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Article

Chemical Characteristics and Antioxidant Activity of Astaxanthin Extracted from Shrimp Residues Using Soybean Oil

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Shrimp residues generated by the seafood industrial processing are generally discarded in the environment without any further treatment, leading to a loss of nutritional value such as proteins, lipids, chitin, carotenoids, and minerals. This practice can lead to environmental problems due to the decomposition of these substances in the air. The objective of this study was to extract astaxanthin-rich materials from shrimp (*Litopenaeus vannamei*) industrial residue (WO) and smaller-scale residue meal (MO) in processing shrimp with soybean oil to evaluate their physicochemical characteristics, fatty acid content, and antioxidant potential. WO and MO were found to contain 27.48 and 33.34 μg g^{-1} of astaxanthin, respectively. The physicochemical properties of pigmented oils are established by legislation. The MO material showed significantly higher antioxidant activity compared to the soybean oil (control), especially when based on the oxygen radical absorbance capacity (ORAC) test, which showed antioxidant activity of 0.484 and 0.264 μmol eq Trolox g^{-1} for the MO and soybean oil (control), respectively, possibly accompanying their respective astaxanthin contents in the MO sample. The pigmented oils from both the shrimp residues showed significant potential for being used in the food industry due to their affordability and their high antioxidant activity.

Keywords: fatty acid profile, physicochemical characterization, ORAC, antioxidant activity, waste material

Introduction

Brazilian shrimp production has been significantly increasing in the last years. The Northeast region is the largest regional producer, and corresponds to 99.4% of the Brazilian national production of the *Litopenaeus vannamei* species.[1] The residues generated from shrimp industrial processing are formed by the shell and cephalothorax, which correspond to 50-60% of the crustacean weight.[2] These residues are generally discarded secretly in the sea and rivers or are buried, thereby causing environmental problems, especially in places without strict environmental inspection.[3] Eliminating waste generates expenses for the beneficiary companies, lowering their profits and increasing the industry's waste of compounds.[4]

This residue contains a considerable amount of functional substances such as proteins, lipids, chitin, and carotenoid pigments,[5] including astaxanthin. Studies about industrial waste use which would reduce the consequences of environmental accumulation and the recovery of valuable compounds such as astaxanthin have increased.[6]

Astaxanthin (3,3-dihydroxy-β, β-carotene-4,4-dione) is the primary carotenoid present in shrimp residue, which can be found in its free or esterified form.[7] There are several scientific and commercial applications for astaxanthin because it is considered a bioactive natural compound

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and can be used in several areas. For example, as cellular markers and antioxidant in the pharmaceutical industry; a coloring and antioxidant agent in the cosmetic area, and as a supplement or additive for feeding salmonids, shrimp, and lobsters to intensify the meat pigmentation,[8] and in chicken feed to improve the yellow color of the egg yolk in the food industry.[9]

The search for new natural astaxanthin sources has resulted in developing several methods to extract the pigment from shrimp waste, for example, enzymatic hydrolysis,[10] fermentation process,[11] the use of organic solvents[12] and ultrasound.[13] However, these methods are expensive and may promote a structural change, leading to a loss in the functionality of astaxanthin.[5]

Therefore, it is necessary to carry out studies using alternative extraction techniques. Astaxanthin extraction using vegetable oil does not require eliminating solvent as in conventional extraction,[14] thereby avoiding thermal pigment degradation. Moreover, the obtained oil can be added to industrialized products to intensify coloration and provide health benefits due to the presence of carotenoids.[15]

In view of the above, this study had the objective to extract astaxanthin from shrimp (*Litopenaeus vannamei*) residues using soybean oil to determine the amount of astaxanthin present in each oil and evaluate their physicochemical characteristics and antioxidant potential.

Experimental

Raw material

Litopenaeus vannamei shrimp residues (cephalothorax) were kindly provided by the Enseg Indústria Alimentícia Ltda., which is located in the city of Macaíba (Rio Grande do Norte, Brazil). The shrimp residues (3 kg) were packed and stored under refrigeration and carried to the Food Analysis Laboratory of the Nutrition Department of the Federal University of Rio Grande do Norte (UFRN). They were split into two portions. The first portion was chopped in a blender (Philips Walita, Mod. 6000W, São Paulo, Brazil) and stored at −20 °C until use. The second was dried at 70 °C in a ventilated oven (Tecnal, Mod. TE-394/1, Piracicaba, SP, Brazil) for 8 h, and then chopped to obtain shrimp residue meal according to the procedure described by Seabra *et al*.[16] and then stored at 10 °C until use.

Astaxanthin extraction from shrimp waste using soybean oil

Astaxanthin was extracted from both shrimp residue and residue meal according to the procedure by Sachindra and Mahendrakar.[17] First, 10 g of each sample were homogenized in 20 and 40 mL soybean oil, respectively, for the shrimp industrial residue and residue meal. Next, the samples were heated in a water bath (Marconi, Dubinoff, Mod. MA-093/1, Zhejiang, China) at 70 °C for 2 h. The mixture was subsequently filtered using gauze and centrifuged (Fanem, Excelsa 4, Mod. 280R, São Paulo, Brazil) at 1600 RPM for 10 min at 25 °C, to separate the pigment (supernatant), thus obtaining the pigmented oil of the industrial residue (WO), and the residue meal (MO).

Physicochemical characterization of the pigmented oils

The density analyses were performed with a densimeter (Anton Paar, Mod. DMA 4500M, Champaign, IL, USA) at 25 °C. The absolute viscosity was determined by a viscometer (Brookfield, Mod. R/S Rheometer, Middleboro, MA, USA) at 25 °C. The refractive index was evaluated using a refractometer.[18]

Next, the acidity, peroxide, saponification, and iodine indexes were also evaluated at 25 °C.[18] All these analyses were performed in triplicate. The original soybean oil was used as a control.

Determination of the fatty acid profile by gas chromatography (GC)

The fatty acid profile was determined via the formation of fatty acid methyl esters, as described by Hartman and Lago.[19] The fatty acid determination was adapted from reported protocols in the scientific literature.[20,21] They were quantified by normalizing the peak areas and identified by the mass spectra database library (NIST) using a GCMS-QP2010 (Shimadzu, Kyoto, Japan) equipped with a Durabound DB-23 column (30 × 0.25 mm × 0.25 μm). The injection port and detector temperature were fixed at 230 °C, whereas the column temperature was set at 90 °C. The elution gradient in the column was 90 to 150 °C (10 °C min^{-1}), 150 to 200 °C (2 °C min^{-1}), and 200 to 230 °C (10 °C min^{-1}) in a total run of 42 min with a split of 100. The carrier gas was He.[20]

Quantification of astaxanthin in pigmented oils

The pigmented oils (WO and MO samples) were analyzed by high-performance liquid chromatography diode-array detector (HPLC-DAD) to determine the astaxanthin concentration and absorption spectra at 450 nm, according to Ranga *et al*.[22] First, 1 mL of pigmented oil and 3 mL of dichloromethane:methanol (1:2, v:v) were mixed for 2 min using a vortex mixer. Next, 1.5 mL hexane was added, mixed, and centrifuged at 1000 g for 15 min. The hexane/dichloromethane upper phase was collected. The

extraction procedure was repeated twice with 1 mL of dichloromethane and 1.5 mL of hexane. The pooled extracts were evaporated under a nitrogen stream.

The HPLC (Shimadzu, Kyoto, Japan) presented a UV-visible detector model SPD-10AV using a reverse phase C18 column (4.6 × 25 mm, Shimadzu, Japan). The extract was solubilized in the mobile phase (1 mg mL^{-1}) for the injection containing dichloromethane:acetonitrile: methanol (20:70:10, v:v:v). The analysis was performed under an isocratic condition and was injected with 20 µL of solution. The flow used in the column was 1.0 mL min^{-1}. A calibration curve for the astaxanthin (Sigma-Aldrich, Saint Louis, MO, USA) was previously constructed.

Antioxidant capacity of the pigmented oils

Extract preparation

The WO and MO extracts and the soybean oil (control) were obtained according to Espín *et al.*[23] with modifications: first, 5 mL of each oil were mixed with 5 mL of methanol and vigorously stirred (ACB Labor, Mod. AC-045, São Paulo, Brazil) for 20 min and centrifuged (Fanem, Excelsa 4, Mod. 280R, Piracicaba, Brazil) at 3000 × g for 10 min at 25 °C. Next, the methanolic layer 1 (ML 1, supernatant) was collected and stored. Then, 5 mL of methanol were added into the lipid layer (LL, precipitated) and the extraction procedure was repeated. The methanolic layer 2 (ML 2) was again collected and mixed with ML 1, forming a methanolic extract. Two extracts of each pigmented oil were obtained (ML and LL) and stored at –20 ºC until the analyses were performed (Figure 1).

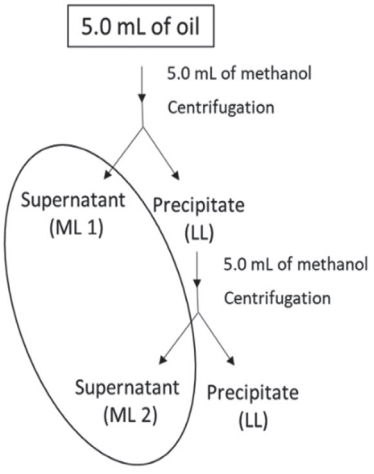

Figure 1. Extract preparation chart flow.

Two or more techniques are usually used to analyze the antioxidant activity in vegetable oils as there are several types of free radicals and different action sites.[24] Thus, evaluation tests of the total antioxidant capacity (TAC), reducing power, hydroxyl radical sequestration (OH), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical sequestration activity, and the oxygen radical absorbance capacity (ORAC) method were used to increase the effectiveness of the results.

Total antioxidant capacity (TAC) determination

The method proposed by Prieto *et al.*[25] was used for the TAC evaluation: first, 100 µL of extracts were combined with 100 µL of the 4 mM ammonium molybdate:0.6 M sulfuric acid solution, 100 µL 28 mM sodium phosphate and 700 µL distilled water. Distilled water was used as the blank instead of the extract. The tubes were incubated at 95 °C for 90 min, and the absorbance was then measured at 695 nm using a spectrophotometer (Biospectro UV-VIS SP-220, Curitiba, Brazil) after cooling to room temperature. The antioxidant activity was expressed in milligrams of ascorbic acid *per* gram of sample (mg AA g^{-1}) using a standard curve constructed for different ascorbic acid concentrations (25-250 mg g^{-1}).

Reducing power test

The reducing power test was conducted according to the method of Wang *et al.*[26] First, 200 µL of samples and 100 µL of potassium ferricyanide (1% m:v) were mixed and incubated at 50 °C for 20 min. Next, 180 µL of 10% (m/v) trichloroacetic acid (TCA), 20 µL of ferric chloride (0.1% m:v) and 1.5 mL of phosphate buffer (0.2 M, pH 6.6) were added to the mixture. The tubes were shaken and the absorbance was measured at 700 nm with a spectrophotometer (Biospectro UV-VIS SP-220, Curitiba, Brazil). The antioxidant activity was expressed in milligrams of ascorbic acid *per* gram of sample (mg AA g^{-1}) using a standard curve constructed with different ascorbic acid concentrations (100-1000 mg g^{-1}).

Hydroxyl radical sequestration

This parameter was evaluated according to the methodology proposed by Smirnoff and Cumbes.[27] First, 750 µL of reagent was added to all tubes with the samples, including the blank and control. Next, 50 µL of the extract was added, and 50 mL of 150 mM phosphate buffer pH 7.4 was added to the blank and control. Then, 200 µL of 30% hydrogen peroxide and 200 µL of phosphate buffer, respectively, were added into the tubes with the samples and control. The contents of each tube were mixed and incubated in a water bath (Quimis, Mod. Q334M-28, Diadema, SP, Brazil) at 37 °C for 60 min, and its absorbance was measured at 510 nm in triplicate. Results were expressed as inhibition percentage (%).

DPPH· (2,2-diphenyl-1-picrylhydrazyl) activity on the radical elimination activity

The antioxidant activity was determined according to the method of Nóbrega *et al*.,[28] with modifications for the use of 96-well microplates. The absorbance was measured at 517 nm using a BioChrom ASYS UVM 340 spectrophotometer (Cambridge, UK), and the calibration curve was constructed with concentrations of 30 to 200 μM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The results are expressed in micromoles of Trolox equivalents *per* gram of sample (μmol eq Trolox g^{-1}).

Oxygen radical absorption capacity (ORAC)

The antioxidant activity by the ORAC method was determined according to Ganske *et al*.[29] with modifications. First, 1 mL of extracts were diluted into 1 mL of 7% (m:v) methylated β-cyclodextrin in acetone:water (1:1 v:v) as a solubility enhancer, with subsequent stirring for 10 s. In 96-well microplates, 20 μL of diluted extracts were mixed with 120 μL of fluorescein (10 mM phosphate buffered saline (PBS) buffer, pH 7.4). The microplates were incubated for 10 min at 37 °C and 60 μL of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) 10.85 g L^{-1} in PBS buffer were added. The fluorescence intensity (485 nm excitation and 528 nm emission) was evaluated using a spectrophotometer (BMG LABTECH, Fluostar Optima, Ortenberg, Germany) every 3 min until 180 min. The antioxidant activity was expressed in micromoles of Trolox equivalents *per* gram of sample (μmol eq Trolox g^{-1}) using a standard curve constructed with different concentrations of Trolox (3.125-50.000 μg mL^{-1}).

Statistical analysis

An analysis of variance (ANOVA) with Tukey's post-hoc test was performed at a significance level of 5% to verify whether there was a significant difference between the three analyzed oils. The results were organized in tables and submitted to descriptive statistics using the XLStat® software program.[30]

Results and Discussion

Pigmented oil physical-chemical characterization

The density regarding the physical-chemical parameters ranged significantly ($p < 0.05$) between the samples, and the viscosity was considered the same for the WO, MO and SO samples (Table 1).

In addition, the WO and MO samples did not show any significant difference in the refractive index compared to the soybean oil.

The acidity of the two pigmented oil samples (WO and MO) was significantly higher ($p < 0.05$) than in the reference standard (soybean oil). The MO sample presented a higher acidity index ($p < 0.05$) than the WO sample (Table 1).

However, no significant change was found in the peroxide level (Table 1), suggesting that astaxanthin extraction did not alter the peroxidation degree of the pigmented oils. Similar data were found by Pu *et al*.[31] in astaxanthin extraction using linseed oil. In terms of identity characteristics, MO had a significantly higher saponification value ($p < 0.05$) than the other oils, mainly due to the thermal process used in the shrimp residue meal preparation. No significant differences were found in the iodine values (Table 1), suggesting that there was no difference in the unsaturation degree of the assessed oils.

The increase in the acidity index in MO and WO may be due to lipases present in the oils (fermentation). Fermentation is caused by microorganism contamination which can develop in the oil.[32] In addition, no treatment was performed on these oils after the astaxanthin extraction process. Vegetable oils which go through the refining process, as in the case of soybean oil (SO), have low acidity due to the neutralization process which they are submitted

Table 1. Physical-chemical characterization of pigmented oils containing astaxanthin (WO and MO) and soybean oil (SO) according to the different methods

Analysis	WO	MO	SO
Density / (kg m^{-3})	916.2	918.0	916.1
Viscosity / (Pa s)	0.05	0.05	0.05
Refraction value	1.5	1.5	1.5
Acidity content / (mg KOH g^{-1})	0.6	1.1	0.3
Peroxide index / (mEq 1000 g^{-1})	0.5	0.6	0.5
Saponification index / (mg KOH g^{-1})	219.2^{b} (1.9)	227.2^{a} (1.3)	216.7^{b} (1.1)
Iodine index / (g I_2 100 g^{-1})	121.4^{a} (2.0)	122.5^{a} (1.5)	122.9^{a} (2.0)

^{a,b}Values are shown as mean ± standard deviation in triplicates. Means on the same line, followed by different letters differ significantly ($p < 0.05$) according to the Tukey's test (0.05). WO: pigmented oil of the waste; MO: shrimp waste meal oil; SO: soybean oil.

to.[33] In contrast, some authors[34] did not observe changes in acidity in oil containing astaxanthin extracted from algae (*Haematococcus pluvialis*) and submitted to heating at 70 and 90 °C, or with linseed oil to extract astaxanthin from shrimp (*Litopena eussetiferus*) residue under heating at 70 °C.[31]

The low peroxide level is related to the protective action of natural antioxidants, such as astaxanthin, against peroxide formation, and therefore against oxidative rancidity by reducing peroxide formation and consequently increasing oxidation stability.[34] This result is consistent with the findings of Pu *et al*.,[31] who evaluated the stability of linseed oil containing astaxanthin and found no changes in peroxide levels.

The iodine value tends to decrease when the oil undergoes oxidation due to the decrease in the proportion of polyunsaturated fatty acids. However, this process is delayed due to the natural antioxidant action of the astaxanthin.[35]

Fatty acids profile

The fatty acid composition of pigmented oils and soybean oil (control) are presented in Table 2.

When compared to the soybean oil, the pigmented oil shows an increase in palmitic (C16:0), stearic (C18:0), linoleic (C18:1), and linolenic (C18:2) acids, while the oleic acid (C18:1) content is lowered.

The amount of polyunsaturated fatty acids found in WO and MO (62.01 and 61.43% for WO and MO, respectively) was greater than the soybean oil values.

Gómez-Estaca *et al*.[36] reported that the fatty acids in the shrimp residue corroborate that the increase of fatty acids in soybean oil. The reduction of oleic acid may have occurred due to the oxidation of some of the oil since it is very susceptible to degradation. The amount of saturated (15.69 and 15.96% for WO and MO, respectively) and polyunsaturated fatty acids (62.01 and 61.43% for WO and MO, respectively) found in this study are higher than those found by Goméz-Estaca *et al*.[36]

Roy *et al*.[37] obtained shrimp astaxanthin from *Penaeus monodon* by simultaneous extraction using supercritical CO_2 and found monounsaturated fatty acids (MUFA) values (21.02%) close to those found in the present study (22.29 and 22.62% for WO and MO, respectively). However, the results found for polyunsaturated fatty acids (PUFAs) were 38.94% lower[37] compared to the extraction with soybean oil (62.01 and 61.45% for WO and MO, respectively). Thus, several factors may be associated with this difference in fatty acids as an extraction method in shrimp species.[38,39] According to Takeungwongtrakul *et al*.,[40] the high concentrations of PUFAs are because they are the primary fatty acids found in white shrimp.

Quantification of astaxanthin by HPLC

The HPLC analysis of the WO and MO astaxanthin content showed 27.48 and 33.34 μg g^{-1}, respectively.

Carotenoid content mainly varies due to the heat treatment used to obtain the shrimp from residue meal, which also makes the astaxanthin more accessible because of the breakage of the bonds with proteins. Increasing the temperature leads to an irreversible denaturation of the carotene-protein complexes, resulting in a more intense orange color. The resulting sample has an astaxanthin concentration of 1.2 times the samples which did not undergo this process.[41]

Takeungwongtrakul *et al*.[40] extracted the astaxanthin present in the cephalothorax and hepatopancreas from shrimp using acetate as a solvent, yielding results of 3.10 and 1.89 μg g^{-1} of lipids, respectively. Several factors are related to the variation in astaxanthin contents, for example, the extraction method, type of solvent used (its

Table 2. Profile of fatty acids of pigmented oils and soybean oil

Fatty acid	Soybean / %	WO / %	MO / %
C16:0 (palmitic acid)	11.01	12.21	12.26
C18:0 (stearic acid)	3.22	3.48	3.70
C18:1n9c (oleic acid)	26.16	20.90	21.38
C18:1n9t (elaidic acid)	1.72	1.39	1.24
C18:2n6c (linoleic acid)	52.83	56.16	55.58
C18:3(9, 12, 15)n3c (linolenic acid)	5.05	5.85	5.85
Saturated fatty acids	14.23	15.69	15.96
Monounsaturated fatty acids (MUFA)	27.88	22.29	22.62
Polyunsaturated fatty acids (PUFA)	57.88	62.01	61.43

WO: pigmented oil of the waste; MO: shrimp waste meal oil.

polarity and solubility of astaxanthin), the size of the residue particles, the ratio of the residue to oil, and the shrimp species used.[5,42]

Extracting astaxanthin using vegetable oil has the advantage of not needing any solvent elimination, which could lead to the thermal degradation of the pigment. In addition, the obtained oil can not only be used as a dye, but also to increase the oxidative stability of the product.[36]

Astaxanthin extraction using vegetable oils contributes to its stability because it provides a protective barrier against oxygen, delaying the oxidation processes. Furthermore, the extraction oil serves as an excellent transporter of carotenoids when it is applied to food supplements.[7,31] Shrimp residue is already used in several industry sectors as a source of carotenoids for different purposes.[8] Still, there are few reports in the literature regarding the use of pigmented oils with this carotenoid. Thus, because of the astaxanthin concentration in pigmented oils, our product has significant potential for use in food as a natural antioxidant.

Pigmented oil antioxidant capacity

The MO samples and its fractions (lipid layer (MO_{LL}) and methanolic layer (MO_{ML})) showed the best antioxidant activity ($p < 0.05$) (Tables 3-5) in most of the tests performed.

According to Table 3, the WO samples showed higher antioxidant activity for the TAC, reducing power, and ORAC tests, which can be explained by the higher astaxanthin concentration in the oil. The antioxidant activity results in the DPPH and hydroxyl radical tests were similar for the three samples (WO, MO and SO). This corroborates the data of Zhong and Shahidi,[43] who observed that the antioxidant concentration in non-polar samples must be higher to achieve the optimal concentration of antioxidant activity.

The antioxidant activity in these samples is due to the astaxanthin concentration obtained from the residue meal. The heating extraction process was sufficient to make astaxanthin more available in the food matrix, and consequently improved its extraction.

Despite presenting lower astaxanthin content, WO, lipid layer (WO_{LL}), and methanolic layer and (WO_{ML}) showed higher DPPH radical scavenging capacity than the MO, MO_{LL}, and MO_{ML} samples (Tables 3-5). The methanolic extract (polar fraction) of all oils achieved the highest antioxidant activity values overall (Table 4).

Exposing shrimp residues to heat through cooking and drying processes subsequently improves the astaxanthin recovery in different solvents.[9] Thus, some researchers have chosen to lyophilize[11] or to cook[44] samples before extract preparation to achieve the best results.

Table 3. Antioxidant capacity of pigmented oils containing astaxanthin (WO and MO) and soybean oil (SO) according to different methods of antioxidant activity analysis

Analysis	WO	MO	SO
TAC / (mg AA g^{-1})	0.028^{c} (0.001)	0.073^{a} (0.002)	0.055^{b} (0.002)
Reducing power / (mg AA g^{-1})	0.147^{c} (0.007)	1.335^{a} (0.014)	0.178^{b} (0.004)
Hydroxyl radical / (% of inhibition)	1.937^{a} (0.317)	2.238^{a} (0.083)	2.154^{a} (0.228)
DPPH / (μmol eq Trolox g^{-1})	0.039^{a} (0.000)	0.038^{b} (0.000)	0.038^{c} (0.000)
ORAC / (μmol eq Trolox g^{-1})	0.496^{a} (0.084)	0.484^{a} (0.064)	0.264^{b} (0.051)

[a,b,c]The values are shown as mean ± standard deviation of triplicates. Means on the same line, followed by different letters differ significantly ($p < 0.005$) according to the test of Tukey (0.05). WO: dry pigmented oil; MO: pigmented oil of the shrimp residue meal; SO: soybean oil; TAC: determination of total antioxidant capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical elimination activity; ORAC: oxygen radical absorption capacity.

Table 4. Antioxidant capacity of the methanolic layers of pigmented oils with astaxanthin (WO_{ML} and MO_{ML}) and soybean oil (SO_{ML}) according to different methods of antioxidant activity analysis

Analysis	WO_{ML}	MO_{ML}	SO_{ML}
TAC / (mg AA g^{-1})	0.045^{c} (0.028)	0.224^{a} (0.042)	0.095^{b} (0.070)
Reducing power / (mg AA g^{-1})	1.399^{b} (0.552)	2.957^{a} (0.231)	1.151^{c} (0.097)
Radical hydroxyl / (% of inhibition)	30.730^{b} (0.900)	52.746^{a} (3.745)	3.841^{c} (0.583)
DPPH / (μmol eq Trolox g^{-1})	0.039^{a} (0.000)	0.038^{b} (0.000)	0.037^{c} (0.000)
ORAC / (μmol eq Trolox g^{-1})	0.819^{a} (0.104)	0.786^{a} (0.089)	0.264^{b} (0.035)

[a,b,c]Values are shown as mean ± standard deviation in triplicates. Means on the same line, followed by different letters differ significantly ($p < 0.005$) according to the test of Tukey (0.05). WO_{ML}: a methanolic layer of the shrimp waste pigmented oil; MO_{ML}: a methanolic layer of the shrimp waste meal pigmented oil; SO_{ML}: methanolic layer of soybean oil; TAC: determination of total antioxidant capacity; DPPH: elimination activity of 2,2-diphenyl-1-picrylhydrazyl radical; ORAC: oxygen radical absorption capacity.

Table 5. Antioxidant capacity of the lipid layer in pigmented oils containing astaxanthin (WO_{LL} and MO_{LL}) and soybean oil (SO_{LL}) according to different methods of antioxidant activity analysis

Analysis	WO_{LL}	MO_{LL}	SO_{LL}
TAC / (mg AA g^{-1})	0.021[b] (0.002)	0.055[a] (0.005)	0.026[b] (0.000)
Reducing power / (mg AA g^{-1})	0.090[b] (0.008)	0.155[a] (0.018)	0.097[b] (0.000)
Radical hydroxyl / (% of inhibition)	1.000[c] (0.143)	2.793[a] (0.564)	1.903[b] (0.093)
DPPH / (μmol eq Trolox g^{-1})	0.035[a] (0.000)	0.034[b] (0.000)	0.032[c] (0.000)
ORAC / (μmol eq Trolox g^{-1})	0.279[b] (0.055)	0.380[a] (0.076)	0.255[b] (0.046)

[a,b,c]Values are shown as mean ± standard deviation in triplicates. Means on the same line, followed by different letters differ significantly ($p < 0.005$) according to the test of Tukey (0.05). WO_{LL}: lipid layer in shrimp waste pigmented oil; MO_{LL}: lipid layer in shrimp waste meal pigmented oil; SO_{LL}: soybean oil lipid layer; TAC: determination of total antioxidant capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; ORAC: oxygen radical absorption capacity.

Although well established, the HPLC method may be influenced by the absorption spectra of the analyzed carotenoids because they overlap those of the DPPH radical.[45] Therefore, it can be concluded that data interpretation becomes complex, as this interference may lead to overestimating the antioxidant activity of the pigmented oils.

The highest antioxidant activity values in the methanolic extracts (polar fraction) can be attributed to the polarity paradox: lipophilic antioxidants are more efficient in polar media (methanol). However, further studies are needed to confirm this effect.[46] In addition, astaxanthin is a water-soluble compound as it contains O_2 and OH groups in its chemical structure (xanthophyll),[47] and probably some of it was extracted with methanol.

These results are similar to those in other studies on the antioxidant capacity of astaxanthin. Sowmya and Sachindra[48] analyzed the extract of shrimp (*Penaeus indicus*) residues and their respective fractions, particularly the astaxanthin-rich fraction, and observed intense antioxidant activity in different trials. Shashindra and Bhaskar[11] reported on the antioxidant activity of carotenoids in lyophilized protein isolates of shrimp (*Penaeus monodon*) residues, and potent antioxidant activity was detected.

It should be emphasized that there are few studies which have evaluated the astaxanthin antioxidant capacity in vegetable oil. The current scientific literature only presents information about determining the antioxidant activity of shrimp (*L. vannamei*) muscle[49] or cooked and enzymatically hydrolyzed shrimp (*Pandalopsis dispar*) by-products,[44] for example. Thus, it can be said that shrimp residue contains effective natural antioxidants, regardless of the extraction method.

Shrimp residue contains other antioxidants such as phenolics and tocopherols which influence the antioxidant potential of carotenoids, as antioxidants are known to have a synergistic action, and they may also be responsible for eliminating free radicals.[11,50] However, as the primary carotenoid found in shrimp residues is astaxanthin, and the pigmented oils showed high concentrations of carotenoids, it is possible to infer that the detectable antioxidant activity observed is mainly attributable to astaxanthin.

These results on the antioxidant capacity revealed that the MO generally has higher antioxidant activity than the other oils, and this phenomenon is likely to be caused by the higher astaxanthin concentration. The antioxidant capacity of astaxanthin is attributed to its chemical structure characterized by a long chain of double-bonded hydrocarbons (polyester chain) with an aromatic benzene ring at each terminal containing a hydroxyl group (OH) and a carbonyl/ketone (=O) group.[47]

In this regard, in the last years we have witnessed a growing number of studies on the use of natural antioxidants such as pure antioxidants or extracts rich in antioxidants (essential oils) to protect oils and fats from thermal oxidation.[37,51] Thus, astaxanthin may serve as a natural antioxidant in vegetable oils, delaying lipid oxidation, and increasing its shelf life; at the same time, it may add functional characteristics and benefits to human health.

Conclusions

Extracting astaxanthin using soybean oil shows the sustainable potential of using residues from the shrimp industry, which will decrease the amount of organic waste discarded to the environment. In addition, these results show a technological alternative for using oil with natural antioxidant potential in the food industry. The use of shrimp residues to recover bioactive compounds is crucial to reduce the environmental impact and generate a product with higher added value.

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Author Contributions

Denise M. L. e Silva and Penha P. C. Ribeiro were responsible for the project, participated in all planning, execution, and preparation of the manuscript; Thaís S. Passos and Karla S. F. S. C. Damasceno were co-advisor and supervisor of the project and were responsible for the statistical analysis and revision of the text; Maristela A. Alcântra and Angela M. T. M. Cordeiro supervised the analysis for the determination of fatty acids; Francisco C. de Sousa Jr. supervised the analyses of astaxanthin and performed the analysis of the results obtained; Cristiane F. de Assis was advisor and supervisor the project.

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