Biomacromol. J., Vol. 7, No. 3, 77-85, December 2021.

Biomacromolecular Journal
www.bmmj.org

Extraction of Astaxanthin from *Penaeus Semisulcatus* and *Penaeus Merguiensis* Waste Using Vegetable Oils: Process Optimization

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(Received 21 October 2022, Accepted 13 November 2021)

ABSTRACT

The marine environment has been recognized as a source of diverse natural compounds. Astaxanthin is the main carotenoid in crustaceans, including shrimp. Shrimp waste is considered a cheap source of natural astaxanthin. A study was performed to compare the extraction yield of astaxanthin in *Penaeus semisulcatus* and *Penaeus merguiensis* waste using sunflower and sesame oils. Furthermore, the Box-Behnken design optimized the astaxanthin extraction conditions of sunflower oil from *Penaeus semisulcatus* waste. The results showed that the extraction yield of astaxanthin with sunflower oil from *Penaeus semisulcatus* wastes (13.43 ± 0.17 μg g^{-1}) was significantly different ($p < 0.001$) compared to sesame oil and *Penaeus merguiensis*. Moreover, it was shown that the maximum yield of astaxanthin extracted (14.74 ± 1.6 μg g^{-1} of waste) with sunflower oil from *Penaeus semisulcatus* waste was produced under ideal circumstances comprising heating duration of 120 min, oil: waste ratio (v/w) of 2, the heating temperature of 70 °C, and waste size 80 mesh. A regression equation for astaxanthin yield was obtained as a function of heating temperature, time of heating, oil-to-waste ratio, and size of shrimp waste.

Keywords: *Penaeus semisulcatus*, *Penaeus merguiensis*, Astaxanthin, Box-Behnken design

INTRODUCTION

Oceans and marine ecosystems have a wide variety of animals and plants, making them one of the resources of new chemical and bioactive compounds [1-3]. Marine organisms produce specific secondary metabolites to protect themselves against various environmental pressures and stresses, such as predators, parasites, and competitors in their habitats [4,5]. This evolutionary process has produced countless natural compounds with unique chemical structures and a wide range of biological activities [6,7]. Therefore, the extraction of natural products from marine organisms for various uses was increasingly studied [8,9].

Undoubtedly, shrimp is one of the most important commodities in the world fisheries industry, which accounts for almost 20% of the world's total exports of fish and fishery products [10]. Global fisheries and aquaculture output has surpassed 160 million tons per year, however, a significant portion of these resources are lost as by-catches or processing waste [9]. Head and body carapace account for around 40-50% of the total weight of the raw material used to produce shrimp, but there are other byproducts as well [10-12]. Most of the shrimp waste is buried on land or dumped in seawater, which leads to significant surface pollution and unpleasant odors in coastal areas. Furthermore, it has become a significant concern in terms of the adverse environmental effects it creates around coastal areas [11]. The bioactive compounds in shrimp waste consist mainly of 30-50% mineral salts, 30-40% protein, and 13-42% chitin, also lower amounts of lipids and pigments, all of which have a market value [12,13]. Shrimp waste could provide a rich and cheap source of natural astaxanthin [14,15]. The red pigment astaxanthin, a member of the xanthophyll family and oxygenated derivative of carotenoids, is the main carotenoid in marine organisms, including shrimp, zooplankton, and salmon, which plays an

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important role in their pigmentation. This compound has various biological properties, such as antioxidant, and anti-inflammatory [16-19]. Moreover, the studies showed that it is extremely effective to reduce oxidative damage to the skin [20]. Thus, astaxanthin is used in different industries, such as pharmaceuticals, animal feed, and cosmetics [21, 22]. Recovery of natural astaxanthin from shrimp waste improves the economy for shrimp processors [23]. Several methods of astaxanthin extraction from crustacean byproducts were reported, such as chemical, biological, and mechanical methods [14,24-26]. Vegetable oils are employed in the production of astaxanthin in the food and cosmetic sectors because of the pigment's high solubility in oil and the benefits of vegetable oils, such as their widespread availability, cheap cost, and good environmental features [27-29]. The green tiger (*Penaeus semisulcatus*) prawns and Banana (*Penaeus merguensis*) prawns are important species of the genus Penaeus. These two species are important commercial and economic species of coastal waters of the Bushehr and Hormozgan provinces, respectively. Moreover, they comprise approximately 70 percent of the total catch of these provinces [25,30]. For environmental and economic reasons, appropriate and optimized technology should be used to prevent decay and convert the biological material into valuable products in this waste [31]. Earlier, the optimization of the extraction conditions of astaxanthin with oil from shrimp shells was presented [32-34]. However, it is necessary and important to specify the best conditions for the extraction to get a higher astaxanthin yield.

The present study was carried out to compare the extraction yield of astaxanthin using different oils from the processing by-products of two important commercial prawns waste (*Penaeus semisulcatus* and *Penaeus merguiensis*) on the south coast of Iran using response surface methodology. Besides, optimization of extraction conditions was designed based on the effects of four factors such as waste particle size, oil-to-waste ratio, heating time, and heating temperature on the yield of astaxanthin.

MATERIALS AND METHODS

Materials

Freshly caught shrimp (*Penaeus semisulcatus* and *Penaeus merguiensis*) were purchased from Bushehr and Bandar Abbas City's seafood central market, a district located in the south of Iran. The samples were kept at -4 °C while being sent to the lab. After being dried at 40 °C in the oven for 24 h, the shrimp peelings and waste (including the head and carapace) were homogenized in a mixer (Depose, Moulinex, Italy) to a fine powder and kept in the fridge at -20 °C until utilized. Sunflower oil and sesame oil were purchased locally and were used in the oil extraction experiments. Astaxanthin standard (HPLC purity ≥ 92%, standard A9335) was procurement from Sigma Company.

Extraction Yield of Astaxanthin by Different Oils from *Penaeus Semisulcatus* and *Penaeus Merguiensis* Waste

Based on Sachindra and Mahendrakar (2005), astaxanthin was extracted from shrimp waste using oils with slight modification [33]. 10 g of shrimp waste in size 60 mesh was mixed with 20 ml of oils and heated in the water bath for 120 min at 60 °C. The mixture was centrifuged at 9000 g for 15 minutes to remove particles and separate the pigmented oil (Sigma, model 2-16KC). The astaxanthin content of pigmented oil was examined by spectrophotometry (BioQuest CE2501, UK) at 487 nm against the particular oil as blank. The carotenoid yield was calculated and presented as total carotenoid (presented as astaxanthin), based on Eq. (1) as astaxanthin yield (μg g^{-1} waste):

$$Y = \frac{A \times V \times D \times 10^6}{100 \times W \times E} \quad (1)$$

A = absorbance at 487 nm, V = volume of pigmented, oil recovered, D = dilution factor, W= weight of waste in grams, and E = extinction coefficient of standard astaxanthin in each vegetable oil at 487 nm (Sunflower oil: 2290 and Sesame oil: 2266) [33].

Experimental Design to Optimize the Extraction Conditions of Astaxanthin by Sunflower Oil

Since the carotenoid yield in *Penaeus semisulcatus* with sunflower oil was the highest, optimization studies were performed using sunflower oil in *Penaeus semisulcatus*. The optimization conditions were the same conditions used in the initial experiments. Four main factors required to create a model for optimization extraction yield of astaxanthin were incorporated in the Box-Behnken design (BBD) [35] which consisted of 28 experimental runs, including 4 replications at the center point. The response pattern was determined based on the single factor experiments, four independent variables: heating temperature (50-70 °C), oil: waste ratio (1-3 v/w), heating time (60-180 min), and shrimp waste particle size (40-80 mesh).

The four variables X1, X2, X3, and X4 were the coded variables for the temperature of heating, time of heating, oil: waste ratio, and particle sizes of shrimp waste, respectively. Each factor was coded as -1, 0, and +1; while the response value was the extraction yield. The factors, their levels, and codes for the levels are listed in Table 1.

The mathematical model for the optimization of dependent variables was based on the following equation (Eq. (2)):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j \quad (2)$$

Where Y is the extraction carotenoid yield; Xi, and Xj are the coded values of independent variables; while β0, βi, βii and βij are the regression coefficients of intercept, linearity, quadratic, and interaction effect respectively.

Statistical Analysis

Statistical analysis to compare mean differences among the groups was performed with a two-way ANOVA test using the GraphPad Prism (Version 8.4.3.686.x64) statistical software. Data were reported as the mean value of astaxanthin extraction yield ± SD. P-value < 0.05 was considered statistically significant. Furthermore, the optimization data for the effect of each factor and their interaction on astaxanthin yield were analyzed using analysis of variance (ANOVA) by Design Expert (version v11.0.4.x64) software package.

RESULTS AND DISCUSSION

Astaxanthin Extraction Yield by Different Oils in *Penaeus Semisulcatus* and *Penaeus Merguiensis* Waste

The statistical analysis showed that the highest astaxanthin extraction yield (13.43 ± 0.17 μg g^{-1}) (Table 2) was obtained using sunflower oil from *Penaeus semisulcatus* waste with a significant difference (p < 0.001) compared to sesame oil, as shown in Fig. 1. Extraction yield differed significantly (p < 0.001) between *Penaeus semisulcatus* and *Penaeus merguiensis* with each oil (Fig. 1). The extraction yield for Penaeus semisulcatus by sunflower oil had a greater astaxanthin yield, according to an analysis of sunflower and sesame oil for recovering astaxanthin from *Penaeus semisulcatus* and *Penaeus*

Table 1. The Factors, their Levels and Codes that Each Factor was Coded -1, 0, and +1

Factors	Code	Level		
		-1	0	+1
Temperature of heating (°C)	X1	50	60	70
Time of heating (min)	X2	60	120	180
Oil: Waste ratio (v/w)	X3	1	2	3
Particle sizes of shrimp waste (mesh)	X4	40	60	80

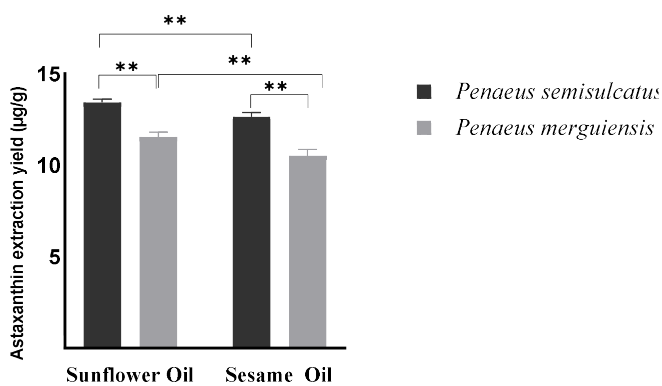

Fig. 1. Extraction yield of astaxanthin from *Penaeus semisulcatus* and *Penaeus merguiensis* wastes in different vegetable oils (n = 6). **Asterisks describe a significant difference in extraction yield of astaxanthin from *Penaeus semisulcatus* and *Penaeus merguiensis* by each vegetable oil (p < 0.001). * and ** indicates p < 0.05 and p < 0.001 respectively.

Table 2. Data are Expressed as Mean ± SD of Astaxanthin Yield from *Penaeus Semisulcatus* and *Penaeus Merguiensis* Wastes in Sunflower and Sesame oils (n = 6). The Highest Astaxanthin Extraction Yield was Obtained Using Sunflower Oil from *Penaeus Semisulcatus* Waste

Shrimps	Oils	Astaxanthin yield (µg g^{-1} waste)
Penaeus semisulcatus	Sunflower oil	13.43 ± 0.17
	Sesame oil	12.65 ± 0.22
Penaeus merguiensis	Sunflower oil	11.54 ± 0.26
	Sesame oil	10.51 ± 0.35

merguiensis waste.

The results indicated higher astaxanthin extraction efficiency using sunflower oil in *Penaeus semisulcatus*. The difference in the yield of extracted carotenoids between the two species can be in terms of the type of species, environmental conditions, and the carotenoid content of their diet [36]. Data analysis showed that the efficiency of sunflower oil extraction was significantly ($p < 0.001$) improved relative to sesame oil in both species. Based on this result, it was previously reported that solvents determine a crucial section of the performance of extraction processes, which affects the recovery efficiency [37]. Sachindra and Mahendrakar (2005) showed that using sunflower oil resulted in higher carotenoid yields (26.3 µg g^{-1}) of shrimp waste (*P. indicus*) compared with other oils [33]. Furthermore, in a similar study, Hooshmand *et al.* (2016) extracted astaxanthin using vegetable oils from shrimp waste (*Penaeus semisulcatus*) and concluded that sunflower oil had a higher carotenoid yield (5.553 µg g^{-1}) than soy, sesame, and rice bran oils [32]. Chen and Meyers (1984) indicated that extraction of astaxanthin yield with vegetable oil was superior to fish oils [38]. In their study, El-Bialy and El-Khalek (2020) showed that corn oil (5.60 µg g^{-1}), flaxseed oil (4.89 µg g^{-1}), and sesame oil (4.69 µg g^{-1}) could be used to recover carotenoids from shrimp. They reported that the amphiphilic derivatives content of vegetable oils and polyunsaturated triglyceride levels were important for increasing recovery pigments [39].

Optimization of Astaxanthin Extraction Conditions with Sunflower Oil in *Penaeus Semisulcatus* Waste

Box-Behnken design optimized the astaxanthin extraction conditions of sunflower oil from *Penaeus semisulcatus* waste. The experiments planned by Box-Behnken design and the analysis results of the models were summarized in Tables 3, 4, and 5. The results displayed that a second-order polynomial regression model was able to accurately interpret the experimental data. The polynomial regression model includes these features: the heating temperature (X1), heating time (X2), oil: waste ratio (X3), and particle sizes of shrimp waste (X4). It was found that all four factors and their interactions significantly affected the astaxanthin yield. Besides, the lack of fit test was not significant ($p > 0.05$); R2 of the astaxanthin extraction model was 0.9915; the adeq precision was 42.9437 and had a low-value coefficient of 1.28 of the coefficient of variation (CV), which means that the model can be used for prediction (Table 4). The regression equation yielded predictions for carotenoids that were quite close to the observed values (Table 3).

The coefficients showed that the experimental results were very valid and reliable. According to Table 3, it was found that the yield of astaxanthin ranged between 9.65 and 14.74 µg g^{-1} of waste. The regression equation was obtained using the constant, linear, and quadratic regression coefficients of main variables and the linear equation by linear regression coefficients of interactions (Table 5) for the astaxanthin yield (Y) as a function of four independent variables (X1, X2, X3, X4) and their interactions as follows (Eq. (3)):

$$Y = 13.43 + 0.4917\,X1 - 0.1008\,X2 - 0.4958\,X3 + 0.5267\,X4 + 0.4529\,X1^2 - 1.40\,X2^2 - 1.36\,X3^2 + 0.1629\,X4^2 + 0.2250\,X1X2 + 0.4800\,X1X3 - 0.1950\,X1X4 - 0.2450\,X2X3 + 0.2325\,X2X4 - 0.4975\,X3X4 \tag{3}$$

Figure 2 indicates three-dimensional (3D) response surface plots with the effects of two independent variables on the response, while the other two factors are kept constant. It was observed that the temperature of heating and the size of shrimp waste particles significantly affected the extraction

Table 3. Box-Behnken Design Experimental and Observed Values and Predicted Values of Extraction Yield of Astaxanthin in Sunflower Oil

Std	Run	Factor 1 A:Temperature of heating (°C)	Factor 2 B:Time of heating (min)	Factor 3 C:Oil:Waste ratio (v/w)	Factor 4 D:size (mesh)	Response 1 Y-observed (μg g^{-1})	Y-predicted (μg g^{-1})
24	1	60	180	2	80	12.8	12.85
23	2	60	60	2	80	12.65	12.58
14	3	60	180	1	60	11.31	11.31
20	4	70	120	3	60	12.98	13
6	5	60	120	3	40	11.67	11.71
17	6	50	120	1	60	13	13.01
7	7	60	120	1	80	13.9	13.75
21	8	60	60	2	40	12.01	11.99
11	9	50	120	2	80	14.1	14.27
5	10	60	120	1	40	11.68	11.71
1	11	50	60	2	60	12.18	12.31
8	12	60	120	3	80	11.9	11.77
10	13	70	120	2	40	14.3	14.20
4	14	70	180	2	60	13.33	13.09
2	15	70	60	2	60	12.87	12.84
26	16	60	120	2	60	13.42	13.43
22	17	60	180	2	40	11.23	11.33
3	18	50	180	2	60	11.74	11.66
13	19	60	60	1	60	11.12	11.02
25	20	60	120	2	60	13.47	13.43
27	21	60	120	2	60	13.34	13.43
15	22	60	60	3	60	10.44	10.52
9	23	50	120	2	40	12.88	12.83
12	24	70	120	2	80	14.74	14.87
19	25	50	120	3	60	11.24	11.06
28	26	60	120	2	60	13.48	13.43
18	27	70	120	1	60	12.82	13.03
16	28	60	180	3	60	9.65	9.83

yield and the response surface maximized when the temperature is approximately 70 °C and the shrimp waste's particle size was 80 mesh (Fig. 2E). Furthermore, by increasing the heating time by 120 min and the ratio of oil to waste (v/w) by 2, the extraction yield first increased and then decreased (Fig. 2A). It is well known that the extraction yield is affected by all factors. Meanwhile, the oil: waste ratio (v/w) of 2 and temperature at 70 °C showed significant performance ($p < 0.001$) compared to other temperatures and ratios (Fig. 2C).

Box-Behnken design results for the optimization showed that all four factors (including the heating temperature, heating time, oil: waste ratio, and particle sizes of shrimp waste) and their interactions significantly affected

Table 4. Analysis of Variance (ANOVA) Results for the Box-Behnken Design. All Four Factors and their Interactions Significantly Affected the Astaxanthin Yield; the Model was Significant, Lack of Fit Test was not Significant ($p > 0.05$), R2 of the Astaxanthin Extraction Model was 0.9915, Standard Deviation was 0.16, the Adeq Precision was 42.9437 and the Coefficient of Variation (CV) was 1.28. (* and ** Indicates $p < 0.05$ and $p < 0.001$ Respectively)

Source	Sum of Squares	df	Mean square	F-value	p-value
Model	39.09	14	2.79	108.59	< 0.0001**
A-Temperature of heating	2.90	1	2.90	112.82	< 0.0001**
B-Time of heating	0.1220	1	0.1220	4.75	0.0484*
C-Oil:Waste ratio	2.95	1	2.95	114.74	< 0.0001**
D-waste size	3.33	1	3.33	129.46	< 0.0001**
AB	0.2025	1	0.2025	7.88	0.0148*
AC	0.9216	1	0.9216	35.84	< 0.0001**
AD	0.1521	1	0.1521	5.92	0.0302*
BC	0.2401	1	0.2401	9.34	0.0092**
BD	0.2162	1	0.2162	8.41	0.0124*
CD	0.9900	1	0.9900	38.51	< 0.0001**
A^2	1.23	1	1.23	47.87	< 0.0001**
B^2	11.82	1	11.82	459.56	< 0.0001**
C^2	11.03	1	11.03	428.98	< 0.0001**
D^2	0.1593	1	0.1593	6.19	0.0272*
Residual	0.3342	13	0.0257		
Lack of Fit	0.3220	10	0.0322	7.87	0.0581
Pure Error	0.0123	3	0.0041		
Cor Total	39.42	27			

$R^2 = 0.9915$, C.V. % = 1.28, Std. Dev. = 0.16

Adjusted $R^2 = 0.9824$, Predicted $R^2 = 0.9524$, Adeq Precision = 42.9437

Table 5. Results of Regression Coefficients for Variables and their Interactions

Factor	Coefficient estimate	df	Standard error	VIF
Intercept	13.43	1	0.0802	
A-Temperature of heating	0.4917	1	0.0463	1.00
B-Time of heating	-0.1008	1	0.0463	1.00
C-Oil:Waste ratio	-0.4958	1	0.0463	1.00
D-waste size	0.5267	1	0.0463	1.00
AB	0.2250	1	0.0802	1.00
AC	0.4800	1	0.0802	1.00
AD	-0.1950	1	0.0802	1.00
BC	-0.2450	1	0.0802	1.00
BD	0.2325	1	0.0802	1.00
CD	-0.4975	1	0.0802	1.00
A^2	0.4529	1	0.0655	1.14
B^2	-1.40	1	0.0655	1.14
C^2	-1.36	1	0.0655	1.14
D^2	0.1629	1	0.0655	1.14

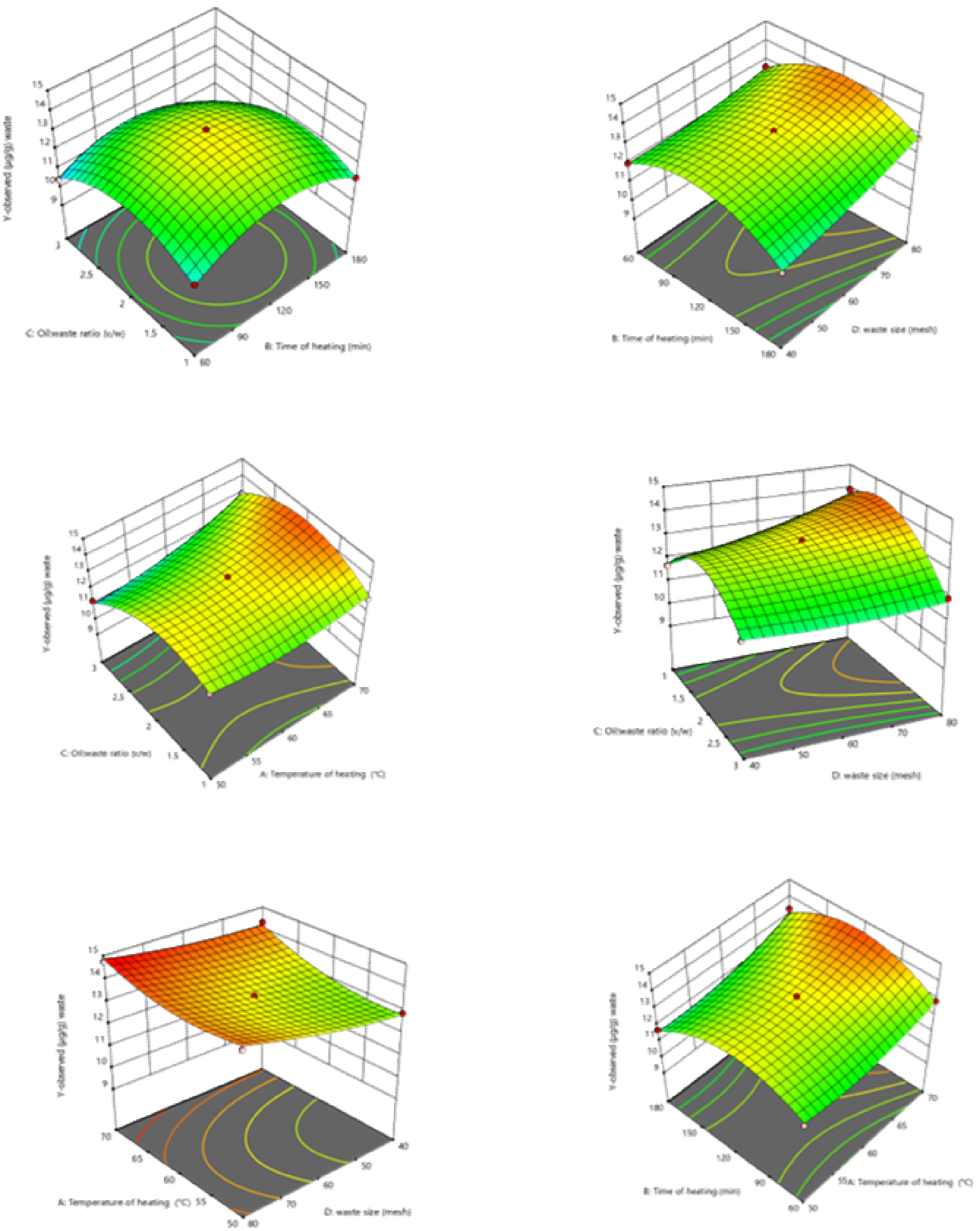

Fig. 2. Indicates three-dimensional response surface plots with the effects of two independent variables on the response (extraction yield (Y-observed)), while the other two factors are kept constant.

the astaxanthin. The regression equation yielded predictions for carotenoids that were quite close to the observed values (Table 3). The coefficients showed that the experimental results were very valid and reliable. They reported that extraction of astaxanthin by sunflower oil at 70 °C, oil: waste ratio of 2, and heating time of 120 min from shrimp waste (*Penaeus indicus*) led to optimal results (26.3 μg g^{-1}) [33]. Getachew *et al*. (2022) reported that increasing the temperature could facilitate extraction performance by increasing solubility and breaking down the carotenoid-protein complex; however, high temperatures can reduce the extraction efficiency by a higher degree of hydrolysis [40]. As shown in this study, the optimum temperature significantly affects the extraction performance of astaxanthin.

Hooshmand *et al.*, (2016) achieved the highest extraction efficiency of astaxanthin (5.553 μg g^{-1}) from shrimp (*Penaeus semisulcatus*) waste using sunflower oil at 40 °C for 180 min and an oil: shell waste ratio of 5:1 [32]. In addition, the results of the present study showed that the size of the waste particle had a significant effect on extraction performance, and a positive relationship was found between waste particle size and extraction efficiency (Figs. 2E, D, B). Handayani *et al.*, (2008) explained that the length of the diffusion pathways decreases by decreasing particle size, and thus the astaxanthin mass transfer rate increases [41].

In another study, Sharayei *et al.*, (2021) optimized the extraction conditions of astaxanthin from shrimp (*Penaeus semisulcatus*) using nonpolar/polar solvents by Box-Behnken design [25].

CONCLUSIONS

The method of extracting astaxanthin with oil is a sustainable extraction method that protects the pigment from oxygen and consequently retards the degradation rate of astaxanthin extract. Although extraction efficiency is not very great, extracting astaxanthin from shrimp waste using oil might be acceptable owing to its favorable environmental effects, low toxicity, and widespread availability. To improve the astaxanthin extraction conditions, we have created a model using BBD. The highest yield of extracted astaxanthin was 14.74 ± 1.6 μg g^{-1} of waste under a heating time of 120 minutes, oil to shell waste ratio (v/w) of 2, the heating temperature at 70 °C, and waste the size of 80 mesh. In addition, *Penaeus semisulcatus* waste has the highest yield of astaxanthin extraction using sunflower oil compared to *Penaeus merguiensis*.

ACKNOWLEDGMENTS

The authors express their gratitude to the research council of the University of Hormozgan for financial support during the course of this project.

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