# Drying and extraction of astaxanthin from pink shrimp waste (*Farfantepenaeus subtilis*): the applicability of spouted beds

Aline Kazumi Nakata da SILVA<sup>1</sup>, Breno Diniz RODRIGUES<sup>1</sup>, Luiza Helena Meller da SILVA<sup>1</sup>, Antonio Manoel da Cruz RODRIGUES<sup>1\*</sup>

# Abstract

(CC) BY

In this study, the spouted bed drying technique was used to obtain powder from shrimp wastes, with high quality for subsequent extraction of astaxanthin using vegetable oil, as alternative to organic solvents. Shrimp waste paste was dried in three inlet air temperatures (70, 80 and 90 °C), and air velocity used in the experiments was 100% over minimum spouting velocity. The minimum spouting, the velocities were 1.23, 1.33 and 1.39 m/s, for temperatures of 90, 100 and 110 °C respectively. The powder obtained in this work shows properties comparable to of the commercial products, indicating that the spouted bed is an alternative technique to obtain powdered products with quality good, at low cost and suitable for subsequent extraction of astaxanthin. The extractions of astaxanthin from shrimp waste were performed using palm olein at three different temperatures (50, 60, and 70 °C). At these conditions, maximum extraction of astaxanthin was  $29.814\mu g/g$  of dried waste. The extraction kinetics were modeled using a simplified mass transfer kinetic model which showed a good agreement ( $0.9685 < r^2 < 0.9912$ ), between the experimental and calculated data.

Keywords: shrimp waste; oil extraction; carotenoid; palm olein.

**Practical Application:** Obtain vegetable oil enriched with bioactive compounds (astaxanthin) from pink shrimp waste associated with greener technologies.

# **1** Introduction

Astaxanthin (3,3-dihydroxy- $\beta$ , $\beta$ -carotene-4,4-dione) is a ketocarotenoid, oxidized form of  $\beta$ -carotene being responsible for the pink to red pigmentation and widely distributed in aquatic animals such as shrimp, salmon, trout, and lobster (Stepnowski et al., 2005; Higuera-Ciapara et al., 2006; Pu et al., 2011; Ambati et al., 2014). Astaxanthin has important applications in pharmaceutical, cosmetic and food industries and as a source of pigmentation in aquiculture feed (Higuera-Ciapara et al., 2006; Sachindra et al., 2007; Dhankhar et al., 2012; Prameela et al., 2017). The pigment is also considered in medical and biomedical studies and applications due to its biological function as a vitamin A precursor and its high antioxidative effects (Lorenz & Cysewski, 2000; Guerin et al., 2003; Yang et al., 2016; Prameela et al., 2017). It has been reported that Astaxanthin has up to 10 times the antioxidant activity of other carotenoids such as zeaxanthin, lutein, canthaxanthin and  $\beta$ -carotene; and 100 times more that of a-tocopherol (Miki, 1991; Naguib, 2000; Silva et al., 2015).

Numerous studies have reported the recovery of astaxanthin from crustacean byproducts such as snow crab (Shahidi & Synowiecki, 1991; Yang et al., 2015), shrimp (Chakrabarti, 2002; Sachindra et al., 2006; Handayani et al., 2008; Mezzomo et al., 2016) and crawfish (Chen & Meyers, 1984; Omara-Alwala et al., 1985; Cremades et al., 2001; Pu et al., 2011; Pu & Sathivel, 2011). The byproduct from pink shrimp (*Farfantepenaeus subtilis*) is a good source of high quality to extract astaxanthin (Ogawa et al., 2007; Sánchez-Camargo et al., 2011; Sila et al., 2015). The processing of pink shrimps is one of the important marine industries in the State of Pará (Brazil). Shrimp processing involves the removal of head and hard carapace, which account for about 40-48% of a whole shrimp. Normally, these byproducts are discarded as waste (a significant source of natural astaxanthin), which is a loss of potentially valuable byproducts but it can also cause serious environmental problems, due to their high content of organic material. Nevertheless, these residues can be recycled to produce additives by extracting the components of "valued added".

As most carotenoids, astaxanthin is a highly unsaturated molecule and thus, can easily be degraded by thermal or oxidative processes and lose its bioactive properties during the manufacture and storage of foods. Generally, carotenoids are found in nature as all-trans molecules in which all the double bonds are in the trans configuration (Rodriguez & Rodriguez-Amaya, 2007). It is also well known that high temperature and light conditions may promote the isomerization to the cis forms. The cis isomers of the provitamin A carotenoids have less activity than their corresponding all-trans carotenoids (Stahl & Sies, 2003; Rodriguez & Rodriguez-Amaya, 2007).

Drying is a widely used technology for the production of various materials of organic origin dehydrated such as foods and bio products. Besides, this type of dehydration is the usual drying

Received 07 Mar., 2017

Accepted 02 Apr., 2018

<sup>&</sup>lt;sup>1</sup> Universidade Federal do Pará – UFPA, Belém, PA, Brasil

<sup>\*</sup>Corresponding author: amcr@ufpa.br

process for sub-products. The drying operation is important in astaxanthin production in order to guarantee necessary moisture content for product extraction, without causing alterations in the material (Anderson & Sunderland, 2002; Niamnuy et al., 2008).

Drying processes performed in spouted beds with inert particles has been an emerging technology, very popular used for the drying of liquid materials such as pastes, suspensions and solutions including heat sensitive products and has been presented as an alternative to spray drying, in an attempt to obtain powdered products with the same quality, at low cost (Shuhama et al., 2003; Kutsakova, 2004; Souza & Oliveira, 2005; Benali & Amazouz, 2006; Cunha et al., 2006; Oliveira et al., 2008; Bezerra et al., 2013; Araújo et al., 2014; Serowik et al., 2017). However, in literature, spouted bed drying of astaxanthin under different conditions has not been studied.

With the aim to investigate the possibility astaxanthin recovery from pink shrimp waste, the present research proposed: (1) evaluate the application of spouted bed in drying of the shrimp waste paste, avaluating the effects of temperature on the physicochemical characteristics and nutritional properties, (2) investigate the viability of extraction of astaxanthin using vegetable oil (palm olein), as alternative to organic solvents and also evaluate the kinetic of extraction of astaxanthin from shrimp waste using palm olein. A comprehensive understanding of both, the drying and extraction steps is necessary to design the extraction process, avoid the denaturation of the product and determine feasible operating parameters

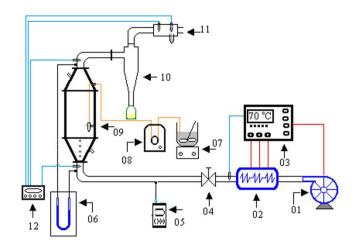
## 2 Materials and methods

#### 2.1 Preparation of shrimp waste

Waste from processing pink shrimp (*Farfantepenaeus subtilis*), comprising of head and carapace, was collected from a shrimp processing plant situated in the city of Belém (Pa, Brazil) and transported to the laboratory under frozen (-4 °C) conditions. The frozen shrimp waste was washed in running water, then ground and homogenized in an electric grinder (WALITA, RI 3148 SP, Brazil), until obtaining a homogeneous paste. The resulting paste was stored at -20 °C until use and analyzed.

### 2.2 Drying equipment

Shrimp waste paste was dried in a conical spouted bed with continuous feeding. A scheme of the experimental stand is presented in Figure 1. The dryer consists of a conical base, which has an inlet orifice diameter of 5.0 cm, included angle of 60°, made of stainless steel. Connected the conical base of the dryer a cylindrical column of glass with diameter and height of 20.0 cm and 40.0 cm respectively. The upper part of the equipment is composed of another cone and a cyclone. The air was supplied to the system through a radial blower (IBRAN, CR-8, Brazil) with power of 7.0 hp. It was heated in a system of four electric resistances of 1000 W each. The air temperature was measured by a type-K thermocouple and controlled by a PID controller (Contemp, IDO2B, Brazil). The air velocity was measured by a pitot tube, which was connected to a multifunction measuring device (Delta, HD2114P.O Italy). Gate valves were used to adjust the spouting air velocity. The inert particles used in the spouted



**Figure 1**. Spouted bed dryer. Blower (01); air heater (02); controller of electrical cur-rent (03); valve to regulation of the inlet air (04); flow rate indicator (05); differential pressure indicator (06); suspension (07); peristaltic pump (08); spouted bed (09); cyclone (10); psychrometer (11) and thermocouples (12).

bed were polyethylene pellets (diameter  $3.60 \pm 0.02$  mm, sphericity 0.850, density 905.23  $\pm$  3.82 kg/m<sup>3</sup>). The drying chamber was loaded with 2.0 kg of inert particles.

#### 2.3 Drying of shrimp waste

Shrimp waste paste was dried in three inlet air temperatures (70, 80 and 90 °C), and air velocity used in the experiments was 100% over minimum spouting velocity, as recommended by Mathur & Epstein (1974) for pastes drying. When a steady velocity regime and the desired temperature were established, the feed of the shrimp waste paste, was introduced into drying chamber (in the annular sliding layer), at a constant rate of  $7.0 \pm 0.15$  mL/min using a peristaltic pump (MILAN, BP-200, Brazil). In continuous operation, the droplets of the shrimp waste paste cover the surface of inert particles. This wet coat is dried by convective heat transfer from the upward hot air stream and by conduction of heat stored in the Polyetylene particles. The drying process continues until the moisture content drops to a critical value, the coating then becomes friable and fractures due to the attrition produced by the colliding between particle and particle collisions with the dryer walls. Dried shrimp waste in powder form was transported pneumatically by the drying air stream and collected in a cyclone. All the drying experiments were carried out in triplicate for each temperature.

#### 2.4 Characterization of paste and powder

The moisture content was determined gravimetrically using a oven at 105 °C (method n°. 934.01; Association of Official Analytical Chemists, 1995). The crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25 (method n°. 993.13; Association of Official Analytical Chemists, 1995). The lipid content was analysed gravimetrically following Soxhlet extraction (method n°. 920.39; Association of Official Analytical Chemists, 1995). The crude ash content was estimated by incineration in a muffle furnace at 550 °C (method n°. 942.05; Association of Official Analytical Chemists, 1995). The astaxanthin content in the shrimp paste was determined using the organic solvent extraction methodology (mixture isopropyl alcohol:n-hexane, 40:60 v/v), using the conditions optimized for maximum extraction yield as reported by Sachindra et al. (2006). Powder grain-size analysis was carried out in standardized mesh screen. The average diameter was calculated by definition of Sauter (Equation 1):

$$D_{PS} = \frac{1}{\sum_{i} \left( \frac{\Delta X}{D_{P}} \right)_{i}} \tag{1}$$

Where,  $D_{p_s}$  is the average diameter of Sauter,  $D_p$  is the arithmetic average diameter between two screens and  $\Delta X$  is the weight fraction of particles size  $D_p$ .

### 2.5 Fatty acids analysis

The fatty acid composition was determined by conversion to fatty acid in methyl esters (FAMEs) based on the suggested method by Lepage & Roy (1984) and detected using gas chromatography (Varian model CP 3380, USA) equipped with a flame ionization detector and with a CP-Sil 88 capillar column (length 60 m, internal diameter 0.25 mm, film thickness 0.25 µm; Varian Inc., USA). Operating conditions were: helium as carrier gas a flow rate of 0.9 mL/min, a FID detector at 250 °C, a injector (split ratio 1:100) at 250 °C, an injection volume of 1 µL. The temperature programmed of the column was 175 °C for 8 min, followed by 2.0 °C/min to 180 °C for 28 min and then 2.0 °C/min to 250 °C for 10 min. The individual fatty acid peaks were identified by comparison of retention times with those of known mixtures of standard fatty acids (Nu-check-prep, Inc., USA) run under the same operating conditions. Retention time and area of each peak were computed using the Varian Star 3.4.1. software. The results were expressed as relative percentages of total fatty acids.

#### 2.6 Extraction experiments

The extractions of astaxanthin from dried shrimp waste were carried out using methodology described by Handayani et al. (2008). The extractions were performed using palm olein at three different temperatures (50, 60, and 70 °C). 90 mL of palm olein were introduced in jacketed glass reactor connected to a thermostatic controlled water heater and with magnetic stirring. After the desired temperature reached, 2.5 g of shrimp waste in powder was added to palm oil and stirred. Following extraction, 3mL the suspension was centrifuged under 1520g (or 4500 rpm) for 5 min at 20 °C. The supernatant was removed and was used for the determination of concentration of astaxanthin. The supernatant was collected at regular intervals of 15 min, until the concentration become constant.

### 2.7 Yield of astaxanthin

The concentration of total carotenoid (presented as astaxanthin) in palm olein was measured spectrophotometrically using Spektrophometer, Shimadzu, UV-1200 series, operated at 485 nm wavelength as described by Sachindra & Mahendrakar (2005). The astaxanthin content was reported as µg astaxanthin/g waste using Equation 2:

Astaxanthin yield=
$$\frac{A \times V \times D \times 10^{6}}{100 \times W \times E}$$
(2)

Where, A = absorbance at  $\lambda_{max}$ , V = volume of pigmented oil recovered, D = dilution factor, W = weight of waste in grams and E = extinction coefficient.

#### 2.8 Kinetic models

Many studies have been conducted to describe the kinetics and the mechanism of the extraction process. Even if the principle of solid-liquid extraction is relatively simple, when this technique is applied for bioactive compounds, it entails a complex mechanism, because of the structure these compounds. Many mathematical models have been proposed by several authors to describe the extraction of different bioactive compounds from natural resources. In this work the mathematical model used was the one proposed by Handayani et al. (2008).

This model proposes that main mechanism that controls the rate of bioactive compounds extraction is mass transfer of bioactive compounds from solid to bulk liquid. Rate of mass transfer of astaxanthin (bioactive compound) from solid to bulk liquid (palm oil) can be expressed by the Equation 3:

$$\overline{N}_A = A_T k_L (C_{Ae} - C_A) \tag{3}$$

Where  $\bar{N}_A$  is the rate of astaxanthin mass transfer (µg/s),  $C_A$  and  $C_{Ae}$  are concentration of astaxanthin in bulk liquid and at equilibrium (µg/L), respectively. Here  $k_L$  is mass transfer coefficient and A is surface area for mass transfer process. Since the extraction was taken in batch process and its volume was kept constant during process, therefore

$$\overline{N}_{A} = \frac{VdC_{A}}{dt} \tag{4}$$

Substitution of Equation 4 into Equation 3 gives the following result (Equations 5, 6, 7):

$$\frac{VdC_A}{dt} = k_L A \left( C_{Ae} - C_A \right) \tag{5}$$

$$\frac{dC_A}{dt} = k_L \frac{A}{V} \left( C_{Ae} - C_A \right) \tag{6}$$

$$\frac{dC_A}{dt} = k_L . a \left( C_{Ae} - C_A \right) \tag{7}$$

Where  $k_L.a$  is volumetric mass transfer coefficient. Integration of Equation 7 considering at beginning of extraction process (t=0), the concentration of astaxanthin in bulk liquid is zero, CA = 0 and for any time the concentration of astaxanthin in bulk liquid is CA = CA. With those the initial and boundary conditions, integration of Equation 7 gives the following result:

$$C_A = C_{Ae} \left[ 1 - Exp(-k_L a.t) \right] \tag{8}$$

Equation 8 can be written in term of yield per mass of shrimp waste:

$$Y = Y_{eq} \left[ 1 - Exp(-k_L a.t) \right] \tag{9}$$

Where Y and  $Y_{eq}$  are yield of astaxanthin in bulk liquid and at equilibrium per mass of shrimp waste, respectively.

## 2.9 Statistical analysis

The one-way Analysis of variance (ANOVA) was conducted to determine the effect of variable factors on parameters of both, the drying and extraction steps using Microsoft excel<sup>®</sup>. The parameters of analytical model proposed (Equation 9), were estimated using the software Statistica for Windows 7.0 (STATSOFT Inc., USA). The fit quality of the proposed models for the extraction kinetics data was estimated by means of the correlation coefficient (r<sup>2</sup>).

## 3 Results and discussion

## 3.1 Shrimp waste paste composition

Shrimp waste paste obtained showed moisture content 71.90  $\pm$ 1.29 wt%, ashes 4.31 $\pm$ 0.52%, protein 15.92  $\pm$ 1.19%. These values are similar to values previously reported in the literature for shrimp waste (Babu et al., 2008; Brasileiro et al., 2012). Fat content obtained 4.43  $\pm$  0.19%, showed that the pink shrimp residues captured in Brazil are rich in this component and are in agreement with values reported in the literature (Bragagnolo & Rodriguez-Amaya, 1997; Ibrahim et al., 1999; Sánchez-Camargo et al., 2011), since the fat deposit is localized in the hepato-pancreas, which is in the head region. The yield of carotenoids from fresh waste was 38.91  $\pm$  1.18µg/g waste.

The results of the composition realized for the characterization of shrimp waste after drying are listed in Table 1. The results show that the moisture content of the powder obtained decrease from 10.85 to 8.70% (wb) as the increase of inlet air temperatures from 70 to 90 °C. These values were less than those reported by Niamnuy et al. (2008), around 15–16% (wb) in drying of white shrimp (Penaeus indicus) in jet spouted bed dryer. Devahastin et al. (2006) investigated the hydrodynamic behavior of a jet spouted bed, in drying of white shrimp (Penaeus indicus), reported that shrimp should therefore be dried to moisture content around 20% (wb). On the other hand, studies emphasize the influence this parameter on extraction efficiency of bioactive compounds (Dunford et al., 1997; Anderson & Sunderland 2002; Obeng et al., 2010; Wijngaard et al., 2012; Azmir et al., 2013). In general the higher the moisture content, lower the extraction yield of bioactive compounds such as carotene (Wijngaard et al., 2012). This can be explained by the fact that water can act as a barrier to diffusion of bioactive compounds out of the matrix decreasing the extraction efficiency.

It was also observed that the values of moisture content of the powder obtained in this work when comparing the commercial moisture content (around 10% w.b) the product obtained has good conditions for preservation and storage.

**Table 1**. Various quality attributes of dried shrimp waste at different drying conditions.

Property								
Moisture	Protein	Lipids	Ash	D <sub>ps</sub> (µm)				
()	()	()	( )	$\frac{(\mu m)}{124 \pm 60}$				
10100 - 1102	01100 - 1100	/110 = 0120	21110 - 0107	$124 \pm 0.0$ $155 \pm 10.0$				
510 I <u></u> 010 <u></u>	0 110 1 2 010 1	/ // 0 = 0.000	2101 2000	$135 \pm 10.0$ $186 \pm 8.0$				
	Moisture (%) 10.85±1.02 9.51±0.82 8.70±0.54	(%)         (%)           10.85±1.02         51.66±1.00           9.51±0.82         54.84±0.54	Moisture         Protein         Lipids           (%)         (%)         (%) $10.85 \pm 1.02$ $51.66 \pm 1.00$ $7.48 \pm 0.20$ $9.51 \pm 0.82$ $54.84 \pm 0.54$ $7.78 \pm 0.63$	Moisture         Protein         Lipids         Ash           (%)         (%)         (%)         (%)           10.85±1.02         51.66±1.00         7.48±0.20         21.48±0.87           9.51±0.82         54.84±0.54         7.78±0.63         21.31±0.90				

Values of lipids, protein and ash (Table 1) of the powder obtained show that, no significant differences with drying conditions. Compared to the data found in literature the shrimp waste proven to be a good source protein (Guerard et al., 2007; Babu et al., 2008; Sánchez-Camargo et al., 2011).

Other fundamental quality aspects are particle size. The temperature increase caused an increase in powder particle size (p < 0.05) (Table1). This behavior can be explained due to the modifications material proprieties with temperature increase, bigger particle sizes were obtained at higher temperature. Similar behavior was obtained by Dotto et al. (2011) in experimental production of chitosan powder in spouted bed. In this case, the temperature increase from 90 °C to 110 °C caused an increase in particle size from 100 to 200 µm. Studies emphasize the influence this parameter on extraction efficiency of bioactive compounds. In the study of Sun & Temelli (2006), the total carotenoid yield increased from 1110 to 1370 and 1504  $\mu$ g/g dry carrot with particle sizes of 1.0-2.0 mm to 0.5-1.0 mm and 0.3-0.5 mm respectively. Reducing the particle sizes obviously, increases the surface/volume ratio of the sample and consequently increases the contact between solid and fluid phase.

## 3.2 Drying operation

Through pressure drop velocity curves, the air drying velocity used in the experiments to guarantee spouted stability was determined. The pressure drop velocity curves obtained were similar to the generic pressure drop velocity curve showed by Mathur & Epstein (1974). The minimum spouting, the velocities found were 1.23 ms<sup>-1</sup>, 1.33 ms<sup>-1</sup> and 1.39 ms<sup>-1</sup> for temperatures of 70, 80 and 90 °C, respectively.

Due to the peculiarities of spouted beds, their application to industrial processes requires a clear vision of their fluid-dynamic since scale-up from laboratory experience is required. The fluid-dynamic behaviour of spouted beds was evaluated by determining the minimum spouting velocity through pressure drop velocity curves, the air drying velocity showed by Mathur & Epstein (1974).

Figure 2 illustrates the changes caused in outlet air temperature by feeding of the shrimp waste paste relative to the dry bed. All of the results show a sudden decrease in outlet air temperature at moment when the paste was added to the bed. The decrease can be explained by energy spent to vaporize the liquid films formed upon the surface of particle by adhesion of the paste; this in turn causes the particle temperature to also decrease as they experience evaporative cooling. This behavior was more pronounced in the first 7 min of drying. The outlet air temperature is not restored to its initial value but stabilizes at a lower and remains nearly constant along the drying. The same effect was observed by Dotto et al. (2011), in drying of chitosan in a spouted bed and Oliveira et al. (2008), in production of dried extract of Spirulina platensis in a spouted bed. The moment when the temperature became stable were considered to steady state condition. This behavior remained constant, until the end of the operation (60 min).

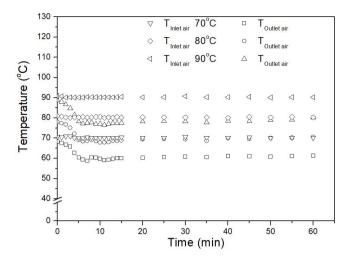


Figure 2. Outlet air drying temperature in Conical-cylindrical spouted.

The residence times of shrimp waste in the bed observed in this study, were 3.1, 2.8 and 2.4 min at 70, 80 and 90 °C, respectively. These values were less than those reported by Tacon & Freitas (2007), from 12.2 to 17.7 min in drying of the pastes in conventional spouted bed dryers. However, are higher than those reported in spray dryers (Kieviet & Kerkhof, 1995; Caparino et al., 2012). On the other hand, these results demonstrate that applying the spouted bed may have beneficial effects on the stability of carotenoids and other macromolecules from the residue, such as proteins and fatty acids by combination of the moderate temperature with reduced drying times.

#### 3.3 Fatty acid composition

The fatty acids profiles present in the shrimp waste dry in spouted bed are illustrated in Figure 3. Fourteen fatty acids were identified. No significant differences (p < 0.05), were found in the fatty acid composition of oil present in the shrimp residue when comparing between at different drying temperatures applied. The fatty acid composition found in this study was similar with a reported by Sánchez-Camargo et al. (2012). The results show that the percentages of saturated and polyunsaturated fatty acids were 43.65% and 56.35%, respectively. Of the saturated fatty acids, the most common were palmitic acid (22.05%) and stearic acid (15.95%) of the monounsaturated fatty acids, the most predominate were oleic acid (11.34%) and palmitoleic acid (8.90%). With respect to the polyunsaturated fatty acids, the eicosapentaenoic (EPA) and the docosahexanoic (DHA) components, fatty acids from  $\omega$ -3 group, are the most representative with 12.85% and 10.03%, respectively. Marine products constitute an important source of  $\omega$ -3 polyunsaturated fatty acids, mainly EPA and the DHA. They are essential for the development and function of certain organs and for several biochemical and physiological responses of the organism and cannot be synthesized by humans and must be obtained from the diet. Thus, the lipids present in pink shrimp residues constitute an important source of  $\omega$ -3 fatty acids, offering benefits to human health and in the enrichment of animal feeds.

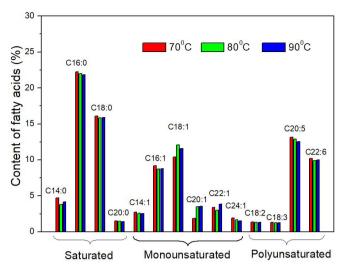


Figure 3. Fatty acids profile of pink-shrimp (*Farfantepenaeus brasiliensis*) waste.

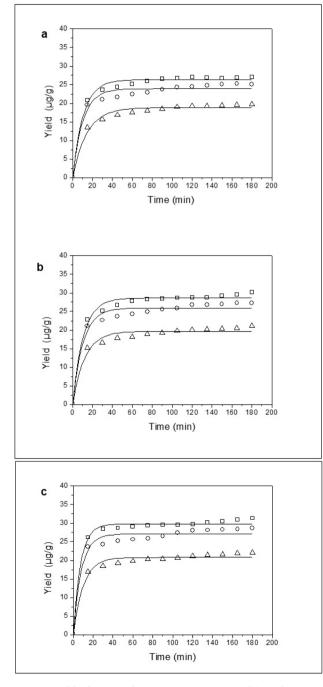
#### 3.4 Influence of temperature on kinetics of extraction

The evolution of the extraction yield of astaxanthin at different temperature of extraction and drying is shown in Figure 4. As can be seen, the yield of astaxanthin decreased with the increase of inlet air temperatures. This can be explained by the fact that as most carotenoids, astaxanthin is a highly unsaturated molecule and thus it is highly sensitive to high temperature, light, and oxidative conditions which may promote the isomerization of astaxanthin into cis form which possesses less activity than their corresponding trans configuration. The mathematical model described by Equation 9 shows a good fit to the experimental data, as can be seen in Figure 4. The model gave consistently high coefficient of determination (r<sup>2</sup>) values in the range 0.9685-0.9912. This indicates that the model could satisfactorily describe the extraction of astaxanthin at different temperature conditions.

The model constants are summarized in Table 2 the parameters Y<sub>e</sub> and k<sub>1</sub>.a were estimated by nonlinear least squares fit of Equation 9 to experimental kinetic data. It can be seen that the yield of astaxanthin in equilibrium per mass of shrimp waste (Y<sub>a</sub>), increases with increasing the extraction temperature, on the other hand, decreased with increasing of inlet air temperatures in spouted bed. The influence of drying temperature and extraction temperature on parameters Y and k, .a was analyzed by a one-way ANOVA, which revealed significant influence of both temperatures (p < 0.05) on the parameters Y and k.a. The increase in the extraction yield may be attributed to cleavage of carotenoprotein complex by thermal treatment, which resulted in increased uptake of pigments by the extraction medium. Sachindra & Mahendrakar (2005) obtained maximum carotenoids yield from shrimp waste using a sunflower oil process involving 2:1 ratio of oil to waste, heating the waste with oil at a temperature of 70 °C. The authors observed that increase in the extraction temperature above 70 °C resulted in decrease in carotenoid yield. As carotenoids are thermolabile compounds, it is advisable to use lower temperature for both processes (drying and extraction) for optimum extraction yield of carotenoids from

Extraction	Drying Temperature											
	70 °C			80 °C			90 °C					
T (°C)	Υ <sub>e</sub> fitted (µg/g)	Y <sub>e</sub> experimental (µg/g)	k <sub>L</sub> a (1/min)	R <sup>2</sup>	Υ <sub>e</sub> fitted (µg/g)	Y <sub>e</sub> experimental (µg/g)	k <sub>L</sub> a (1/min)	R <sup>2</sup>	Υ <sub>e</sub> fitted (µg/g)	Y <sub>e</sub> experimental (µg/g)	k <sub>L</sub> a (1/min)	R <sup>2</sup>
50	26.375	27.635	0.0936	0.9878	23.941	25.098	0.0949	0.9702	18.829	19.722	0.07	0.9785
60	28.623	30.087	0.0944	0.986	25.914	27.314	0.0968	0.9716	19.662	21.064	0.0824	0.9685
70	29.814	31.308	0.1347	0.9912	27.084	28.672	0.1228	0.9708	20.801	22.035	0.0983	0.9776

Table 2. Parameters Y<sub>e</sub> and k<sub>L</sub>.a at various temperatures for processes of drying and extraction calculated using mass transfer kinetic model.



**Figure 4.** Yield of astaxanthin at 50 °C (a), 60 °C (b), and 70 °C (c) and mass transfer kinetic model fit at various inlet air temperatures in a spouted bed dryer. Drying temperature of ( $\Box$ ) 70 °C; (o) 80 °C and ( $\Delta$ ) 90 °C.

shrimp waste. The value of parameter  $k_{L}$  a increases with increasing the extraction temperature and remained approximately constant with an increase in the inlet air temperatures in spouted bed (Table 2). This can be explained by the fact that the diffusivity is affected by temperature. As temperature increase, the diffusivity coefficient also increase leading to increase rate of mass transfer of astaxanthin from solid to bulk liquid of palm olein.

## **4** Conclusion

Drying of shrimp waste in spouted bed with inert particles aiming an astaxanthin recovery proved a good alternative. The product obtained has good conditions for preservation and storage with final moisture content around 10.85-8.70% (wb) and a high content of EPA and DHA fatty acids, but it was also observed that the yield of astaxanthin was influenced with increasing inlet air drying temperature. This is an interesting result for the industrial processing of this material. In the present work the best condition for drying of shrimp waste in spouted bed with inlet air drying temperature of 70 °C. In this condition, the vield of astaxanthin was around 50.42% in relation to value total astaxanthin in the fresh waste. It was observed that mass transfer kinetic model showed a good agreement (0.9685<r<sup>2</sup><0.9912), between the experimental and model calculated data, which allows the application of the above mentioned model for the purpose of modeling and optimization of the process of solid-liquid extraction of astaxanthin from shrimp waste using palm olein under different extraction temperature.

## Acknowledgements

The authors are thankful to CNPq (Process 408761/2006-7, 557479/2009-6), CAPES (PROCAD-NF Process 107/2009) and CNPQ (Universal Project process 477183/2013-1).

## References

- Ambati, R. R., Phang, S. M., Ravi, S., & Aswathanarayana, R. G. (2014). Astaxanthin: sources, extraction, stability, biological activities and its commercial applications–a review. *Marine Drugs*, 12(1), 128-152. http://dx.doi.org/10.3390/md12010128. PMid:24402174.
- Anderson, J. S., & Sunderland, R. (2002). Effect of extruder moisture and dryer processing temperature on vitamin C and E and astaxanthin stability. *Aquaculture (Amsterdam, Netherlands)*, 207(1-2), 137-149. http://dx.doi.org/10.1016/S0044-8486(01)00787-6.
- Araújo, A. D. A., Coelho, R. M. D., Fontes, C. P. M. L., Silva, A. R. A., Costa, J. M. C., & Rodrigues, S. (2014). Production and spouted bed drying of acerola juice containing oligosaccharides. *Food and*

*Bioproducts Processing*, 94, 565-571. http://dx.doi.org/10.1016/j. fbp.2014.08.005.

- Association of Official Analytical Chemists AOAC. (1995). Official methods of analysis of the Association of Official Analytical Chemists (Vol. 1, 16th ed.). Arlington: AOAC.
- Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 4(4), 426-436. http://dx.doi.org/10.1016/j.jfoodeng.2013.01.014.
- Babu, C. M., Chakrabarti, R., & Surya Sambasivarao, K. R. (2008). Enzymatic isolation of carotenoid-protein complex from shrimp head waste and its use as a source of carotenoids. *Lebensmittel-Wissenschaft + Technologie*, 41(2), 227-235. http://dx.doi.org/10.1016/j. lwt.2007.03.006.
- Benali, M., & Amazouz, M. (2006). Drying of vegetable starch solutions on inert particles: quality and energy aspects. *Journal of Food Engineering*, 74(4), 484-489. http://dx.doi.org/10.1016/j.jfoodeng.2005.01.045.
- Bezerra, C. V., Amante, E. R., Oliveira, D. C., Rodrigues, A. M. C., & Silva, L. H. M. (2013). Green banana (Musa cavendishii) flour obtained in spouted bed e effect of drying on physicoechemical, functional and morphological characteristics of the starch. *Industrial Crops and Products*, 41, 241-249. http://dx.doi.org/10.1016/j.indcrop.2012.04.035.
- Bragagnolo, N., & Rodriguez-Amaya, D. B. (1997). Optimization of cholesterol determination by HPLC and levels of cholesterol, total lipids and fatty acids of the pink shrimp (Penaeus brasiliensis). *Food Science and Technology (Campinas)*, 17(3), 275-280. http://dx.doi. org/10.1590/S0101-20611997000300016.
- Brasileiro, O. L., Cavalheiro, J. M. O., Prado, J. P. S., Anjos, A. G., & Cavalheiri, T. T. B. C. (2012). Determination of the chemical composition and functional properties of shrimp waste protein concentrate and lyophilized flour. *Ciência e Agrotecnologia*, 36(2), 189-194. http://dx.doi.org/10.1590/S1413-70542012000200007.
- Caparino, O. A., Tang, J., Nindo, C. I., Sablani, S. S., Powers, J. R., & Fellman, J. K. (2012). Effect of drying methods on the physical properties and microstructures of mango (Philippine 'Carabao' var.) powder. *Journal of Food Engineering*, 111(1), 135-148. http://dx.doi.org/10.1016/j.jfoodeng.2012.01.010.
- Chakrabarti, R. (2002). Carotenoprotein from tropical brown shrimp shell waste by enzymatic process. *Food Biotechnology*, 16(1), 81-90. http://dx.doi.org/10.1081/FBT-120004202.
- Chen, H. M., & Meyers, S. P. (1984). A rapid quantitative method for determination of astaxanthin pigment concentration in oil extracts. *Journal of the American Oil Chemists' Society*, 61(6), 1045-1047. http://dx.doi.org/10.1007/BF02636215.
- Cremades, O., Ponce, E., Corpas, R., Gutierrez, J. F., Jover, M., Alvarez-Ossorio, M. C., Parrado, J., & Bautista, J. (2001). Processing of Crawfish (Procambarus clarkii) for the Preparation of Carotenoproteins and Chitin. *Journal of Agricultural and Food Chemistry*, 49(11), 5468-5472. http://dx.doi.org/10.1021/jf0104174. PMid:11714345.
- Cunha, R. L., de la Cruz, A. G., & Menegalli, F. C. (2006). Effects of operating conditions on the quality of mango pulp dried in a spout fluidized bed. *Drying Technology*, 24(4), 423-432. http://dx.doi. org/10.1080/07373930600611869.
- Devahastin, S., Tapaneyasin, R., & Tansakul, A. (2006). Hydrodynamic behavior of a jet spouted bed of shrimp. *Journal of Food Engineering*, 74(3), 345-351. http://dx.doi.org/10.1016/j.jfoodeng.2005.03.012.
- Dhankhar, J., Kadian, S. S., & Sharma, A. (2012). Astaxanthin: A potential carotenoid. *International Journal of Pharmaceutical Science and Research*, 3, 1246-1259.

- Dotto, G. L., Souza, V. C., & Pinto, L. A. A. (2011). Drying of chitosan in a spouted bed: The influences of temperature and equipment geometry in powder quality. *Lebensmittel-Wissenschaft + Technologie*, 44(8), 1786-1792. http://dx.doi.org/10.1016/j.lwt.2011.03.019.
- Dunford, N. T., Temelli, F., & Le Blanc, E. (1997). Supercritical CO2 extraction of oil and residual proteins from atlantic mackerel (Scomber scombrus) as affected by moisture content. *Journal of Food Science*, 62(2), 289-294. http://dx.doi.org/10.1111/j.1365-2621.1997.tb03987.x.
- Guerard, F., Sumaya-Martinez, M. T., Laroque, D., Chabeaud, A., & Dufosse, L. (2007). Optimization of free radical scavenging activity by response surface methodology in the hydrolysis of shrimp processing discards. *Process Biochemistry*, 42(11), 1486-1491. http://dx.doi.org/10.1016/j.procbio.2007.07.016.
- Guerin, M., Huntley, M. E., & Olaizola, M. (2003). Haematococcus astaxanthin: applications for human health and nutrition. *Trends in Biotechnology*, 21(5), 210-216. http://dx.doi.org/10.1016/S0167-7799(03)00078-7. PMid:12727382.
- Handayani, A. D., Sutrisno, Indraswati, N., & Ismadji, S. (2008). Extraction of astaxanthin from giant tiger (Panaeus monodon) shrimp waste using palm oil: studies of extraction kinetics and thermodynamic. *Bioresource Technology*, 99(10), 4414-4419. http:// dx.doi.org/10.1016/j.biortech.2007.08.028. PMid:17911016.
- Higuera-Ciapara, I., Felix-Valenzuela, L., & Goycoolea, F. M. (2006). Astaxanthin: a review of its chemistry and applications. *Critical Reviews in Food Science and Nutrition*, 46(2), 185-196. http://dx.doi.org/10.1080/10408690590957188. PMid:16431409.
- Ibrahim, H. M., Salama, M. F., & El-Banna, H. A. (1999). Shrimp's waste: chemical composition, nutritional value and utilization. *Die Nahrung*, 43(6), 418-423. http://dx.doi.org/10.1002/(SICI)1521-3803(19991201)43:6<418::AID-FOOD418>3.0.CO;2-6.
- Kieviet, F., & Kerkhof, P. J. A. M. (1995). Measurements of particle residence time distributions in a co-current spray dryer. *Drying Technology*, 13(5-7), 1241-1248. http://dx.doi.org/10.1080/07373939508917019.
- Kutsakova, V. E. (2004). Drying of liquid and pasty products in a modified spouted bed of inert particles. *Drying Technology*, 22(10), 2343-2350. http://dx.doi.org/10.1081/DRT-200040018.
- Lepage, G., & Roy, C. C. (1984). Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *Journal of Lipid Research*, 25(12), 1391-1396. PMid:6530596.
- Lorenz, R. T., & Cysewski, G. R. (2000). Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. *Trends in Biotechnology*, 18(4), 160-167. http://dx.doi.org/10.1016/ S0167-7799(00)01433-5. PMid:10740262.
- Mathur, K. B., & Epstein, N. (1974). *Spouted beds*. New York: Academic Press.
- Mezzomo, N., Martínez, J., Maraschin, M., & Ferreira, S. R. S. (2016). Pink shrimp (P. brasiliensis and P. paulensis) residue: supercritical fluid extraction of carotenoid fraction. *The Journal of Supercritical Fluids*, 74, 22-33. http://dx.doi.org/10.1016/j.supflu.2012.11.020.
- Miki, W. (1991). Biological functions and activities of animal carotenoids. *Pure and Applied Chemistry*, 63(1), 141-146. http://dx.doi.org/10.1351/pac199163010141.
- Naguib, Y. M. A. (2000). Antioxidant activities of astaxanthin and related carotenoids. *Journal of Agricultural and Food Chemistry*, 48(4), 1150-1154. http://dx.doi.org/10.1021/jf991106k. PMid:10775364.
- Niamnuy, C., Devahastin, S., Soponronnarit, S., & Vijaya Raghavan, G. S. (2008). Kinetics of astaxanthin degradation and color changes of dried shrimp during storage. *Journal of Food Engineering*, 87(4), 591-600. http://dx.doi.org/10.1016/j.jfoodeng.2008.01.013.

- Obeng, G. Y., Adjaloo, M. K., & Donkor P. (2010). Effect of temperature, moisture content, particle size and roasting on shea butter extraction efficiency. *International Journal of Food Engineering*, 6(2), 1-11.
- Ogawa, M., Maia, E. L., Fernandes, A. C., Nunes, M. L., Oliveira, M. E. B., & Freitas, S. T. (2007). Waste from the processing of farmed shrimp: a source of carotenoid pigments. *Food Science and Technology (Campinas)*, 27(2), 333-337. http://dx.doi.org/10.1590/ S0101-20612007000200022.
- Oliveira, E. G., Rosa, G. S., Moraes, M. A., & Pinto, L. A. A. (2008). Phycocyanin content of Spirulina platensis dried in spouted bed and thin layer. *Journal of Food Process Engineering*, 31(1), 34-50. http://dx.doi.org/10.1111/j.1745-4530.2007.00143.x.
- Omara-Alwala, T., Chen, H. M., Ito, Y., Simpson, K. L., & Meyers, S. P. (1985). Carotenoid pigment and fatty acid analysis of crawfish oil extracts. *Journal of Agricultural and Food Chemistry*, 33(2), 260-263. http://dx.doi.org/10.1021/jf00062a026.
- Prameela, K., Venkatesh, K., Immandi, S. B., Kasturi, A. P. K., Rama Krishna, C., & Murali Mohan, C. (2017). Next generation nutraceutical from shrimp waste: The convergence of applications with extraction methods. *Food Chemistry*, 237, 121-132. http://dx.doi.org/10.1016/j. foodchem.2017.05.097. PMid:28763972.
- Pu, J., & Sathivel, S. (2011). Kinetics of lipid oxidation and degradation of flaxseed oil containing crawsifh (Procambarus clarkii) astaxanthin. *Journal of the American Oil Chemists' Society*, 88(5), 595-601. http:// dx.doi.org/10.1007/s11746-010-1713-8.
- Pu, J., Bankston, J. D., & Sathivel, S. (2011). Production of microencapsulated crawfish (Procambarus clarkii) astaxanthin in oil by spray drying technology. *Drying Technology*, 29(10), 1150-1160. http://dx.doi.or g/10.1080/07373937.2011.573155.
- Rodriguez, E. B., & Rodriguez-Amaya, D. B. (2007). Formation of apocarotenals and epoxycarotenoids from β-carotene by chemical reactions and by autoxidation in model systems and processed foods. *Food Chemistry*, 101(2), 563-572. http://dx.doi.org/10.1016/j. foodchem.2006.02.015.
- Sachindra, N. M., & Mahendrakar, N. S. (2005). Process optimization for extraction of carotenoids from shrimp waste with vegetable oils. *Bioresource Technology*, 96(10), 1195-1200. http://dx.doi.org/10.1016/j. biortech.2004.09.018. PMid:15683912.
- Sachindra, N. M., Bhaskar, N., & Mahendrakar, N. S. (2006). Recovery of carotenoids from shrimp waste in organic solvents. *Waste Management (New York, N.Y.)*, 26(10), 1092-1098. http://dx.doi. org/10.1016/j.wasman.2005.07.002. PMid:16129592.
- Sachindra, N. M., Bhaskar, N., Siddegowda, G. S., Sathisha, A. D., & Suresh, P. V. (2007). Recovery of carotenoids from ensilaged shrimp waste. *Bioresource Technology*, 98(8), 1642-1646. http://dx.doi. org/10.1016/j.biortech.2006.05.041. PMid:16828548.
- Sánchez-Camargo, A. P., Martinez-Correa, H. A., Paviani, L. C., & Cabral, F. A. (2011). Supercritical CO<sub>2</sub> extraction of lipids and astaxanthin from Brazilian redspotted shrimp waste (Farfantapenaeus paulensis). *The Journal of Supercritical Fluids*, 56(2), 164-173. http://dx.doi. org/10.1016/j.supflu.2010.12.009.
- Sánchez-Camargo, A. P., Meireles, M. A. A., Ferreira, A. L. K., Saito, E., & Cabral, F. A. (2012). Extraction of ω-3 fatty acids and astaxanthin from Brazilian redspotted shrimp waste using supercritical CO2 + ethanol mixtures. *The Journal of Supercritical Fluids*, 61, 71-77. http://dx.doi.org/10.1016/j.supflu.2011.09.017.

- Serowik, M., Figiel, A., Nejman, M., Pudlo, A., Chorazyk, D., & Kopec, W. (2017). Drying characteristics and some properties of spouted bed dried semi-refined carrageenan. *Journal of Food Engineering*, 194, 46-57. http://dx.doi.org/10.1016/j.jfoodeng.2016.09.007.
- Shahidi, F., & Synowiecki, J. (1991). Isolation and characterization of nutrients and value added products from snow crab (Chinoecetes opilio) and shrimp (Pandalus borealis) processing discards. *Journal* of Agricultural and Food Chemistry, 39(8), 1527-1532. http://dx.doi. org/10.1021/jf00008a032.
- Shuhama, I. K., Aguiar, M. L., Oliveira, W. P., & Freitas, L. A. P. (2003). Experimental production of annatto powders in spouted bed dryer. *Journal of Food Engineering*, 59(1), 93-97. http://dx.doi.org/10.1016/ S0260-8774(02)00433-8.
- Sila, A., Kamoun, Z., Ghlissi, Z., Makni, M., Nasri, M., Sahnoun, Z., Nedjar-Arroume, N., & Bougatef, A. (2015). Ability of natural astaxanthin from shrimp by-products to attenuate liver oxidative stress in diabetic rats. *Pharmacological Reports*, 67(2), 310-316. http://dx.doi.org/10.1016/j.pharep.2014.09.012. PMid:25712656.
- Silva, F. O., Tramonte, V. L. C. G., Parisenti, J., Lima-Garcia, J. F., Maraschin, M., & Silva, E. L. (2015). Litopenaeus vannamei muscle carotenoids versus astaxanthin: a comparison of antioxidant activity and in vitro protective effects against lipid peroxidation. *Food Bioscience*, 9, 12-19. http://dx.doi.org/10.1016/j.fbio.2014.11.001.
- Souza, C. R. F., & Oliveira, W. P. (2005). Spouted bed drying of Bauhinia forficate link extract: the effects of feed atomizer position and operating conditions on equipment performance and product properties. *Brazilian Journal of Chemical Engineering*, 22(2), 239-247. http:// dx.doi.org/10.1590/S0104-66322005000200011.
- Stahl, W., & Sies, H. (2003). Antioxidant activity of carotenoids. *Molecular Aspects of Medicine*, 24(6), 345-351. http://dx.doi.org/10.1016/ S0098-2997(03)00030-X. PMid:14585305.
- Stepnowski, P., Olafsson, G., Helgason, H., & Jastorff, B. (2005). Recovery of astaxanthin from seafood wastewater utilizing fish scales waste. *Chemosphere*, 54(3), 413-417. http://dx.doi.org/10.1016/S0045-6535(03)00718-5. PMid:14575754.
- Sun, M., & Temelli, F. (2006). Supercritical carbon dioxide extraction of carotenoids from carrot using canola oil as a continuous co-solvent. *The Journal of Supercritical Fluids*, 37(3), 397-408. http://dx.doi. org/10.1016/j.supflu.2006.01.008.
- Tacon, L. A., & Freitas, L. A. A. (2007). Paste residence time in a spouted bed dryer. III: effect of paste properties and quality interactions. *Drying Technology*, 25(5), 841-852. http://dx.doi. org/10.1080/07373930701370225.
- Wijngaard, H., Hossain, M. B., Rai, D. K., & Brunton, N. (2012). Techniques to extract bioactive compounds from food by-products of plant origin. *Food Research International*, 46(2), 505-513. http:// dx.doi.org/10.1016/j.foodres.2011.09.027.
- Yang, S., Zhang, T., Xu, J., Li, X., Zhou, Q., & Xue, C. (2015). Chiral separation and analysis of astaxanthin stereoisomers in biological organisms by high-performance liquid chromatography. *Food Science*, 36, 139-144.
- Yang, Y., Bae, M., Kim, B., Park, Y. K., Koo, S. I., & Lee, J. Y. (2016). Astaxanthin prevents and reverses the activation of mouse primary hepatic stellate cells. *The Journal of Nutritional Biochemistry*, 29, 21-26. http://dx.doi.org/10.1016/j.jnutbio.2015.11.005. PMid:26895661.