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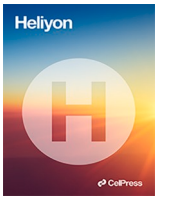

Sustainable production of astaxanthin from *Dilocarcinus pagei* crab and optimisation of its extraction with edible oils

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ABSTRACT

Dilocarcinus pagei is a South American crab commonly found in fishponds. As crabs are a source of astaxanthin (AST) and food inputs, this preliminary research aimed at studying the composition of females and males of this species to assess its potential for commercial applications, and at optimising the extraction of AST with edible oils to promote its use as an ingredient for the nutraceutical, pharmaceutical and feed industries. The chemical composition differed between males and females only in moisture, and the values obtained were: 65.4 ± 1.0% and 72.5 ± 3.1% moisture, 45.7–40.3% d.m. minerals, 22.0–24.1% d.m. fibres, 18.2–17.4% d.m. proteins, and 10.4–11.1% d.m. lipids. The Box-Behnken design was applied and validated for extraction with soya bean and sunflower oils, adjusting the oil:crab ratio, temperature, and extraction time. The optimal conditions found consisted of 140 mL/g, 90 °C and 170 min for soya bean oil, reaching an accumulation of AST of 50 ± 5 μg/g crab d.m. For sunflower oil, the conditions were 60 mL/g, 90 °C and 161 min, reaching 31 ± 3 μg/g crab d.m. Finally, the amounts of AST obtained using soya bean oil were higher than those obtained using sunflower oil; thus, soya bean oil would be recommended as a solvent to extract the pigment.

1. Introduction

The world's population growth together with the increasing demand for food in the context of climate change require the search for non-conventional production strategies that increase sustainability and reduce the carbon footprint [1]. After global capture fisheries stagnated, aquaculture has undeniably established its crucial role in food security, with its production largely meeting the supply-demand gap in aquatic food [2]. As the demand for fish for human consumption rises, the development of the aquaculture sector has great potential for expansion in any region of the world. Thus, it is important that this growth is accompanied by technological innovations that guarantee social, environmental, and economic sustainability [3].

Aquaculture production, as most food production, has shown undesirable environmental effects [4,5]. Generally, this industry uses large amounts of water and it can cause contamination of nearby aquatic environments due to the discharge of effluents with a high content of organic matter and chemicals [3]. Integrated multitrophic aquaculture is a suitable approach to reduce this problem through bio-mitigation since the co-cultured species are used as biofilters, and each level has its own independent commercial value, providing both economic and environmental sustainability [6]. Species that integrate a multitrophic system must be chosen considering their

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trophic habit, ecosystem function, commercial value, and impacts on the environment [7,8].

Dilocarcinus pagei (Decapoda: brachiura) is a South American crab that has generalist and opportunistic trophic habits [9] and it is commonly found in fishponds in Argentina. These crabs could be cultured like a secondary species with fishes to improve water quality as well as to increase profitability [10] since several added-value components have been recovered from crab species [11]. Like other crustaceans [12,13], crabs are a source of protein, chitin, minerals, lipids, flavour compounds, and pigments among which astaxanthin (AST) is found [11]. Calvo et al. [10] found that *D. pagei* had a higher amount of AST than other crab species [14]. Knowing the composition of this species is essential to assess its potential for commercial applications. AST is a red-orange carotenoid pigment of the xanthophyll family with a wide range of physiological benefits as it can remove free radicals [15,16]. It presents a conjugated olefinic structure with a hexatomic ring at each end. It has two chiral carbon atoms and thirteen polyene double bonds that can exist as cis or trans geometric isomers [17]. AST, therefore, presents several conformational and configurational stereoisomers [18]. Its physicochemical properties are: a density of 1.071 g/mL, a melting point of 216 °C, and a boiling point of 774 °C. AST has very low solubility in water and it is slightly soluble in organic reagents, such as acetone (0.20 mg/mL), DMSO (0.5 mg/mL), chloroform (10 mg/mL), and dichloromethane (30 mg/mL) [17]. It has been reported that the antioxidant activity of AST is ten times higher than that of beta-carotene and one hundred times higher than that of α-tocopherol [19]. Moreover, it has been noted that this pigment has the potential to prevent and treat several health problems, such as inflammation, hepatic dysfunction, diabetes, cardiovascular and neurodegenerative diseases, cancers, gastrointestinal disorders, and ocular problems, as well as to improve fertility [20], skin and muscle conditions [17]. Due to this, natural AST has been included in varied market products such as food and beverages, nutritional supplements, biomedicals and cosmetics [20]. In animal production, AST is used to improve survival, growth performance, reproductive capacity, stress tolerance, disease resistance and immune-related gene expression [21]. Moreover, it is an effective pigmentation agent when incorporated in salmonids, crustaceans, and chicken diets, and it also serves to intensify the colour of egg yolk, skin, and meat [22]. Although it has been possible to obtain synthetic AST, its high cost and the request for the use of natural additives have promoted the search for new sources [21]. Nowadays, the main sources of AST that are used in aquaculture and poultry industry are microalgae such as *Haematococcus pluvialis* [17]. However, due to the high value of natural AST on the market (ranging from USD $2500 to USD $7000/kg) [20,23], several research has been conducted to obtain this pigment from other natural sources by employing organic solvents and edible oils as well as the enzymatic and fermentation processes [22]. During the extraction process, AST can lose its bioactivity since it is unstable and sensitive to many environmental factors such as temperature, light, and acidity [22,24]. While the methods of AST extraction with vegetable oil [25,26] and with organic solvents [27] are similar, the extraction process using vegetable oil is easier and less dangerous to employ and it can be directly applied to feed and food products [28], as dietary oils may enhance its absorption [15]. Numerous authors have used vegetable oils for the extraction of AST from different substrates [29]. In addition, some vegetable oils, such as sunflower and soya bean oils, proved to be the solvents that exhibited the highest rates for maintaining and stabilising AST isomers during storage [30]. Since Argentina is positioned among the three major producers of sunflower and soya bean oils in the world [31], there is access to abundant and inexpensive supplies of good quality. The economic feasibility of the commercial use of different solvents to extract AST from shrimp waste showed that sunflower oil was the most suitable due to its low cost and easiness of processing for its use in feed production [32]. On the other hand, AST degradation follows first order kinetics and it can also be prevented by the presence of antioxidants in the food matrix [33]. Calvo et al. [10] demonstrated that AST dissolved in sunflower or in soya bean oils with BHT (0.1%) is stable in varied temperature conditions (from 25 to 120 °C), which are compatible with food processing temperatures. The free forms of AST are susceptible to oxidation, but the interaction of these carotenoids with other food constituents would prevent oxidation reactions. The hydroxyl groups present in its structure can form esters with fatty acids. These are the predominant forms in natural sources and they show better thermal stability and bioavailability [34].

The design proposed by Box and Behnken [35] is used to estimate a response, in this case extraction yield, affected by at least three factors with three levels per factor (−1, 0, +1). The independent variables are tested simultaneously, giving an idea of their influence on the final yield of the extraction using the response surface methodology. The number of experiments required for this design is given by the central point together with the combinations of the different levels of the selected factors. Since Box-Behnken designs usually have few experimental points, they are less expensive and less time-consuming to carry out with the same number of factors.

The objectives of this research are: to study the composition of females and males of *Dilocarcinus pagei* crabs, to assess the potential of the species for commercial applications, and to optimise the extraction of AST with edible oils in order to promote its use as an ingredient for the nutraceutical, pharmaceutical and feed industries. These preliminary results could serve as a basis for understanding the extraction parameters in this species and as an exploratory study for a future scaling up of the process.

2. Materials and methods

2.1. Chemicals

Acetone, chloroform and methanol were purchased from Merck; butylhydroxytoluene (BHT) from Biopack (Argentina); and D(+) glucose, sodium carbonate anhydrous, copper sulphate, sodium hydroxide, sodium potassium tartrate, and sulfuric acid from Cicarelli (Argentina). All these chemicals were analytical grade. Astaxanthin standard, purity ≥97% from *Hematoccocus pluvialis* was obtained from Sigma Aldrich (*S*ML0982, China), and bovine serum albumin ≥96% and anthrone 97% were purchased from Sigma Aldrich (MO 63103, USA). Sunflower oil and soya bean oil were purchased from local supermarkets in Buenos Aires, Argentina. Both were refined edible oils with antioxidants: sunflower oil naturally has vitamin E (0.54 mg/mL, as stated in the label), and soya bean oil has added tertiary BHT (100 ppm) and citric acid (5 ppm).

2.2. Collection and processing of crab samples

Adult males and females of *D. pagei* were collected in CENADAC (National Centre for Aquaculture Development, Corrientes, Argentina) from tilapia (*Oreochromis* sp.) ponds. All crabs were sacrificed in iced water and transported to the laboratory where they were washed with clean water, dried and immediately frozen to −20 °C until processing. A meat mincer was used to grind and homogenise each crab (Moulinex, model Ad6011, Bagnolet, France). Finally, they were lyophilised in a freeze dryer (Flexi-dry FD-3-85A-MP, FTS Systems, PA USA) and stored separately at −20 °C until analysis.

2.3. Experimental design

The experiment was conducted in three stages: analysis of proximate chemical composition of crabs, quantification of AST content in crab using acetone as an extraction solvent, and statistical optimisation of AST extraction with soya bean and sunflower oils using Box-Behnken design (BBD) [35].

2.4. Analysis of chemical composition of Dilocarcinus pagei crabs

Thirty-six lyophilised crabs were taken, and six individuals were randomly assigned to each Falcon tube: three tubes had females and three males. Each pool was lyophilised, ground and sieved through a 700 μm mesh. Proximate analyses were performed as follows: the percentage of moisture was calculated by weight difference from the raw samples dried at 105 °C for 16 h [36]. The ash content was determined at 550 °C for 4 h after flame combustion [37]. The total lipid content was extracted by using chloroform:methanol (2:1) following the Folch method [38]. The protein content was determined by the method of Lowry et al. [39], using Bovine serum albumin as standard. Carbohydrate content was quantified with a solution of anthrone in concentrated sulfuric acid, as described by Dreywood [40]. The fibre content was calculated by difference. All assays were performed in triplicate. All values were expressed as mean ± standard deviation, and those that correspond to ash, lipid, protein, carbohydrates, and fibre as percentage of dry matter. After assumptions of normality and homoscedasticity were met, the chemical compositions of both sexes were compared using one-way ANOVA test at 95% confidence level. The statistical analysis was done using the R environment provided by R Core Team [41].

2.5. Astaxanthin quantification from crab samples

Astaxanthin was extracted and quantified from each crab pool defined in section 2.4 using acetone with BHT (0.1 g/kg), as previously published by Calvo et al. [10]. Briefly, 3 mL of acetone containing 0.1 g/kg of BHT were added to approximately 50 mg of the sample. The mixture was shaken in a vortex (MSI Minishaker IKA, USA) for 20 s and was sonicated (Branson Ultrasonic, Mexico) for 15 min at room temperature. After that, it was centrifuged at 3500×*g* (Rolco, Argentina) for 10 min and the supernatants were gathered, evaporated to dryness in a nitrogen stream, and resuspended in 1 mL of methanol. The extract was injected in a High-Performance Liquid Chromatography (HPLC). The analysis was conducted in triplicate and darkness conditions. HPLC analysis was performed using a module separation (Waters, Alliance 2695, MA, USA) with a diode array detector (Waters, 2998, CA, USA). An analytical column Hyperclone C18, 5 μm of particle size, 4.6 mm of inner diameter and 250 mm of length (Phenomenex, 00G-4308-E0, CA, USA) fitted with a guard column C18, 5 μm of particle size and 3.0 mm of inner diameter and 40 mm of length (Phenomenex, K10-4282, CA, USA) was used. The column temperature was set at 25 °C and the injection volume was 20 μL. Elution was carried out with methanol as a mobile phase and the flow was 1 mL/min. The wavelength determination was performed with a resolution of 1.2 nm and a wavelength scanning range from 350 to 600 nm. Astaxanthin was identified by comparing its spectrum and retention time with the astaxanthin standard.

2.6. Optimisation of astaxanthin extraction

2.6.1. Experimental design

In this work, Box-Behnken design consisting of three variables at three levels was used, and then response surface methodology analysis was applied. The design was first performed using soya bean oil as a solvent for the extraction, and then using sunflower oil to examine the effectiveness of both oils in the extraction of AST. The independent variables x_1, x_2, and x_3 were set as the oil to crab ratio, temperature, and time of extraction, respectively, at three levels (−1, 0, and +1). The values that the levels take for each variable were chosen from previous bibliography [22,25,26] and they are shown in Table 1.

The experimental design consisted of a central point, which was carried out in triplicate, and 12 combinations of different levels of the variables, giving a total of 15 experimental points. Each design was run three times for each oil.

Table 1
Different experimental conditions corresponding to the independent variables of the Box-Behnken design.

Levels	−1	0	1
Oil:crab ratio (mL/g)	60	100	140
Temperature (°C)	50	70	90
Extraction time (min)	130	150	170

The accumulation of AST obtained in μg/g crab on dry basis was considered the predicted response of the design experiments (y), and the relationship with the independent variables was calculated with the following second-order polynomial equation (1):

$$y = a_0 + \sum_i a_i x_i + \sum_{i<j} \sum_j a_{ij} x_i x_j + \sum_i a_{ii} x_i^2 \quad (1)$$

The coefficients a_0, a_i, and a_{ii} stand for the constant, linear and quadratic effects, respectively, and a_{ij} represents the interaction effects of coded factors (x_i and x_j). The statistical significance of all terms was evaluated according to a p-value of 1%. The goodness of fit of the model equation to experimental data was assessed by the regression coefficient R^2, the adjusted R^2 (R^2 adj.), the evaluation of the residuals, and the lack-of-fit test [42]. The model was validated with an independent set of results, performing extraction at the predicted optimal conditions.

Experimental design, data analysis, and quadratic model building were performed with Statgraphics Centurion XVI software. ANOVA was used to determine the differences between the samples treated with the optimal mixtures obtained by RSM and the control using acetone.

2.6.2. Astaxanthin extraction and quantification with oils

Extraction tests were carried out with soya bean and sunflower oils with 0.1 g/kg of BHT previously added. Approximately 50 mg of homogenate were weighed in a screw-on cap tube and different volumes of each oil were added as indicated by Box-Behnken design (combinations can be seen in Table 3, in section 3.3). These solutions were shaken in a vortex for 20 s. The AST oil solutions and oils with BHT (blanks) were incubated at different temperatures in a heating/stirring module (PIERCE Reacti-Therm IIITM Heating-Stirring Module Nº 18940 Rockford IL, USA). After the suggested heating time, the samples were cooled to room temperature and were measured with a spectrophotometer (Hewlett Packard, G1115A, CA, USA) at 490 nm using oils with BHT as blanks [10]. In order to calculate AST content, calibration curves derived from known pigment concentrations in both oils were used, with sunflower and soya bean oils as blanks. All assays were performed in triplicate.

3. Results and discussion

3.1. Chemical composition of Dilocarcinus pagei crabs

The results of the chemical composition analysis of *D. pagei* are shown in Table 2 alongside crustacean-based value-added food products obtained from bibliography, as a comparison. The main component after moisture content was the percentage of minerals (or ash) followed by fibre, proteins, and lipids, and the lowest percentage corresponded to carbohydrates. The chemical compositions of both sexes of *D. pagei* were similar except for moisture which was significantly higher in females than in males ($p < 0.05$). Contrarily, Soundarapandian et al. [43] found differences between sexes in the biochemical composition of the edible crab *Podophthalmus vigil.* The females of *P. vigil* have a higher level of protein, lipid, and ash contents than ovigerous females and males. Nanda et al. [11] reviewed the biochemical composition variation of many crabs and stated that intra-species differences could be caused by a wide range of factors such as biological characteristics (species, sex, size, stage of maturity, gametogenesis, and spawning cycle), habitat, food source, and environment.

In comparison with similar food inputs, *D. pagei* presented more minerals and lipids and less protein. The processed samples included the carapace and not only brown meat (mainly hepatopancreas and gonads) but also white or edible meat (muscle). Other authors have previously found that edible crab meat is a rich source of water- and fat-soluble vitamins and valuable minerals, especially calcium, copper, selenium, chromium, iron, zinc, potassium, and phosphorus [44,45]. Thus, more studies could be conducted to analyse the mineral compositions of this species to make more complete use of this bioresource. Nanda et al. [11] found that solid and liquid wastes from crab processing contain many biomolecules such as proteins, pigments, chitin, flavour compounds, and

Table 2
Chemical composition of males and females of *Dilocarcinus pagei* crabs and of crustacean-based value-added food products (mean ± standard deviation).

Crustacean species	Input	Moisture (%)	Ash (%d. m.)	Lipid (%d. m.)	Protein (%d. m.)	Fibre (%d. m.)
Dilocarcinus pagei male crabs (present study)	whole crab	65.4 ± 1.0	45.7 ± 2.9	10.4 ± 0.3	18.2 ± 1.6	22.0 ± 0.3
Dilocarcinus pagei female crabs (present study)	whole crab	72.5 ± 3.1	40.3 ± 2.2	11.1 ± 1.5	17.4 ± 1.5	24.1 ± 2.0
Pandalus borealis shrimp (Dave et al., 2020)	whole shrimp	74.85 ± 0.19	15.03 ± 0.02	14.79 ± 1.01	66.42 ± 1.08	–
Litopenaeus vanname shrimp (Karnila et al., 2021)	carapace flour	10,28	33,72	1,56	38,32	25,9
Pandalus borealis shrimp (Kim et al., 2016)	shrimp waste	21,07	17,18	12,19	44,5	–
Litopenaeus vannamei shrimp (Cabanillas-Bojórquez et al., 2021)	liquor produced from fermented waste	2.85 ± 0.27	19.32 ± 0.57	6.29 ± 0.51	25.40 ± 0.47	7.20 ± 0.58
Pandalus borealis shrimp (Dave et al., 2020)	cooked shrimp waste	74.14 ± 0.29	26.57 ± 0.18	8.12 ± 0.72	50.65 ± 0.40	–
Chionoecetes opilio crab (Tremblay et al., 2019)	Crab cooking effluent	5.06 ± 0.87	38.81 ± 0.87	0.70 ± 0.08	58.5 ± 0.2	–

Table 3
Extraction levels of astaxanthin in crab, using soya bean and sunflower oils as solvents, obtained after treatment with the different experimental conditions of the Box-Behnken design. CP: Central point of the design (1–3). Quantification data expressed as the mean ± standard deviation (n = 3). nd: not detected.

Treatment	Independent variables			Astaxanthin concentration (μg/g)					
				Soya bean oil			Sunflower oil		
	Oil:crab ratio (mL/g)	Temperature (°C)	Extraction time (min)	1	2	3	1	2	3
CP1	100	70	150	26 ± 3	26 ± 3	26 ± 3	27 ± 1	21 ± 1	24 ± 1
CP2	100	70	150	24 ± 3	22 ± 3	25 ± 3	22 ± 1	20 ± 1	24 ± 1
CP3	100	70	150	25 ± 3	24 ± 3	22 ± 3	24 ± 1	18 ± 2	20 ± 1
4	60	50	150	24 ± 2	17 ± 2	21 ± 2	5 ± 1	22 ± 1	1 ± 1
5	140	50	150	22 ± 4	26 ± 4	28 ± 4	3 ± 3	nd	6 ± 3
6	60	90	150	33 ± 2	37 ± 2	34 ± 2	25 ± 1	30 ± 1	27 ± 1
7	140	90	150	50 ± 4	41 ± 4	35 ± 4	19 ± 2	17 ± 2	18 ± 2
8	60	70	130	32 ± 2	26 ± 2	27 ± 2	22 ± 1	17 ± 1	18 ± 1
9	140	70	130	27 ± 4	27 ± 4	28 ± 4	13 ± 2	6 ± 3	8 ± 3
10	60	70	170	42 ± 2	42 ± 2	38 ± 2	24 ± 1	25 ± 1	24 ± 1
11	140	70	170	57 ± 4	54 ± 4	52 ± 4	16 ± 2	15 ± 2	14 ± 2
12	100	50	130	17 ± 3	27 ± 3	23 ± 3	6 ± 2	7 ± 2	7 ± 2
13	100	90	130	36 ± 3	35 ± 3	37 ± 3	21 ± 1	19 ± 2	26 ± 1
14	100	50	170	17 ± 3	26 ± 3	21 ± 3	nd	nd	nd
15	100	90	170	40 ± 3	40 ± 3	39 ± 3	28 ± 1	30 ± 1	28 ± 1

peptides that have a variety of uses in almost any scientific area. The biomass generated from *D. pagei*, as from other crabs, can be transformed into a wide range of new bio-based products, reducing environmental pressure.

This species, co-cultured in fishponds, could provide biomitigative services by converting waste organic matter into biomass and contributing to environmental sustainability in a local production cycle. Then, the application prospects of this biological resource in feed, food, pharmaceutical, nutraceutical, and other industries would certainly contribute to the economic development of aquaculture towards a circular economy.

3.2. Total astaxanthin content in crab

The AST content did not show significant differences between males and females. Therefore, the AST extractions with oils were performed using both males and females mixed, in triplicate, using acetone as a solvent. A value of 46 ± 5 μg AST/g crab d.m. (n = 3) was obtained as described in section 2.5 for these mixed pools. The variation among samples could be caused by multiple factors, as discussed in section 1 (i.e. habitat, food source, biological and seasonal factors), but in all cases, crabs were a good biosource of AST.

3.3. Optimisation of extraction of astaxanthin by the Box-Behnken experimental design using edible oils

Regarding AST extraction levels, the results obtained by using soya bean or sunflower oils as solvents are depicted in Table 3. Those values were used to develop the statistical models for each oil.

Concerning the Box-Behnken design with soya bean oil, when applying the conditions that correspond to the central point of the model (100 mL/g, 70 °C, and 150 min), 24 ± 2 μg AST/g crab d.m. was extracted and this represents 53% of the amount obtained with acetone. Regarding sunflower oil, the application of the conditions of the central point permitted the extraction of 22 ± 3 μg/g crab d.m., with 48% of the amount obtained with acetone.

Table 4
Parameter estimates of the polynomial models obtained with Equation (1) for oil:crab ratio (x_1), temperature (x_2), and time (x_3) effect on the extraction of astaxanthin using soya bean oil and sunflower oil: coefficients, R^2 and R^2 adj. of the models. * means significant effect on the response ($p < 0.01$).

Coefficient		Soya bean oil	Sunflower oil
Constant	a_0	451.621	−161.409
Linear	a_1	−31.8755*	5.20882
	a_2	0.086233*	1.14691*
	a_3	−5.17548*	1.51756*
Interaction	a_{12}	0.018133	−0.02123
	a_{13}	0.095063	0.003708
	a_{23}	0.003142	0.008238*
Quadratic	a_{11}	1.78678*	−0.64851*
	a_{22}	−0.00184	−0.01282*
	a_{33}	0.01582*	−0.00681*
Correlation	R^2	81.6	86.3
Model	R^2adj	75.4	81.7

The quadratic regression models that provided the best description of the AST extraction were determined according to Eq. (1) for both conditions using soya bean oil and sunflower oil. The second-order polynomial coefficients together with the statistical significance of the model equations are shown in Table 4. The normal probability of the residuals was validated by a linear pattern (Supplementary Fig. 1). Lack-of-fit tests were non-significant. In soya bean oil designs, regression coefficients R^2 adj. were superior to 0.75, indicating a good correlation between the AST accumulation and the independent variables oil:crab ratio, temperature, and time. Regarding sunflower oil designs, R^2 adj. was 0.82, showing a strong correlation between AST accumulation and the independent variables.

The regression equation of each design was depicted as a 3D mesh graph, showing the variation in the response according to the levels of each of the three independent variables: oil:crab ratio, temperature, and time (Fig. 1).

Regarding soya bean oil, the regions with the highest extraction of AST can be found at higher temperatures and times, regardless of the oil:crab ratio applied (Fig. 1A). As regards sunflower oil, the maximum of AST extraction is predicted at a high time and temperature but at lower oil volumes (Fig. 1B). The studied conditions improved the accumulation of AST extracted from *D. pagei* with both soya bean and sunflower oils (Table 5).

By applying the optimised conditions using response surface methodology, the extraction of AST with soya bean oil reached 50 ± 5 μg/g crab d.m., which are similar values to those obtained using acetone as a solvent ($p > 0.05$). This amount of AST almost doubles that obtained by Sachindra and Mahendrakar [26], applying the same oil at 70 °C during 120 min to shrimp wastes, in an oil to waste ratio of 2:1. Moreover, these extraction levels using soya bean oil are in accordance with those obtained by Handayani et al. [25], which reached 48.5 μg AST/g of *Panaeus monodon* shrimp waste milled at 40/60 mesh size, applying palm oil at 70 °C. On the contrary, the work of Mezzomo et al. [22] obtained lower extraction yield than in this study: 25.15 ± 0.05 μg/g d m when using *P. brasiliensis* and *P. paulensis* residues as raw matter, with soya bean oil at 70 °C during 160 min. The difference in these results could be due to the lack of antioxidants added to the oil, with possible loss of AST during processing. Adding 0.1 g BHT/kg to soya bean oil improves AST stability in high temperature storage conditions ranging from 30 °C to 180 °C, presenting better results when compared with other vegetable oils [10].

With respect to sunflower oil, the amount of AST extracted from the crab was 31 ± 3 μg/g crab d.m. This value is in line with the results previously obtained by Calvo et al. [10]. Also, Mezzomo et al. [22] arrived at a comparable extraction yield of 32 ± 2 μg/g of pink shrimp residues when performing the extraction using sunflower oil in an oil to waste ratio of 4:1 at 70 °C during 160 min. Moreover, this concentration is similar to that obtained by Sachindra and Mahendrakar [26] after optimisation of a comparable Box-Behnken design for the extraction of *P. indicus* shrimp waste. The authors reached 24.8 ± 1.51 μg/g shrimp waste after extraction using sunflower oil in an oil to waste ratio of 2:1 and heating at 70 °C for 150 min.

In the range of operating conditions tested, according to Ref. [46], soya bean oil viscosity ranges from 23.58 mPa s at 50 °C to 8.68 mPa s at 95 °C and its specific heat reaches 1.715, 1.765, 1.822 kJ/kg K at 50, 70 and 90 °C respectively. Regarding sunflower oil, its viscosity varies from 25.02 mPa s at 50 °C to 8.79 mPa s at 95 °C and its specific heat is 2.276, 2.328, and 2.388 kJ/kg K at 50, 70 and

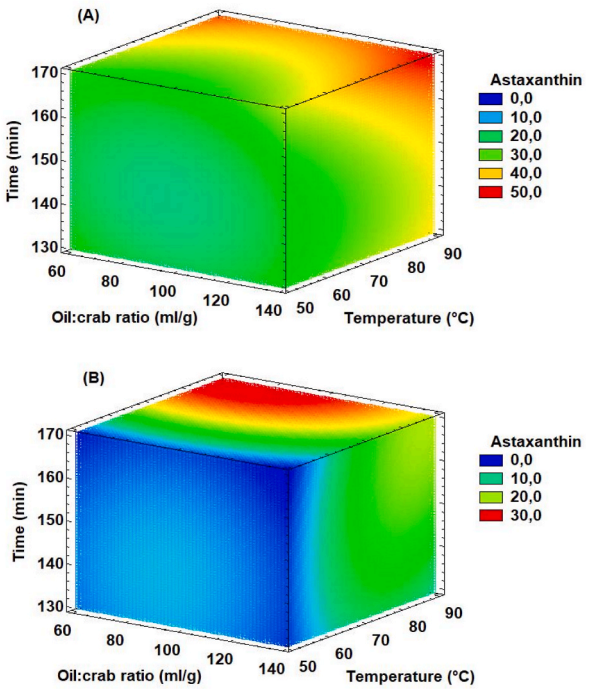

Fig. 1. Response mesh obtained for the extraction of astaxanthin in soya bean (A) and sunflower (B) oils. Colour scale of astaxanthin in μg/g crab d. m. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 5
Optimal conditions to maximise the extraction of astaxanthin using soya bean and sunflower oils. Theoretical extraction levels of astaxanthin predicted by the model for each oil, and experimental extraction obtained after treatment with the optimal conditions. Data expressed as the mean ± standard deviation (n = 3).

Solvent	Optimal conditions			Predicted extraction	Obtained extraction (μg/g)
	Oil:crab ratio (mL/g)	Temperature (°C)	Extraction time (min)		
Soya bean	140	90	170	58±4[a]	50±5[a]
Sunflower	60	90	161	32±1[a]	31±3[a]

90 °C respectively.

The amounts of AST obtained using soya bean oil were higher than those obtained using sunflower oil ($p<0.05$), possibly due to the lower viscosity values at the assayed temperatures. Mezzomo et al. [22] also reported significantly higher AST extraction yields for soya bean oil than sunflower oil at room temperature and at 70 °C HoweverCalvo et al. [10] showed that the degradation rate of AST was higher in sunflower oil than in soya bean oil in the full range studied (25–180 °C). The activation energy of this reaction was 88.7 kJ/mol for soya bean oil and 85.5 kJ/mol for sunflower oil. This difference suggested that astaxanthin is more stable in commercial soya bean oil than in sunflower oil. Furthermore, commercial soya bean oil is a cheap ingredient and a good source of unsaturated fatty acids (ω3, ω6 and ω9). Thus, soya bean oil would be recommended as a solvent to extract the pigment.

Although extraction yield was found to be higher with soya bean oil, AST concentration remaining in sunflower oil was higher (0.52 ± 3 μg/mL) than the amount that remained in soya bean oil (0.36 ± 3 μg/mL), possibly due to the lower oil:crab ratio that was applied in the extraction. In general, the oil extraction method is commonly used to obtain AST with a high extraction rate. Numerous studies have demonstrated that vegetable oils presented good dissolving power of AST, have a high flash point and oxidative stability of their enriched forms. In comparison with organic solvent, these oils have lower toxicity and environmental impact and better organoleptic quality. The selectivity of vegetable oils by AST depends on their origin and lipid profile, resulting in variable extraction efficiency and enrichment factors [47]. A recent study by Honda et al. [30] investigating the stability of AST isomers in 24 different vegetable oils and animal fats found that there was no correlation between the physical properties (viscosity and density) of the oils and the stability of AST isomers. Furthermore, when soya bean and sunflower oils were used as suspension media in the aforementioned study, the AST isomers were hardly degraded [30]. In the case of AST extraction from crayfish waste, the extraction rate rose when the ratio of oil to crayfish increased, as it can be seen in the present study [17]. The ratio is a significant indicator in the extraction process and has a considerable impact on the extraction rate of natural AST. Moreover, temperature proved to be important not only in the extraction but also in the stability of AST, once extracted. At high temperatures (60–90 °C), the oil extraction procedure can obtain a high yield of AST [48], in a compromise situation without exceeding the degradation temperature of AST.

Higher temperature could affect the stability of AST [17]. For example, the AST content in palm oil decreased by 10% when subjected to heat treatment at 80 °C and decreased by 14% at 90 °C after 8 h [25]. The application of BHT to the oil prior to extraction is a useful mechanism to expand the temperature range and overcome the limitations regarding the stability of AST in the studied oils [10].

As for the models obtained for both oils, the predicted results were not significantly different from those obtained experimentally when applying the optimal conditions in the treatment of crab biomass; thus, validating the proposed models (Table 5). They permit estimating the desired extraction outcome, either to maximise the separation of the AST from the crab matrix or to maximise its concentration in the oil used as a solvent, depending on the perspectives of the use of the products.

These results may indicate that, when treated at particular optimal conditions, AST can be extracted using food grade soya bean oil and reach similar yields to those obtained with organic solvents, thus, laying emphasis on the relevance of the selected optimal conditions that will be used. Modelling the accumulation of AST allows understanding of how the process affects these metabolites and it allows for the possibility to compare models using different oils that can be used in food industries or pharmaceutical applications. In relation to this, Weeratunge and Perera [49] evaluated several formulations of goldfish feed, some enriched with AST and either soya bean or coconut oils. They found that fish feed prepared with soya bean or coconut oils and AST extracted from shrimp waste could significantly improve the skin colour of goldfish, in comparison with fish fed with raw shrimp waste powder or fish fed without AST. In addition to the skin colour enhancement, improvement in the health conditions of goldfish was reflected by a higher survival rate.

Regarding the process herein studied, the crab mass remaining from the extraction will still have a certain concentration of AST, depending on the oil used. This biomass represents an interesting food grade ingredient to be applied in the formulation of balanced feed for livestock since it contains a proportion of the oils used in the extraction which may increase the absorption of AST. The administration of AST to layer hen diet increases fertility, improves the overall health status of these animals, and decreases chicken mortality [14]. Egg production and yolk colour also increase, and salmonella infections are reduced dramatically probably due to a stronger membrane formation [50]. AST also provides greater pigmentation to chicken meat, which represents a desirable attribute to some consumers [51]. The fraction of AST that remains unremoved after oil extraction together with the values obtained from the chemical composition make this waste a promising by-product that can be used as poultry supplement.

4. Conclusion

Dilocarcinus pagei resulted in an interesting input, a rich source of minerals, lipids, proteins, and astaxanthin with a similar composition in both sexes.

This species could contribute to economic and environmental sustainability in a local production cycle with promising prospects for the application of both the extraction solvent and the remaining mass in various industries, namely feed, food, pharmaceuticals, and nutraceuticals.

Response surface methodology with Box-Behnken design was used and validated with soya bean and sunflower oils, adjusting the oil:crab ratio, temperature, and extraction time—making this methodology suitable for future food and feed applications.

Since the amounts of AST obtained using soya bean oil were higher than those obtained using sunflower oil, soya bean oil would be recommended as a solvent to extract the pigment.

Further studies are needed to better understand the parameters of matter exchange during extraction, as well as a correct scaling up to take advantage of this bioproduct on large scales.

Author contribution statement

Pok, Paula Sol: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Stefanini, Manuel Ignacio: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Calvo, Natalia Soledad: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17381.

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