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Optimized Solid-Phase Extraction of Astaxanthin from *Portunus trituberculatus* Using Response Surface Methodology and Ionic Liquid-Based Sorbents

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A 17-run Box-Behnken design was used to optimize the extraction conditions of astaxanthin from *Portunus trituberculatus*. The effects of the extraction time (h), extraction temperature (°C) and liquid to solid ratio were investigated. The following optimal conditions were derived using a response surface methodology: extraction time = 2 h, extraction temperature = 83 °C and ratio of liquid to solid = 10:1. The theoretical amount of astaxanthin (29.34 μg g^{-1}) was obtained under the above conditions using Design-Expert software. The solid-phase extraction method was used to purify astaxanthin from *Portunus trituberculatus* using ionic liquid-based silica and polymer, such as SilprMImCl, SilprBimCl, SilprNH_2, PEImCl, PBImCl and PHImCl sorbents. The optimal solid-phase extraction conditions were water as the washing solvent and ethanol as the elution solvent. Under these conditions, 18.3 μg g^{-1} astaxanthin was obtained, which was in good agreement with the value predicted from the Box-Behnken design model.

Keywords: Purification, Solid phase extraction, Astaxanthin, Response surface methodology.

INTRODUCTION

Astaxanthin (3,3'-dihydroxy-β,β'-carotene-4,4'-dione) is present in many types of seafood and is the main carotenoid pigment found in aquatic animals. Astaxanthin is a red pigment common to many marine animals, such as salmon, trout, red sea bream, shrimp, red lobster and fish eggs. Astaxanthin is related to other well-known carotenoids, such as β-carotene, zeaxanthin and lutein. The benzenoid rings at the end of the astaxanthin molecule has two asymmetric atoms. A range of astaxanthin stereoisomers that differ in the configuration of the two hydroxyl groups on the molecule have been found. In the 'R configuration', the hydroxyl group is attached so that it projects above the plane of the molecule (Fig. 1). In contrast, the 'S configuration' is defined when the attached hydroxyl group projects below the plane of the molecule. Therefore, three enantiomers are possible: 3R, 3R'; 3S, 3S' and 3R, 3S' (meso)[1-5]. The response surface methodology (RSM) described by Box and Wilson is an effective optimization tool when many factors and interactions affect the desired response[6]. The RSM can identify the optimal conditions for the response from the designed experiments[7], which will be arranged and interpreted more easily using this efficient design[8-12]. The RSM features two main experimental design methods: the Box-Behnken design (BBD) and Central Composite design (CCD). These experimental designs are fitted to a second-order polynomial using a least squares technique. Box-behnken design has only three levels, requires fewer experiments and is more efficient, which makes it easier to arrange and interpret the experiments compared to other methods[13-15]. The release of various harmful organic chemicals into the environment has attracted considerable interest worldwide because of their toxicity and widespread use. The most widely used methods for analyzing these harmful organic contaminants include chromatographic techniques such as gas chromatography or high performance liquid chromatography[16]. On the other hand, their sensitivity and selectivity are usually too low for a direct determination of these contaminants at very low concentrations in complex matrix environmental samples. Solid-phase extraction (SPE) is the most common technique for environmental water sample pretreatment. Various types of solid-phase extraction sorbents, including C_{18} or C_8 silica, polystyrene-di vinyl benzene polymers and various carbonaceous sorbents, have been used. The potential properties of polymer-based sorbents have been studied using a range of methods. Among them, C_{18} silica is used most widely[17]. Ionic liquid-based materials such as ionic liquid-based silica and ionic liquid-based polymer, have attracted because ionic liquid-based materials have the physical and chemical properties of both ionic liquid and polymer and silica[18]. Ionic liquids are also used as stationary phases in HPLC and solid-phase extraction (SPE) sorbents, bonding them to silica and hence constituting silica-confined ionic liquids

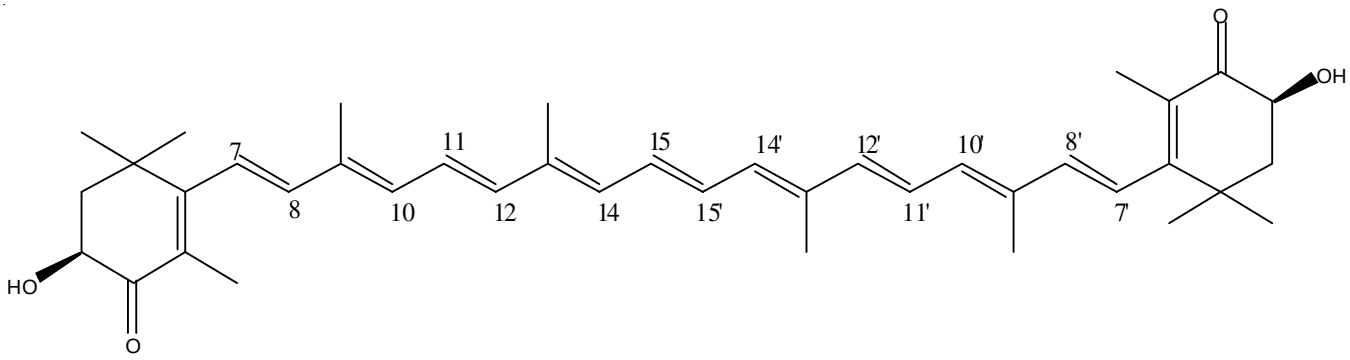


Fig. 1. Molecular structures of astaxanthin

(SiILs)[19]. Imidazolium-based halide is one of the ionic liquid families to well use at studied. dimethylimidazolium-based halide showed the strong hydrogen bonding between anion and cation. Moreover, an extended structure, namely, network, can be formed through hydrogen bonding for dialkylimidazolium chloride[20]. Therefore, in this study focused on the dialkylimidazolium chrolide.

In this study, the significant variables (extraction time, extraction temperature and ratio of liquid/solid) were examined to optimize the process for determining the amount of astaxanthin using a three-level Box-Behnken design. The optimal extraction conditions were found and the purification of astaxanthin from *Portunus trituberculatus* was performed by solid-phase extraction using different solid-phase extraction sorbents materials.

EXPERIMENTAL

Portunus trituberculatus was caught from Yeonpyeong Island on October 2012 (Incheon, Korea). Astaxtaxnthin was obtained from Sigma (St. Louis, Mo., U.S.A). Acetonitrile, methanol and acetic acid were purchased from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). 1-Methylimidazole (99 %) was supplied by Aldrich (Milwaukee, WI, USA). 1-Butylimidazole (> 98 %), 1-vinylimidazole (98 %), ethyl bromide (> 98 %), *n*-butyl bromide (> 97 %) and *n*-hexyl bromide (> 95 %) were acquired from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). (3-Chloropropyl)-trimethoxysilane (97 %) and (3-aminopropyl)trimethoxy silane (97 %), ethyleneglycol dimethacrylate (EDGMA) (> 98 %) were purchased Sigma-Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA) was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). All other reagents used in the experiment were of the highest grade. Double distilled water was filtered using a vacuum pump (Division of Millipore, Waters, USA) and filter (HA-0.45, Division of Millipore, Waters, USA) prior to use. All samples were filtered through a filter (MFS-25, 0.2 mm TF, Whatman, USA) before being injected into the high performance liquid chromatography (HPLC) system.

Chromatography conditions: HPLC was performed using a Waters 600s multisolvent delivery system, Waters 616 liquid chromatography and waters 2487 variable wavelength, dual-channel UV detector (Waters Associates, Milford, MA, USA). A commercial C_{18} column (4.6 × 250 mm, 5 μm) purchased from RStech Co. (Daejeon, Korea) was used. The flavones were separated by HPLC using methanol/dichloromethane-/acetonitrile/water (85:5.5:5:4.5, v/v) as the mobile phase. The flow rate, UV wavelength and injection volume were set to 1 mL min^{-1}, 480 nm and 10 μL, respectively.

Preparation of standard solutions and sample solution: Stock solutions of astaxanthin (0.025, 0.050, 0.250 and 0.500 mg mL^{-1}) were prepared in 1 mL of a methanol/dichloromethane mixture (75:25, v/v). *Portunus trituberculatus* waste was powdered and 1 g of the resulting powder was weighed and extracted with 10 mL of methanol by heating under reflux at 83 °C for 2 h (optimized conditions following RSM). After centrifugation and filtration, the extract was collected and stored for later use.

Response surface methodology design and analysis: A 17-run Box-Behnken design was applied to optimize the extraction conditions, such as the extraction time, extraction temperature and liquid/solid ratio. A Box-Behnken design with three independent variables were used, as listed in Table-1. The *trans* natural astaxanthin is isomerized easily to *cis-trans* by increasing the temperature, exposure to light, or the presence of acid. Therefore, selection of extraction condition level was careful for reduce of astaxanthin isomerized. The three factors were designated as X_1, X_2 and X_3, respectively and divided into three levels, coded +1, 0 and -1, using the following equation:

$$X_i = \frac{X_i - X_o}{\Delta X} \quad i = 1, 2, 3 \tag{1}$$

where x_i is the coded value of the independent variable, X_i is the actual value of the independent variable, X_0 is the actual value of the independent variable at the center point and ΔX is the step change in the independent variable. A second-order polynomial model was fitted to the interaction between the response (amount of monolithic sorbent) and the independent variables (x_i, X_i, X_0, ΔX).

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ij} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} A_{ij} X_i X_j \tag{2}$$

TABLE-1
INDEPENDENT VARIABLES AND LEVELS OF RESPONSE SURFACE ANALYSIS

Variables	Level		
	-1	0	1
Extraction time (X_1)(h)	0.5	1	2
Extraction temperature (X_2)(°C)	75	85	95
Ratio of liquid/solid (X_3)	10	20	30

where Y is the dependent variable (amount of astaxanthin in real value) and A_0, Ai, A_{ii} and A_{ij} are the coefficients estimated by the model. X_i and X_j are the levels of the independent variables representing the linear, quadratic and cross-product effects of the X_1, X_2 and X_3 factors on the response, respectively. The independent variables were used to evaluate the model according to each effect on the response. The experimental design was analyzed and the data was predicted to estimate the response of the independent variables using Design-Expert software (v.8.0.6, Stat-Ease, Inc, Minneapolis, USA). An additional experiment was performed to affirm the validity of the statistical experimental strategies.

Preparation of solid phase extraction sorbent: The SiILs and PolyILs were synthesized using a slight modification of the procedure reported elsewhere[21]. Silica was first immersed in hydrochloric acid for 12 h with constant stirring. Subsequently, the material was washed with deionized water until it was neutral and dried at 100 °C for 8 h. A solution of 5 g activated silica and excess of 3-chloropropyltrimethoxysilane (5 mL) in 50 mL of dry toluene was used to obtain chloropropyl silica (SilprCl). The substrate mixture was heated under reflux for 12 h. After the reaction, the mixture was allowed to cool naturally to room temperature. The particles were washed sequentially with toluene, deionized water and methanol. Finally, the particles were dried at 60 °C for 10 h. The solutions containing 5 g of dry chloropropyl silica and a large excess of 1-methylimidazole, or 1-butylimidazole in 50 mL of dry toluene were prepared to obtain SilprMImCl and SilprBImCl, respectively. Aminopropyl silica ($SilprNH_2$) was obtained by a reaction of 3-aminopropyltrimethoxysilane (5 mL) with activated silica (5 g) using the procedure used to prepare SilprCl. The PolyILs were synthesized using a slight modification of the procedure reported by Bi *et al.*[22]. A 100 mL round-bottom flask loaded with 0.1 mol of 1-vinylimidazole, 0.1 mol of 3-aminopropyl bromide and 30 mL of methanol. The mixture was stirred at 60 °C for 15 h. After cooling, the reaction mixture was added dropwise to 1 L of diethyl ether. The white precipitate was filtered and dried at room temperature until a constant weight was reached. 1-Vinyl-3-butylimidazolium lactate was synthesized using the procedure reported elsewhere.

Solid-phase extraction procedure: Commercial solid-phase extraction cartridges (diameter 0.9 cm, 3 mL) with 0.2 g C_{18}, silica sorbent were purchased from Alltech (Deerfield, IL, USA) and SilprMImCl, SilprBimCl, $SilprNH_2$, PEImCl, PBImCl and PHImCl were packed separately at the bottom of the empty polypropylene cartridge and preconditioned with methanol. The sample solution (0.2 mL) was loaded into the solid-phase extraction cartridge, washed sequentially with methanol, water, *n*-hexane and ethyl acetate and eluted with methanol, ethanol, *n*-hexane and methanol-dichloromethane (75:25, v/v).

RESULTS AND DISCUSSION

Model fitting and statistical analysis: The optimal conditions for the important factors were determined using a 17-run Box-Behnken design. A 17-run Box-Behnken design with three factors (extraction time, temperature and ratio of liquid/solid) and three levels (-1, 0, 1), including five repeats at the center point, was used to fit the second-order response surface to optimize the extraction conditions. Five runs of the center point were carried out to maintain the inherent variability and process stability and the amount of astaxanthin was taken as the response. Table-2 listed the coded variable levels along with the predicted and experimental values of the response. The predicted responses were obtained from a model fitting technique using the software design expert. The predictive equation was obtained by fitting the experimental data to the Box-Behnken design model in eqn. 3. The quadratic polynomial is be expressed as

$$Y=17.66 + 2.42X_1 + 2.03X_2-2.56X_3 + 1.47 X_1X_2-3.11X_1X_3 + 5.34X_2X_3 + 3.08X_1^2-3.28X_2^2 + 0.25X_3^2 \quad (3)$$

The importance of each coefficient was confirmed using the F-value and p value, which are listed in Table-3. The p-value is the probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis was true. One often "rejects the null hypothesis" when $p < 0.05$. The result is said to be statistically significant when the null hypothesis is rejected[23]. In the present study, the F-value of the model was 9.420, which shows that the model

TABLE-2
BOX-BEHNKEN EXPERIMENTAL DESIGN WITH THE INDEPENDENT VARIABLES

Std	Coded variable levels			Amount of astaxanthin (μg g^{-1})		RSDs (%)	
	X_1	X_2	X_3	Actual values	Predicted values	Intra-day	Inter-day
1	-1	0	1	13.11	14.48	2.35	2.30
2	0	0	0	15.75	16.38	1.22	1.19
3	0	0	0	16.21	15.59	1.22	1.19
4	1	0	-1	24.74	23.37	3.54	3.72
5	-1	0	-1	20.01	18.01	1.87	2.01
6	-1	-1	0	30.33	29.08	2.18	2.05
7	0	0	0	17.87	19.12	1.22	1.19
8	0	0	0	15.74	17.74	1.22	1.19
9	0	-1	1	19.87	20.50	1.38	1.52
10	0	1	-1	11.24	13.86	3.35	3.27
11	-1	1	0	7.32	4.70	2.95	3.02
12	0	1	1	20.06	19.43	1.79	1.92
13	1	-1	0	18.12	17.66	3.21	3.20
14	1	0	1	17.25	17.66	2.33	2.52
15	0	0	0	17.55	17.66	1.22	1.19
16	1	1	0	17.25	17.66	1.43	1.23
17	0	-1	-1	18.11	17.66	2.36	2.57

TABLE-3
ANALYSIS OF THE VARIANCE OF THE EXPERIMENTAL RESULTS OF THE BOX-BEHNKEN DESIGN

Source	Sum of squares	DF	Mean square	F-Value	*p*-Value prob > F
Model	374.9368	9	41.660	9.420	0.0037[a]
A-Monomer	46.8512	1	46.851	10.594	0.0140[a]
B-Crosslink	32.805	1	32.805	7.418	0.0296[a]
C-Porogen	52.32645	1	52.326	11.832	0.0108[a]
AB	8.673025	1	8.673	1.961	0.2041
AC	38.75063	1	38.751	8.762	0.0211[a]
BC	114.1692	1	114.169	25.816	0.0014[a]
A2	39.96219	1	39.962	9.036	0.0198[a]
B2	45.41599	1	45.416	10.269	0.0150[a]
C2	0.264739	1	0.265	0.060	0.8137
Residual	30.95742	7	4.422	-	-
Lack of fit	30.1951	3	10.065	52.81	0.0011[a]
Pure error	0.76232	4	0.191	-	-
Cor total	405.8942	16	-	-	-

[a]Means significance (Values of "Prob > F" less than 0.0500)

was significant and noise probability due to the F- value was less than 0.0001 %. A "Lack of Fit F- value" of 52.81 showed that pure error was not significant and the noise probability due to the "Lack of Fit F- value" was 0.11 %. Table-4 lists the coefficient of determination ($R^2 = 0.924$), the adjusted coefficient of determination ($R^2_{Adj} = 0.826$) and the coefficient of variation (C.V = 11.90 %). This suggests that the accuracy and general availability of the polynomial model were adequate and an $R^2P_{red} = 0.193$ showed reasonable agreement with R^2_{Adj}. The "Adeq. Precision" measured the signal to noise ratio and a ratio > 4 is normally desirable. The "Adeq. Precision" of 15.116 indicated that this model could be used to navigate the design space.

Optimization of the procedure: The surface curves of the response were plotted to show the interactions of the variables as well as the optimal level of each variable for the optimal response[24]. The 2D contour plot and 3D response surface plots are provided as graphical representations of the regression equation (Figs. 2-4). Each contour curve shows an infinitive number of combinations of two test variables with the other factors fixed to the zero level. Fig. 2 presents the effects of the extraction temperature, extraction time with the liquid/solid ratio fixed at 20:1 and their reciprocal interactions on the amount of astaxanthin. The amount of astaxanthin increased with increasing extraction time and increasing extraction temperature up to 2 h and 95 °C, respectively. Fig. 3 shows the effects of the extraction time and liquid/solid ratio with an extraction temperature at 85 °C, as well as their reciprocal interaction on the amount of astaxanthin. The amount of astaxanthin increased with increasing extraction time and decreasing liquid/solid ratio up to 2 h and 10:1, respectively. Fig. 4 shows the effects of the extraction temperature, liquid to solid ratio with the extraction time fixed to 1 h and their reciprocal interactions on the amount of astaxanthin. The amount of astaxanthin increased with increasing extraction temperature and liquid/solid ratio up to 95 °C and 10:1, respectively. In addition, the amount of astaxanthin increasing with increased extraction temperature. On the other hand, trans natural astaxanthin isomerizes easily to the *cis-trans* isomer under increased temperature, which can cause the formation of the *cis*-isomers[25,26]. Therefore, extraction at elevated temperatures is undesirable. Fig. 5 shows the normal probability

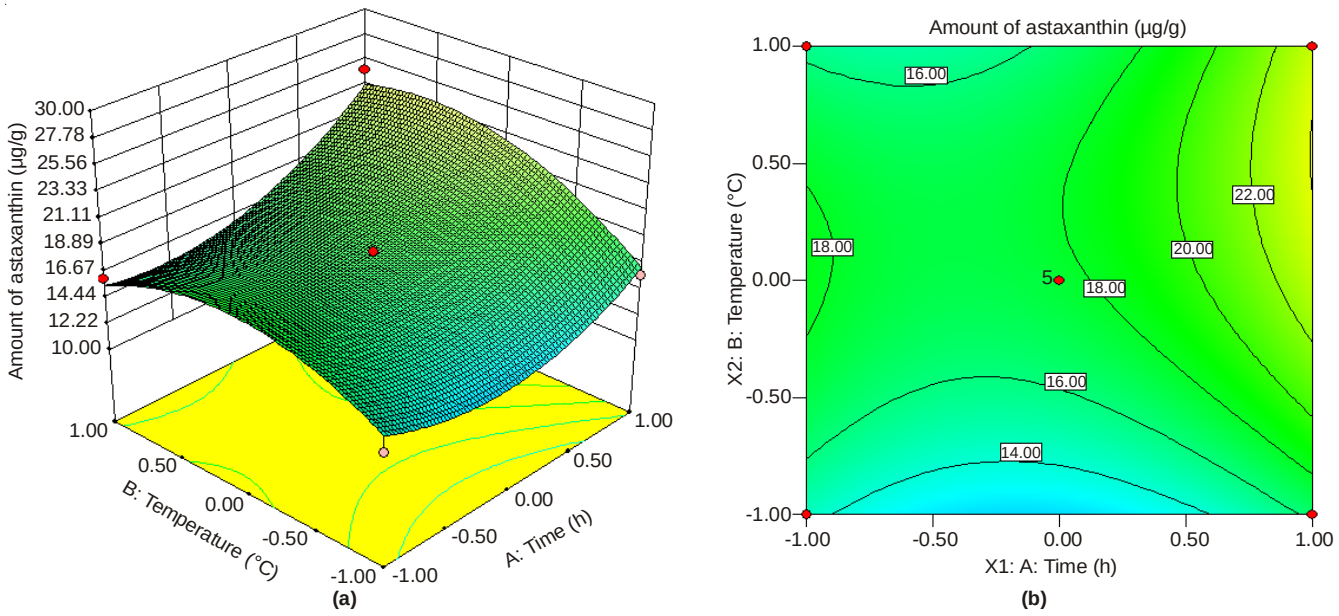


Fig. 2. Effect of the extraction time and extraction temperature. (with ratio of liquid/solid constant at 1: 20) (a) 3D response surface; (b) 2D contour plots)

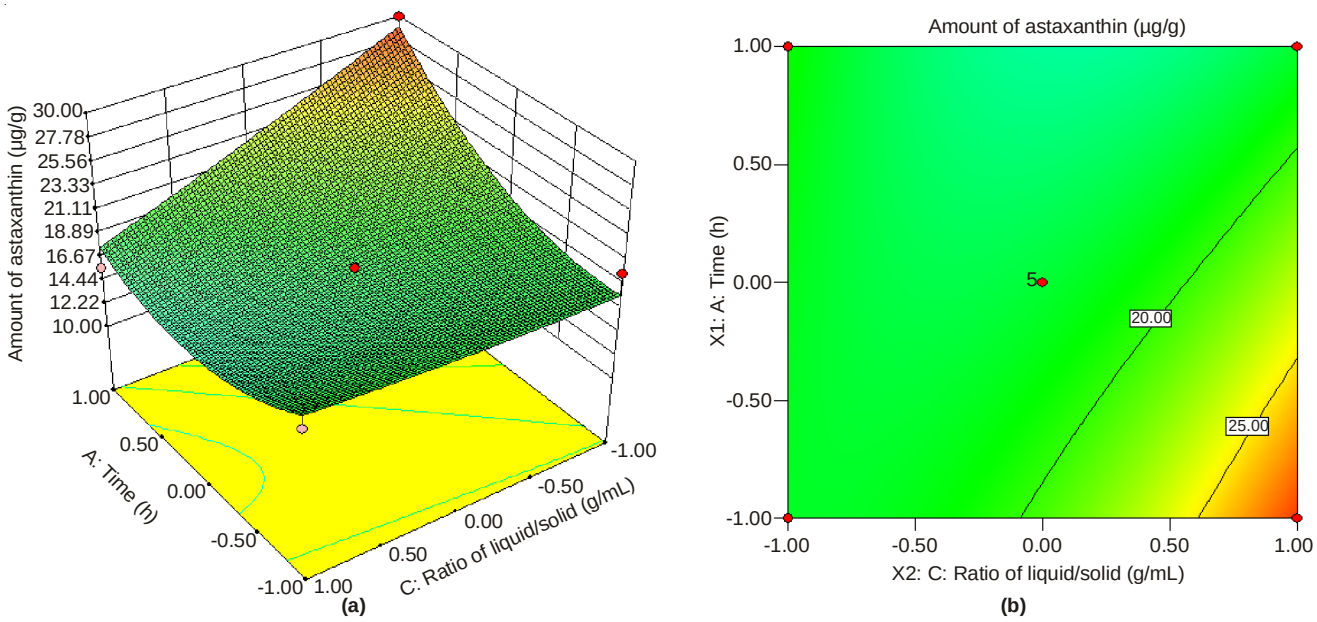


Fig. 3. Effect of the extraction time and extraction ratio of liquid/solid. (with temperature constant at 85 °C) (a) 3D response surface; (b) 2D contour plots)

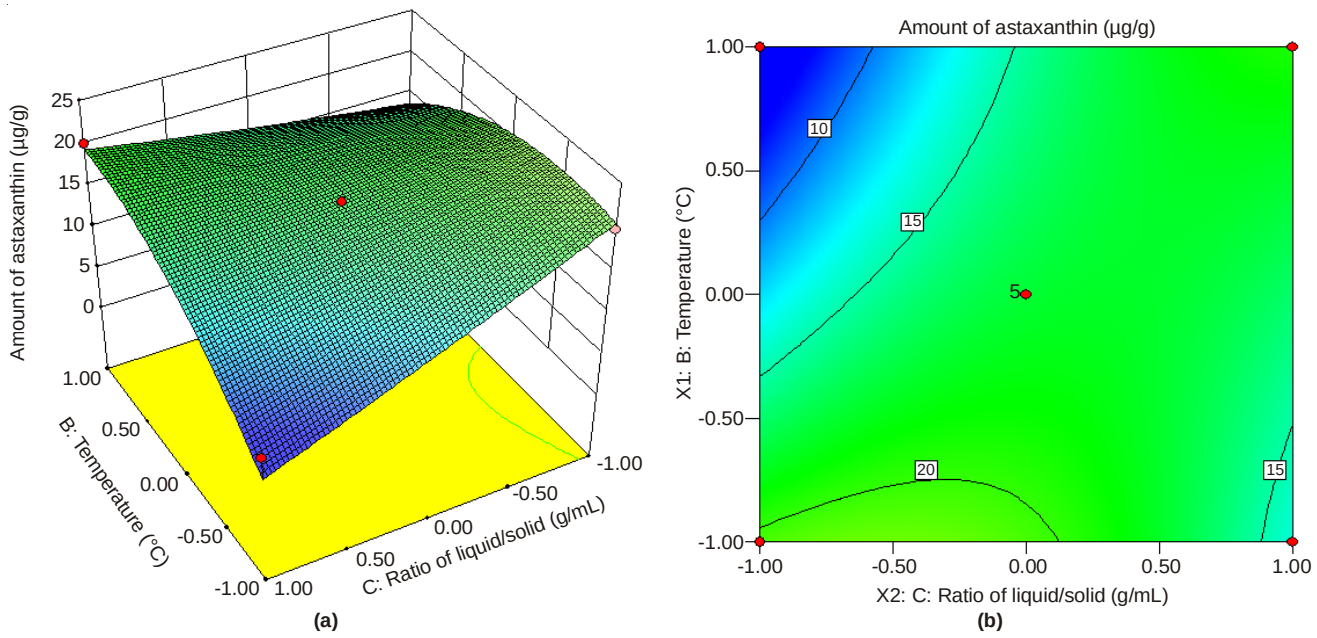


Fig. 4. Effect of the extraction temperature and extraction ratio of liquid/solid. (with time constant at 1 h) (a) 3D response surface; (b) 2D contour plots)

TABLE-4
ANALYSIS OF THE VARIANCE OF THE FITTED F-QUADRATIC POLYNOMIAL MODEL OF EXTRACTION OF ASTAXANTHIN

Item	Std. Dev.	Mean	C.V. %	R^2	R^2_{Adj}	R^2_{Pred}	Adeq. precision
Value	2.10	17.68	11.90	0.924	0.826	0.193	15.116

plot and dot diagram of the residuals. The data points on the plots are reasonably close to a straight line, which means that the underlying assumptions of the analysis were satisfied. Fig. 6 shows the relationship between the actual and predicted values of the amount of astaxanthin. Fig. 6 shows that the residuals were in close proximity to the straight diagonal line. The optimal extraction conditions (X_1 = 2 h, X_2 = 83 °C and X_3 = 10:1) for the amount of astaxanthin were estimated using the model equation by solving the regression equation and analyzing the response surface contour plot. The theoretical amount of astaxanthin (29.34 µg g^{-1}) was obtained under the above conditions using Design-Expert software. The repeatability calculated as the relative standard deviations (RSDs) was assessed by injecting the extraction samples 10 times over a 5-day period. RSDs < 3.72 % showed acceptable precision and accuracy (Table-4).

Determination of astaxantin concentrations: A series of mixtures of standard solutions containing astaxanthin were diluted (0.5, 1.0, 2.0, 4.0 and 5.0 µg mL^{-1}) with methanol-dichloromethane (75:25, v/v). The resulting linear regression equations of the astaxanthin were Y = 4E + 07x-1E + 06 (r^2 = 0.9956), where, Y and X represent the peak area of the

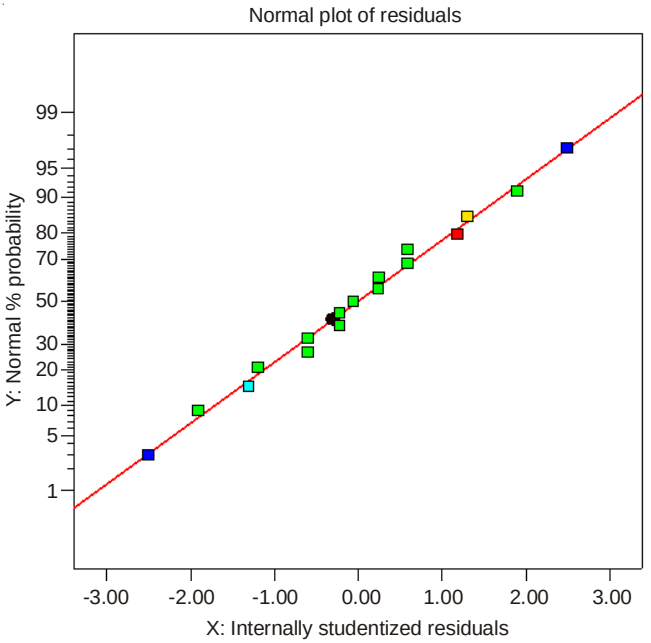


Fig. 5. Normal % probabilities *versus* internally studentized residuals for amount of astaxanthin

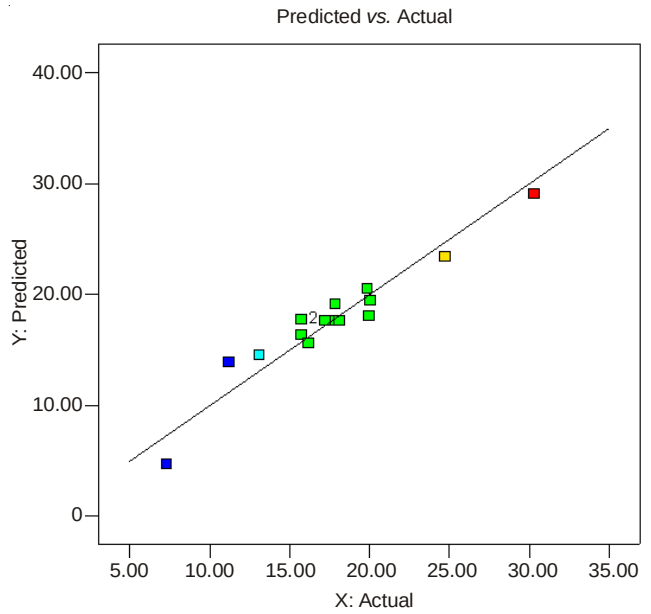


Fig. 6. Scatter diagram of the predicted response *versus* the actual response for the amount of astaxanthin

analytes and concentration of the analytes in the *Portunus trituberculatus* waste.

Purification of astaxanthin by solid phase extraction: Commercial C_{18} and silica sorbent were prepared. The SilprMImCl, SilprBimCl, $SilprNH_2$, PEImCl, PBImCl and PHImCl sorbents were synthesized and the level of adsorption was compared with that of the commercial sorbent. Table-5 presents sorbent structure of ionic liquid-based silica and polymer. Selection of the washing solvent and elution solvent is an important process in solid-phase extraction. The removal of interfering compounds in the washing stage is important for obtaining the target compounds in the elution stage. Table-6 lists the amounts of target compounds in the different washing solvents. The interference from the sorbent structure and

TABLE-5
SORBENT STRUCTURES OF IONIC LIQUID BASED SILICA AND POLYMER

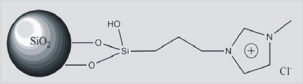
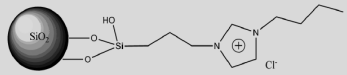
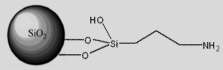
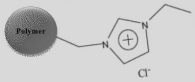
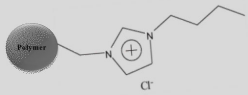
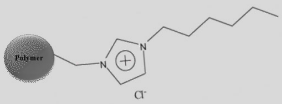
Name	Structure
SilprMImCl	
SilprBImCl	
$SilprNH_2$	
PEImCl	
PBImCl	
PHImCl	

TABLE-6
AMOUNT OF ASTAXANTHIN REMAINING IN THE DIFFERENT WASHING SOLVENTS AND SORBENTS

Washing solvent	Sorbent	Amount (μg g^{-1})
Water	C_{18}	ND
	Silica	ND
	PEImCl	ND
	PBImCl	ND
	PHImCl	ND
	SilprEImCl	ND
	SilprBImCl	ND
	$SilprNH_2Cl$	ND
Methanol	C_{18}	9.0
	Silica	6.0
	PEImCl	17.0
	PBImCl	12.1
	PHImCl	19.0
	SilprEImCl	5.0
	SilprBImCl	8.0
	$SilprNH_2$	11.0
Ethyl acetate	C_{18}	3.3
	Silica	3.0
	PEImCl	1.2
	PBImCl	2.0
	PHImCl	2.0
	SilprEImCl	4.0
	SilprBImCl	5.0
	$SilprNH_2$	ND
Hexane	C_{18}	ND
	Silica	ND
	PEImCl	1.4
	PBImCl	10.0
	PHImCl	2.1
	SilprEImCl	ND
	SilprBImCl	ND
	$SilprNH_2$	ND

ND: Not detected; (Extraction method: heating, time: 0.5 h, Solid/Liquid ratio (g/mL): 1:30 (1g/30 mL); Solvent: Dichloromethane/methanol (25:75, v/v))

pore size could be removed due to the large or weak interactions[17]. Hydrophobic and hydrophilic interference could be washed out without the target compound using *n*-hexane and water, respectively. Astaxanthin was slightly soluble in alcohol and *n*-hexane owing to its two hydroxy groups (-OH) and long carbon chain. Astaxanthin was soluble in organic solvents, such as methanol, ethyl acetate and *n*-hexane but insoluble in water[27]. Therefore, water was selected as the washing solvent and fixed to 2 mL. Table-7 listed the amounts of astaxanthin extracted using the different elution solvents. After the washing stage, the largest amounts of the target compounds were extracted in the elution stage using 2 mL ethanol as the elution solvent. Ethanol was the best reason for dissolution elution, astaxanthin was slightly soluble in alcohol owing to its two hydroxy groups (-OH). The optimal solid-phase extraction conditions were water as the washing solvent and ethanol as the elution solvent. Under these conditions, the amount of astaxanthin extracted was 18.3 μg g^{-1}. Fig. 7 shows a chromatogram of the target compounds purified by the solid-phase extraction process.

TABLE-7
AMOUNT OF ASTAXANTHIN REMAINING IN THE DIFFERENT ELUTION SOLVENT AND SORBENTS

Washing solvent	Eluting solvent	Sorbent	Amount (μg g^{-1})
Water	Methanol/ dichloromethane (75:25 v/v)	C_{18}	17.4
		Silica	7.4
		PEImCl	12.2
		PBImCl	9.0
		PHImCl	8.0
		SilprEImCl	4.0
		SilprBImCl	4.1
		$SilprNH_2$	ND
	Methanol	C_{18}	13.2
		Silica	13.4
		PEImCl	7.0
		PBImCl	7.0
		PHImCl	5.0
		SilprEImCl	8.0
		SilprBImCl	3.0
		$SilprNH_2$	2.0
	Ethanol	C_{18}	2.3
		Silica	2.0
		PEImCl	18.3
		PBImCl	8.0
		PHImCl	8.1
		SilprEImCl	2.0
		SilprBImCl	4.0
		$SilprNH_2$	8.2
	Hexane	C_{18}	ND
		Silica	ND
		PEImCl	ND
		PBImCl	ND
		PHImCl	ND
		SilprEImCl	ND
		SilprBImCl	ND
		$SilprNH_2$	ND

ND: not detected; (extraction method: heating, time: 0.5 h, solid/liquid ratio (g/mL): 1:30 (1g/30 mL); Solvent: dichloromethane/methanol (25:75, v/v))

Effect of ionic liquid based silica and polymer: Ionic liquids have unique properties such as low vapor pressure, thermal stability, non-flammability and miscibility with water and organic solvents[28]. Hydrogen bonding, π-π, or hydrophobic interactions are used for extraction and separation in analytical chemistry[29]. According to previous studies[21,22], ionic liquid-based silica and polymer were excellent materials for the separation of bioactive compounds. An ionic liquid cannot be used as a separation sorbent. On the other hand, ionic liquid can be a possible sorbent by being immobilized on silica or polymer. Although astaxanthin has a hydroxyl group at both ends of the molecule, astaxanthin has non-polar properties due to the longer carbon chain. If astaxanthin interacts with ionic liquid-based silica, that interaction is broken using the water as the washing solvent. In the case of an ionic liquid-based polymer, the imidazolium chain length disturbs the interaction between astaxanthin and ionic liquid-based polymer. Therefore, the optimal sorbent is PEImCl, as shown in Table-7.

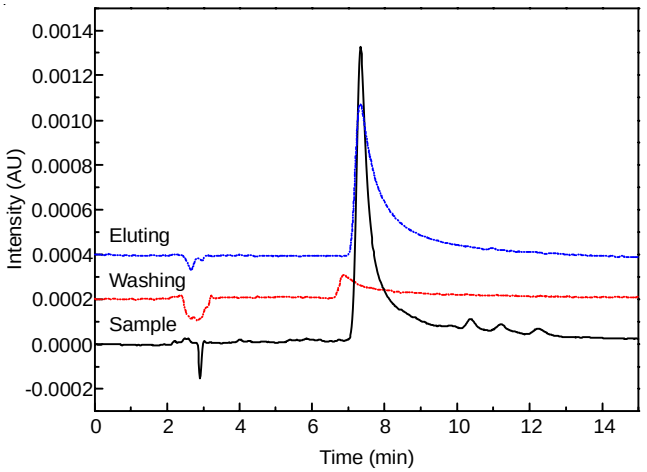


Fig. 7. Chromatogram of the sample after water washing and ethanol elution in PEImCl (mobile phase: methanol/dichloromethane/acetonitrile/water (85:5.5:5:4.5, v/v), column: C18 (4.6 × 250 mm, 5 μm), flow rate: 1 mL min^{-1}, UV: 480 nm)

Conclusion

The RSM is a useful tool for optimizing the extraction of astaxanthin from *Portunus trituberculatus*. The coefficient of determination (R^2) for the model was 0.924 and the probability value ($p < 0.0037$) indicated high significance of the regression model. The optimal conditions (extraction time 2 h, extraction temperature 83 °C and ratio of liquid/solid 10:1) for the amount of astaxanthin extracted were estimated using the model equation. The actual amount of astaxanthin extracted under the above conditions was 29.28 μg g^{-1}, which corresponds well to the predicted value. The solid-phase extraction method was used to purify astaxanthin from *Portunus trituberculatus* using ionic liquid-based silica and polymer. Some interactions between astaxanthin and ionic liquid-based polymer were observed and the optimal sorbent was PEImCl. Under the optimal solid-phase extraction conditions, 18.3 μg g^{-1} of astaxanthin was obtained.

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