids due to oxidation, if antioxidants are not used during and after processing. Environmental issues represent another concern (large volumes of solvent are required) and also the low extraction yields achieved using these types of solvent (almost 50% of the carotenoids are lost), stimulating studies directed at finding alternative extraction processes for this functional food and pharmaceutical supplement (Delgado-Vargas and Paredes-López, 2000). One alternative process currently available is extraction with super-critical carbon dioxide ($SC\text{-}CO_2$), since CO_2 is inert, nontoxic, nonflammable, low cost and ideal for use in the food industry. Moreover, $SC\text{-}CO_2$ extraction does not require high processing temperatures, which is especially appropriate when heat labile compounds such as carotenoids are present (Charest et al., 2001; López et al., 2004; Macías-Sánchez et al., 2005; Careri et al., 2001; Mendes et al., 1995; França et al., 1999) and lipids (Hardardottir and Kinsella, 1988; Yamaguchi et al., 1986; Froning et al., 1990; Tanaka and Ohkubo, 2003). Another advantage is its easy separation from the extract after the process just by reducing the pressure to normal conditions (Del Valle and Aguilera, 1999). Its high rate of processing of the materials should also be mentioned, due to its low viscosity, high diffusivity and high power of solubility for the super-critical solvent, and also its separation efficiency with high selectivity amongst the products extracted, making the process competitive with other earlier technologies (Brunner, 2005; Reverchon and De Marco, 2006). The main purpose of this study was to determine the proximate composition of the redspotted Brazilian shrimp by-products in order to explore the possibilities for their use and compare three methods of extraction of its lipids and carotenoids: traditional solvent extraction (TSE), super-critical fluid extraction ($SC\text{-}CO_2$) and super-critical fluid extraction with entrainer ($SC\text{-}CO_2$ + ethanol) with the intention of assessing the variables (a) the total extract yield, (b) the amount of astaxanthin extracted and (c) the astaxanthin content in the extract.

2. Materials and methods

2.1. Materials

A sample of fresh redspotted shrimp (*Farfantepenaeus paulensis*), type 11/15, from Cananéia city, São Paulo (Brazil) was acquired from a seafood marketplace in Campinas (SP, Brazil). Carbon dioxide (99%) for super-critical fluid extraction and commercial nitrogen (P-4631) were purchased from White Martins Brazil (Praxair Inc., Campinas, SP, Brazil). The astaxanthin standard was purchased from Sigma–Aldrich Co. (⩾92%, A9335, batch 037K1235) and butylated hydroxytoluene (BHT, Lot 88353) from Labsynth Ltda. (Diadema, SP, Brazil). All the solvents used were of analytical grade and obtained from LabSynth Ltda.

2.2. Preparation of shrimp waste

The whole shrimps were peeled manually and the residues, consisting of head, tail and shell, are separated. The waste was packed into 1 L amber pots and stored at −20 °C. About 1.60 kg of the raw waste were freeze-dried (Freeze-Dryer L101, Liobras, São Paulo, Brazil) to a final moisture content of about 5.5 g/100 g (wet basis). The waste was then ground using an electric rotary knife grinder (MARCONI, model MA-340, Piracicaba, Brazil) and sorted according to particle size using vibrating standard Tyler screens of 24 and 400 mesh (BERTEL, São Paulo, Brazil). The average particle size diameter (0.331 mm) was calculated according to the procedure described in the ASAE S319.3 method, as recommended by the ASAE Standards (1997).

2.3. Proximate composition

According to the AOAC (2006) methods, moisture was quantified by oven-drying at 105 °C (AOAC 950.46), total fat by the Soxhlet extraction (AOAC 991.36), crude protein using the micro-Kjeldahl procedure (AOAC 928.08), crude ash by incineration in a muffle furnace at 550 °C (AOAC 920.153) and crude fiber as the organic residue remaining after digesting with H_2SO_4 and NaOH.

2.4. Lipid extraction and fatty acid compositions

Total lipid was extracted according to the Bligh and Dyer method (1959) as modified by Manirakiza et al. (2001). The fatty acid compositions of the extracts were determined by gas chromatography using a GC Agilent 6850 series GC system (Agilent Technologies, Wilmington, USA) with a DB-23 Agilent (50% cyanopropyl)-methylpolysiloxane capillary column (60 m × 0.25 mm × 0.25 μm; Serial No. US2201526H, Agilent Technologies, Wilmington, USA). The carrier gas was helium (1.0 mL/min, White Martins, Campinas, Brazil), using a split ratio of 1:50. The detector and injector temperatures were, respectively, 280 and 250 °C. The column was maintained at 110 °C for 5 min, then raised from 110 to 215 °C at 5 °C/min, and finally maintained at 215 °C for 24 min. One microliter of the methyl esters was injected onto the column, and the fatty acids identified by a comparison of the retention times with those of the methyl ester standards. The analyses were carried out in duplicate using a gas chromatograph equipped with a flame ionization detector (FID) and a column packed with Silar 10C.

2.5. Carotenoid extraction

2.5.1. Traditional solvent extraction (TSE)

The carotenoids in the homogenized freeze-dried shrimp waste were extracted, using organic solvents and solvent mixtures following two methodologies. The first method used was described by Metusalach et al. (1997) and modified by Niamnuy et al. (2008). The carotenoids were extracted repeatedly from 5.00 g of sample using 20 mL of acetone until no further pigment was extracted by the solvent (no color). Four extractions were performed