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Polysaccharide extraction from *Abelmoschus esculentus*: Optimization by response surface methodology

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ABSTRACT

Crude polysaccharide extraction from the Iranian *Abelmoschus esculentus* was performed using water decoction. Response surface methodology (RSM), based on a five level, four variable central composite rotatable design (CCRD), was employed to obtain the best possible combination of extraction time (X_1: 0.5–6.5 h), extraction temperature (X_2: 80–100 °C), number of extraction (X_3: 1–5), and water to the raw material ratio (X_4: 4–28) for maximum polysaccharide extraction. The experimental data obtained were fitted to a second-order polynomial equation using multiple regression analysis and also analyzed by appropriate statistical methods (ANOVA). The optimum extraction conditions were as follows: extraction time of 4.94 h, extraction temperature of 94.97 °C, number of extraction of 4, and the ratio of water to raw material of 21.74. Under these conditions, the experimental yield was 16.895 ± 0.29%, which is well in close agreement with the value predicted by the model 16.916%.

1. Introduction

The thick and slimy texture of okra water-extracts is attributed to its curd polysaccharide content and is of primary technological interest for food and non-food applications and major applications have been previously reviewed (Kontogiorgos, Margelou, Georgiadis, & Ritzoulis, 2012; Whistler & BeMiller, 1993). Such extracts can be used as a natural food-grade emulsifiers (Ndjouenkeu, Akingbala, & Oguntimein, 1997) or thickeners and emulsion stabilizers (Georgiadis et al., 2011) suggesting that can be a promising source of texture modifiers for complex food matrices. In early works, the polysaccharides responsible for viscosity generation were identified as acidic polysaccharides consisting of galactose, rhamnose and galacturonic acid (Whistler & Conrad, 1954) while partial acetylation of the acidic polysaccharides has been also reported (Tomoda, Shimada, Shimizu, Kanari, & Kaneko, 1986). These early findings have been further corroborated by more recent work showing that okra polysaccharides consist of rhamnose, galacturonic acid, galactose, as well as of glucose and glucuronic acid (Deters, Lengsfeld, & Hensel, 2005; Lengsfeld, Titgemeyer, Faller, & Hensel, 2004). Sequential extraction using a series of hot extraction buffers and chelating agents yielded fractions consisting of pectin and highly branched rhamnogalacturonan with high levels of acetyl groups and galactose side chains (Sengkhamparn, Verhoef, Schols, Sajjaanantakul, & Voragen, 2009a, 2009b).

Since the structure of okra polysaccharides contains significant amounts of uronic acids, extraction strategies that could take advantage of this structural detail may yield products of different composition and properties (Kontogiorgos et al., 2012). Okra polysaccharides extracted with water can be characterized as an acidic polysaccharide consisting of galactose, rhamnose and galacturonic acid (Sengkhamparn et al., 2010; Whistler & Conrad, 1954).

The major structural element of okra polysaccharide contains a repeating unit of alternating rhamnose and galacturonic acid residues and carries disaccharide side chains composed of galactose attached to O-4 of half of the rhamnose residues (Sengkhamparn et al., 2010). The acetyl content of the okra polysaccharide was determined to be 5.5% (w/w) (Tomoda, Shimada, Saito, & Sugi, 1980). A detailed study by Sengkhamparn et al. (2009a, 2009b) recently described the different pectic and hemicelluloses (xylan, xyloglucan) structures present in okra.

In the extraction processes, where there are multiple independent variables affecting the responding factors, it is likely to use an optimization method that can determine all the factors. In addition, the possibility of interactions between the independent variables should be considered in order to determine the optimal experimental conditions (Cui, Mazza, Oomah, & Billiaderis, 1994).

Response surface methodology (RSM) has been reported to be an effective tool for optimization of a process when the independent variables have a combined effect on the desired response. RSM is a collection of statistical and mathematical system that has been successfully used for developing, improving and optimizing

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such processes (Bostan, Razavi, & Farhoosh, 2008; Cui et al., 1994; Koocheki et al., 2008; Myers & Montgomery, 2002; Wu, Cui, Tang, & Gu, 2007).

The purpose of the present study was to optimize the process for extraction of polysaccharides from the Iranian okra (*Abelmoschus esculentus*), using response surface methodology (RSM), employing a CCRD (4 factors and 5 levels) to study the effects of extraction time, extraction temperature, number of extraction, and water to the raw material ratio on the extraction yield of okra curd polysaccharides (OCP).

2. Materials and methods

2.1. Materials

The fresh okra pod was purchased from local farmers (Ahvaz, Iran), washed with tap water, rinsed with deionized water, and then air-dried at ambient temperature (25 °C). To inactivate the enzymes naturally present, the pods were heated in a hot air oven at the 90 °C for 90 min.

2.2. Methods

2.2.1. Extraction of okra crude polysaccharides (OCP)

The extraction of crude polysaccharides from okra pods was performed using a method modified from that by Komae and Misaki (1989).

The dried and heated okra pods (20 g) were stirred in deionized water (water to the raw material ratio ranging from 4 to 32) at pH 5.2 (adjusting the suspension pH by sodium acetate buffer), while the temperature of the water bath was kept steady for a given temperature (within ±1.0 °C, extraction temperature ranging from 50 to 100 °C). The slurry in a 2.0 L stainless steel in the hot water bath was stirred with an electric mixing paddle for a given time (extraction time ranging from 0.5 to 8 h) during the entire extraction process. The extracted slurry was centrifuged at 5000 rpm/min for 20 min to collect the supernatant, and the insoluble residue was treated again (extraction times ranging from 1 to 6) as mentioned above. An equal volume of ethanol was added to the resultant viscous solution to precipitate the crude polysaccharide extract. The precipitate was washed successively with 50% ethanol until a clear polysaccharide was obtained. It was then dewatered by washing successively with 80% ethanol and acetone. The extract was air-dried at the 50 °C until its weight was constant, and then was weighted with a balance (BS2202S, Sartorius, Germany). The percentage polysaccharides extraction yield (%) is calculated as follows:

$$\text{Polysaccharides extraction yield \% (w/w)} = \frac{\text{dried crude extraction weight (g)}}{\text{powder weight (20 g)}} \quad (1)$$

2.2.2. Experimental design and statistical analysis

Response surface methodology (RSM) was used to estimate the effect of independent variables (extraction time, X_1; extraction temperature, X_2; number of extraction, X_3 and water to the raw material ratio, X_4) on the extraction yield of OCP (%). A central composite rotatable design (CCRD) was employed for designing the experimental data.

The RSM was applied to the experimental data using a commercial statistical package, Design-Expert version 8.0.7.1 (Minneapolis, USA). Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The experimental design included star points, and six center points to calculate the repeatability of the method

Table 1
Independent variables and their levels used in the response surface design.

Independent variables	Factor level				
	−2	−1	0	1	2
Extraction time (h)	0.5	2	3.5	5	6.5
Extraction temperature (°C)	80	85	90	95	100
Number of extraction	1	2	3	4	5
Ratio of water to raw material	4	10	16	22	28

(Montgomery, 2001). After determining the preliminary range of the extraction variables through the single-factor test, a central composite rotatable design (CCRD) with four independent variables (extraction time, X_1; extraction temperature, X_2; number of extraction, X_3; water to the raw material ratio, X_4) at five levels was performed. The range of independent variables and their levels is presented in Table 1. The independent variables and their ranges were chosen based on preliminary experiment results. The response function (Y) was extraction yield of OCP (%). This value was related to the coded variables (Xi, i = 1, 2, 3 and 4) by a second order polynomial using below equation:

$$Y\ (\%) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 \quad (2)$$

The coefficients of the polynomial model were represented by β_0 (constant term), β_1, β_2, β_3 and β_4 (linear effects), β_{11}, β_{22}, β_{33} and β_{44} (quadratic effects), and β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34} (interaction effects). The statistical significance of the terms in the regression equations was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of the model was checked accounting for R^2 adjusted-R^2 and PRESS in Eqs. (3)–(5), respectively (Gharibzahedi, Mousavi, Khodaiyan, & Hamedi, 2012):

$$R^2 = 1 - \frac{SS_{Residual}}{SS_{Residual} + SS_{Model}} \quad (3)$$

$$R^2_{Adj.} = 1 - \frac{SS_{Residual}/DF_{Residual}}{(SS_{Residual} + SS_{Model})/(DF_{Residual} + DF_{Model})} \quad (4)$$

$$\text{PREES} = \sqrt{\sum_{i=1}^{N} (y_{\text{Pred},i} - y_{\text{Exp},i})^2} \quad (5)$$

Adequate precision compares the range of the predicted values at the design points to the average prediction error. The definition of adequate precision is in Eqs. (6) and (7):

$$\text{Adequate precision} = \frac{Max(\bar{y}) - \min(\bar{y})}{\sqrt{\bar{V}(\bar{y})}} \quad (6)$$

$$\bar{V}(\bar{y}) = \frac{1}{n}\sum_{i=1}^{N} V(\bar{y}) = \frac{P\sigma^2}{n} \quad (7)$$

In Eqs. (3)–(7), SS is the sum of squares, DF is the degrees of freedom, y_i, exp is the experimental responses, Y_i, per is the predicted responses, $\bar{y}$ is the predicted value, p is the number of model parameters, σ^2 is the residual mean square from ANOVA table, and n is the number of experiments.

Numerical and graphical optimization technique of the Design Expert software was used for simultaneous optimization of the multiple responses. The desired goal for variables and response was chosen. All the independent variables were kept within range while the response was maximized.

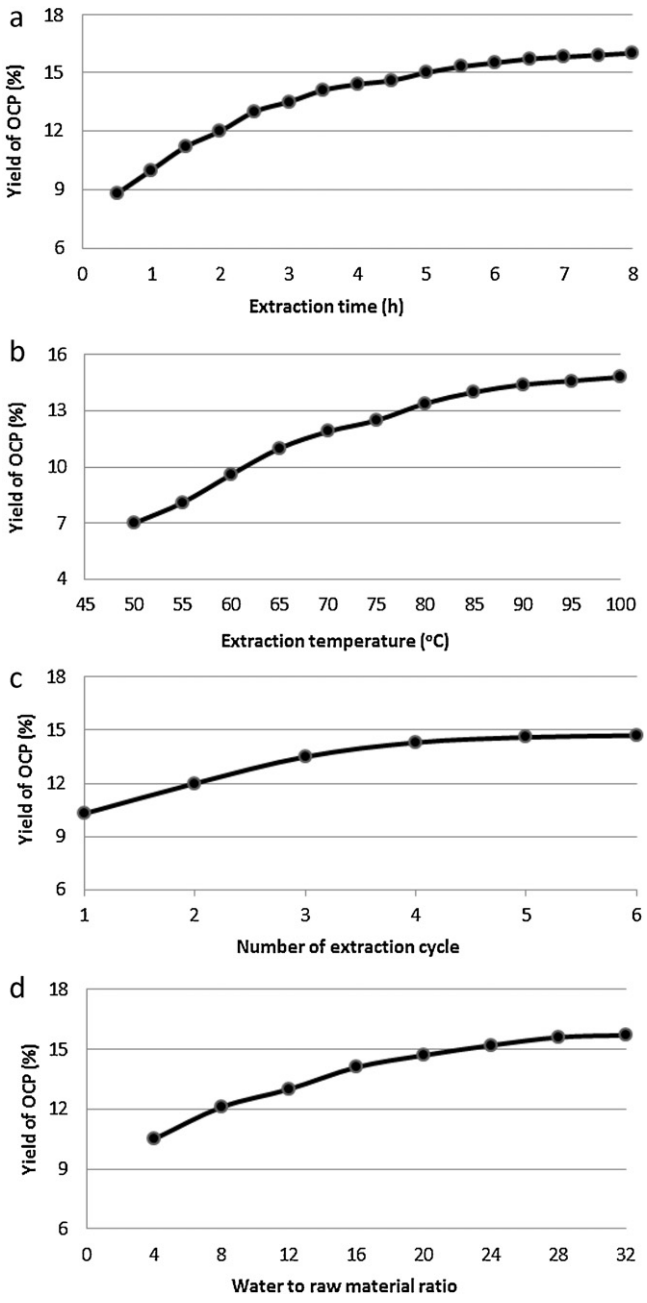

Fig. 1. Effects of different (a) times, (b) temperatures, (c) numbers of extraction cycle, and (d) water to raw material ration on extraction yield of OCP.

3. Results and discussion

3.1. Extraction yield

3.1.1. Effect of different times on extraction yield of OCP

Extraction time is a factor that would influence the extraction efficiency and selectivity of the fluid (Hou & Chen, 2008; Ye & Jiang, 2011). The yield of OCP affected by different number of extraction cycles (0.5–8 times) was seen in Fig. 1a, when other three factors (extraction temperature, number of extraction cycle and water to raw material ratio) were fixed at 90 °C, 3, and 16. The results showed that the extraction yield of COP had an obvious increase within the extraction time (0.5–6.5 h). The extraction yields of the polysaccharides significantly increased from 8.8% to 15.7% as time of extraction increased from 0.5 to 6.5, and then the extraction yield of OCP no longer obviously changed, when the extraction time increasing. This might be due to the time requirement of the exposure of the OCP to the release medium where the liquid penetrated into the raw materials, dissolved the OCP and subsequently diffused out from the raw materials. A longer extraction time presents a positive effect on the yield of OCP. It is reported that a long extraction time favors the production of polysaccharides (Liu, Wei, Guo, & Kennedy, 2006). Sun, Liu, and Kennedy (2010) and Sun, Li, Yan, and Liu (2010) reported that the purity of polysaccharides extracted from the fruiting bodies of *Pleurotus ostreatus* unexpectedly get the value ($58.0 \pm 0.99\%$) when the samples were extracted for 3 times, and when the number of extraction continued to increase, the extraction yields no longer changed. This result indicates that extraction time of 6.5 h is enough to the polysaccharides in the present work.

3.1.2. Effect of different temperatures on extraction yield of OCP

Temperature as an independent variable increases the ability of the solvent to solubilize the compounds and, reduce the viscosity of the liquid solvent which is allowing better penetration of the solvent into the solid matrix. According to the researches of Oosterveld, Beldman, Schols, and Voragen (1996) and Zykwinska, Rondeau-Mouro, Garnier, Thibault, and Ralet (2006), increasing of extraction yield is realized by increasing constant temperature bath time period. Different extraction temperatures were set at 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 °C, respectively, to investigate the influence of extraction temperature on the yield of OCP when the other extraction conditions were set as follows: extraction time 3.5 h, number of extraction 3 and the ratio of water to raw material 16 (Fig. 1b). The extraction yield increases up to its maximum amount at 90 °C. The yield of OCP no longer increased when the extraction temperature continued to rise. This result is in agreement with reports of other authors in extracting polysaccharides (Vinogradov, Brade, Brade, & Holst, 2003; Yin & Dang, 2008). The increase of the polysaccharides diffusion coefficient and the enhanced solubility of the polysaccharides in the extracting solvent at higher temperatures caused the increase of the polysaccharides mass going out from the mushroom particles into the solution (Li et al., 2006; Ye & Jiang, 2011). The extraction coefficient increased with increasing the extraction temperature due to the increase of the polysaccharides solubility (Braga, Moreschi, & Meireles, 2006). It has also been cited that the extraction at the elevated temperature resulted in faster and easier mass transfer of water-soluble polysaccharide from the cell wall into the extract (Tabatabaee Amid & Mirhosseini, 2012). The interaction rate between the raw components and water molecules increases with increasing extraction temperature, thus improving the yield of polysaccharides (Wu et al., 2007). The extraction at the elevated temperature may result in the presence of a broad range of constituents with different molecular mass. It should be noted that a high extraction yield of OCP does not always reflect the desirable response because this may indicate the presence of impurities and insoluble matters in the crude extract (Avallone, Plessi, Baraldi, & Monzani, 1997). Although the extraction yield of polysaccharides was also high at 100 °C, increasing temperature will bring about the increase in cost for the extraction process from the industrialization point of view (Ye & Jiang, 2011). Therefore, the range of 80–100 °C was adopted to be the optimal extraction temperature in this work.

3.1.3. Effect of number of extraction cycles on extraction yield of OCP

The use of the number of extraction cycles is to enter fresh solvent during the extraction process, helping to maintain good extraction equilibrium. The number of extraction is an important factor which influences the yield of polysaccharides during hot water extraction (Ferreira, Mafra, Rosário Soares, Evtuguin, & Coimbra, 2006). The effect of number of extraction cycle on

extraction yield of OCP is shown in Fig. 1c. Extraction was performed at different numbers of extraction cycles (1–6) when other extraction conditions (extraction temperature (90 °C), extraction time (3.5 h), and water to the raw material ratio (16)) were fixed. The extraction yields of OCP significantly increased from 10.3% to 14.3% as number of extraction period increased from 1 to 4. However, when the number of extraction continued to increase, the extraction yields no longer changed. Ye and Jiang (2011) stated that when the number of extraction cycle was increased more than 5, the extraction yield of crude polysaccharides from *Plantago asiatica* L. did not change. The highest extraction yield was observed when the number of extraction was 4. This result is in agreement with reports of other authors in extracting polysaccharides (Liang, 2008; Sun, Liu, et al., 2010; Sun, Li, et al., 2010).

3.1.4. Effect of different ratios of water to raw material on extraction yield of OCP

Water to raw material ratio will significantly affect extract yield (Govender et al., 2005). If ratio of water to raw material is too small, polysaccharides in raw material cannot be completely extracted up. If ratio of water to raw material is too big, this will cause high process cost. Therefore, a suitable ratio of water to raw material should be selected for extraction of polysaccharides (Yin & Dang, 2008). The effect of different ratio of water to raw material (4, 8, 12, 16, 20, 24, 28, and 32) on the yield of OCP was seen in Fig. 1d, when the other three extraction factors were fixed as follows: extraction temperature = 90 °C, number of extraction = 3 times and extraction time 3.5 h. The extraction yields of the OCP significantly increased from 10.5% to 15.6% as the ratio of water to raw material increased from 4 to 28. However, when the ratio continued to increase, the extraction yields no longer changed. A possible explanation is that increase in the ratio of water to raw material may increase diffusivity of the solvent into cells and enhance the desorption of the polysaccharides from the cells (Ray, 2004; Volpi, 2005). Koocheki et al. (2008) and Sepulveda, Sanez, Aliaga, and Aceituno (2007) also reported that when the volume ratio of water to raw materials was increased, a greater mucilage yield obtained from *Alyssum homolocarpum* and *Opuntia* spp. seeds. Conversely, Singthong, Ningsanond, and Cui (2008) stated a higher extraction yield for Yanang leaves gum at a low ratio of solid to water. Therefore, the ratio of water to raw material 4–28 was selected in the work. Singthong et al. (2008) found a higher extraction yield for Yanang leaves gum at low ratios of solid to water. Bendahou, Dufresne, Kaddami, and Habibi (2007) reported that the extraction yield of polysaccharides significantly increases as the ratio of water to raw material was increased, which could be due to an increased driving force for the mass transfer of the polysaccharides.

According to the single-parameter study, extraction time of 0.5–6.5 h, extraction temperature of 80–100 °C, number of extraction of 1–5, and the ratio of water to raw material of 4–28 were adopted for RSM experiments.

3.2. Data analysis and evaluation of the model

The effects of four process variables (i.e. extraction time (X_1), extraction temperature (X_2), number of extraction cycle (X_3) and water to the raw material ratio (X_4)) were studied during experimentation. The response of interest was the extraction yield of OCP. The results of 30 runs using CCRD design are presented in Table 2 that includes the design, observed responses and the predicted values. Results showed that there was a close agreement between experimental and predicted values. In addition, it was observed that the yield extraction ranged from 8.4% to 16.9%. By applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

Table 2
Response surface central composite design (uncoded) and results for extraction yield of OCP.

Run	X_1 (h)	X_3 (°C)	X_2	X_4	Extraction yield (%)	
					Experimental	Predicted
1	2	85	4	10	8.40	8.09
2	5	85	2	10	12.10	12.12
3	2	85	2	22	9.10	9.34
4	2	95	2	10	13.90	13.58
5	3.5	90	3	16	10.70	10.55
6	5	95	2	22	14.00	14.19
7	5	85	4	22	12.80	12.41
8	5	95	4	10	15.50	15.54
9	2	95	4	22	11.10	11.07
10	3.5	90	3	16	14.00	14.31
11	5	85	4	10	12.90	12.63
12	2	85	2	10	15.20	15.35
13	3.5	90	3	16	13.10	13.34
14	3.5	90	3	16	15.70	15.47
15	5	85	2	22	14.80	14.79
16	2	95	4	10	16.90	17.13
17	2	95	2	22	14.00	14.24
18	5	95	4	22	14.50	14.24
19	2	85	4	22	14.00	14.42
20	5	95	2	10	14.50	14.42
21	3.5	100	3	16	8.80	9.11
22	3.5	90	3	28	15.70	15.47
23	3.5	90	3	16	12.10	12.04
24	3.5	90	1	16	14.80	14.94
25	3.5	90	5	16	10.30	10.37
26	0.5	90	3	16	14.60	14.61
27	3.5	90	3	16	10.50	10.81
28	3.5	90	3	4	15.60	15.37
29	3.5	80	3	16	14.10	14.05
30	6.5	90	3	16	14.30	14.05

$$\begin{aligned}\text{OCP extraction yield} = {} & -64.579 + 3.765X_1 + 1.137X_2 + 3.075X_3 \\ & + 0.621X_4 - 0.005X_1X_2 - 0.125X_1X_3 \\ & - 0.032X_1X_4 + 0.012X_2X_3 - 0.004X_2X_4 \\ & - 0.023X_3X_4 - 0.195X_1^2 - 0.006X_2^2 \\ & - 0.389X_3^2 - 0.007X_4^2 \end{aligned} \quad (8)$$

Analysis of variance (ANOVA) was performed to investigate the adequacy of the suggested models and identify the significant factors. A significant lack of fit shows that the models failed to represent the data in the experimental domain at which points were not included in the regression. The ANOVA showed that lack of fit was not significant for response surface model at 95% confidence level, which means that the model represented the data satisfactorily. On the other hand, R^2, adj-R^2, PRESS, coefficient of variation (CV) and adequate precision were calculated to check the model adequacy. The R^2 and adj-R^2 values were 0.989 and 0.978 respectively (Table 3). A high R^2 indicates that the variation could be accounted for by the data satisfactorily fitting the model. However, a large value of R^2 does not always imply that the regression model is a good one. Adding a variable to the model will always increase R^2, regardless of whether the additional variable is statistically significant or not. Thus, it is better to use an adj-R^2 to evaluate the model adequacy. Table 3 shows that R^2 and adj-R^2 values for the model did not differ greatly, indicating non-significant terms have not been included in the model. The CV values were found to be 2.576 for yield extraction of OCP. Since CV is a measure expressing the standard deviation as a percentage of the mean, smaller values of CV give better reproducibility. In general, a CV higher than 10 indicates that variation in the mean value is high and does not satisfactorily

Table 3
Analysis of variance for the fitted models.

	Source	Degree of freedom	Coefficient	Sum of square	Mean square	*F*-Value	*P*-Value
Extraction yield (%)	Model	14		210.2253	15.0161	234.4386	<0.0001
	Residual	13		0.8327	0.0603		
	Lack of fit	10		0.6027	0.0603	0.7861	0.6638 ns
	Pure error	3		0.2300	0.0767		
	Total	29		211.1720			
	R^2		0.9861				
	Adj-R^2		0.9718				
	CV		2.0476				
	PRESS		4.9354				
	Standard deviation		0.2531				
	Adequate precision		54.2391				

develop an adequate response model (Myers & Montgomery, 2002). These values showed a good agreement between the experimental and the predicted values. The low PRESS 9.987 value suggests for the adequacy of the fitted quadratic models for predictive applications (Table 3). Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable (Myers & Montgomery, 2002). For the proposed models, this value was 35.109, a very good signal-to-noise ratio. All these statistical parameters show the reliability of the models.

The regression coefficient values of Eq. (8) were listed in Table 4. The *P*-values were used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. The smaller was the value of *P*, the more significant was the corresponding coefficient. It can be seen from this table that the linear coefficients (X_1, X_2, X_3, X_4), a quadratic term coefficient (X_1^2, X_3^2, X_4^2) and cross product coefficients ($X_1 \times X_3$, $X_1 \times X_4$) were significant, with very small *P* values ($P < 0.05$). The other term coefficients were not significant ($P > 0.05$). The full model filled Eq. (8) was made three-dimensional and contour plots to predict the relationships between the independent and dependent variables.

It can be seen that the variable with the largest effect on extraction yield was a linear term of extraction time (X_1) followed by a linear term of water to the raw material ratio (X_4), a linear term of number of extraction cycle (X_3), extraction temperature (X_2), the quadratic term of extraction time (X_1^2), the quadratic term of number of extraction cycle (X_3^2), the quadratic term of water to the raw material ratio (X_4^2) and interaction term of X_1X_4 and X_1X_3 (Table 4). However, the interaction terms (X_1X_2, X_2X_3, X_2X_4 and X_3X_4) and quadratic term of temperature (X_2^2) were found insignificant ($P > 0.005$). The coefficient of determination (R^2) of the predicted models in this response was 0.9427. This would give a good fit to the mathematic model in Eq. (8).

Fig. 2 shows that the polynomial regression model (Eq. (8)) was in good agreement with the experimental results. In this figure, the observed values are compared to the predicted values calculated from the model. No significant ($P > 0.05$) difference was observed between the experimental and predicted values of extraction yield of OCP. The result suggests that the model used in this research was able to identify operating conditions for selective extraction of crude polysaccharides from okra.

3.3. Optimization of extraction conditions of OCP

The graphical representations of the regression equation (8), called the response surfaces and the contour plots were obtained using Design-Expert, and the results of extraction yield of OCP affected by extraction time, extraction temperature, number of extraction cycle and water to the raw material ratio are presented in Figs. 2 and 3. In the response surface plot and contour plot, the data were generated through keeping two variables at their respective zero level (central value of the testing ranges) and varying the other two within the experimental range.

Figs. 3a and 4a, which give the extraction yield of OCP as a function of extraction time and temperature at fixed number of extraction cycle (3) and water to raw material ratio (16), indicated that the extraction yield increased rapidly with increase of extraction time from 0.5 to 5 h, and increased with the increase of extraction temperature from 80 to 95 °C. The effect of time on yield was more pronounced at lower temperatures (80 °C). At 95 °C, extraction yield reached nearly the equilibrium toward the time (=5 h) and extending the time did not have much effect on mucilage extraction. A similar trend has been reported for flaxseed gum (Cui et al., 1994), Krueo Ma Noy pectin (Singthong, Ningsanond, Cui, & Goff, 2005), boat-fruited sterculia seed polysaccharide (Wu et al., 2007), *Opuntia* spp. mucilage (Sepulveda et al., 2007), *A. homolocarpum* seed gum (Koocheki et al., 2008) and Yanang leaves gum extraction (Singthong, Ningsanond, & Cui, 2008).

The extraction yield of OCP affected by different extraction temperature and extraction time was seen in Figs. 3b and 4b, when the other two variables (extraction temperature and water to raw material ratio) were fixed at 90 °C and 16 respectively. Extraction time and number of extraction cycle had a positive impact on the extraction yield of OCP. From two figures, we can conclude that the extraction yield of OCP increased with increase in number of extraction cycle from 1 to 4, and but beyond 5 h, extraction yield of OCP reached the plateau region where the yield was maximized and did not further increase the yield. The extraction yield of the OCP was found to increase rapidly with increase of extraction time from 0.5 to 5 h. It can be seen that the maximum extraction yield of OCP can be achieved when extraction time and number of extraction cycle are around 5 h and 4, respectively.

Figs. 3c and 4c showed the 3D response surface plot and the contour plot at varying extraction time and water to the raw material

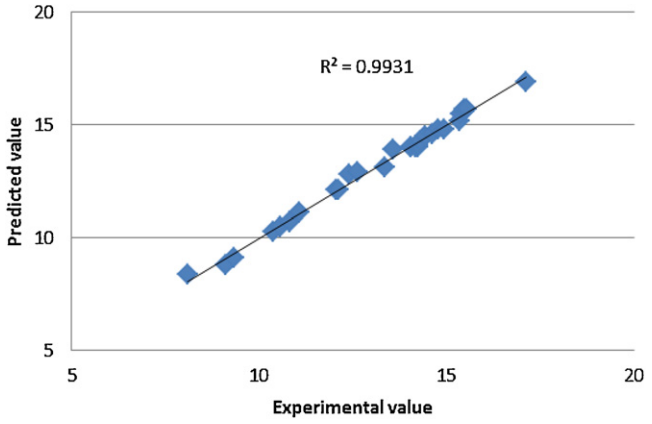

Fig. 2. Experimental extraction yield vs. the predicted extraction yield under optimum extraction conditions.

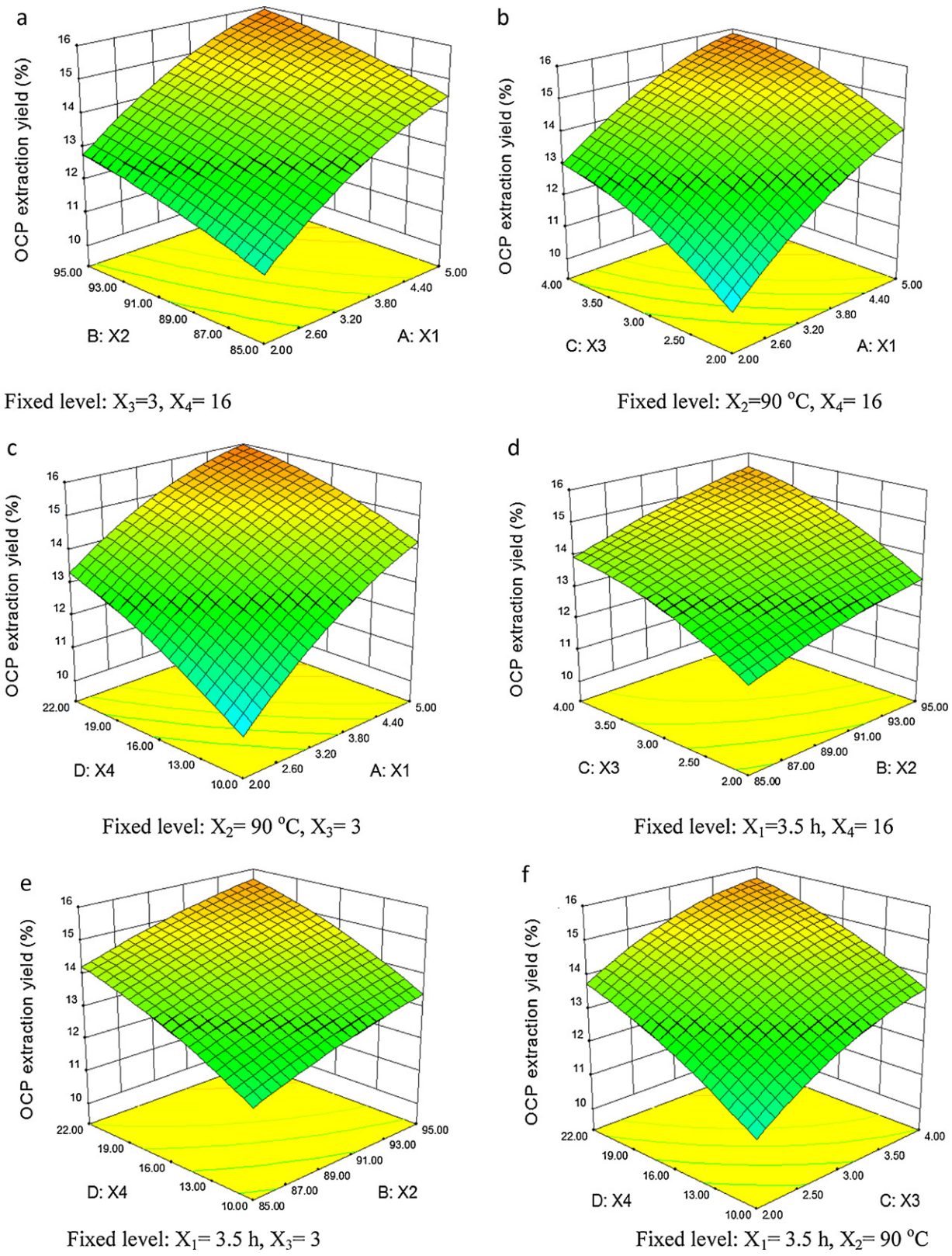

Fig. 3. Response surface (3D) showing the effect of the extraction time (X_1), extraction temperature (X_2), number of extraction cycle (X_3) and water to raw material ratio (X_4) on the OCP extraction yield.

ratio at fixed extraction temperature and number of extraction cycle. It indicated that the maximum extraction yield of OCP can be achieved when extraction time and the ratio of water to raw material at the threshold level of around 5 h and 22, respectively.

Figs. 3d and 4d illustrate the 3D response surface plot and the contour plot at varying extraction temperature and number of extraction at fixed water to the raw material ratio (16) and extraction time (3.5 h). The yield extraction of OCP increased with the

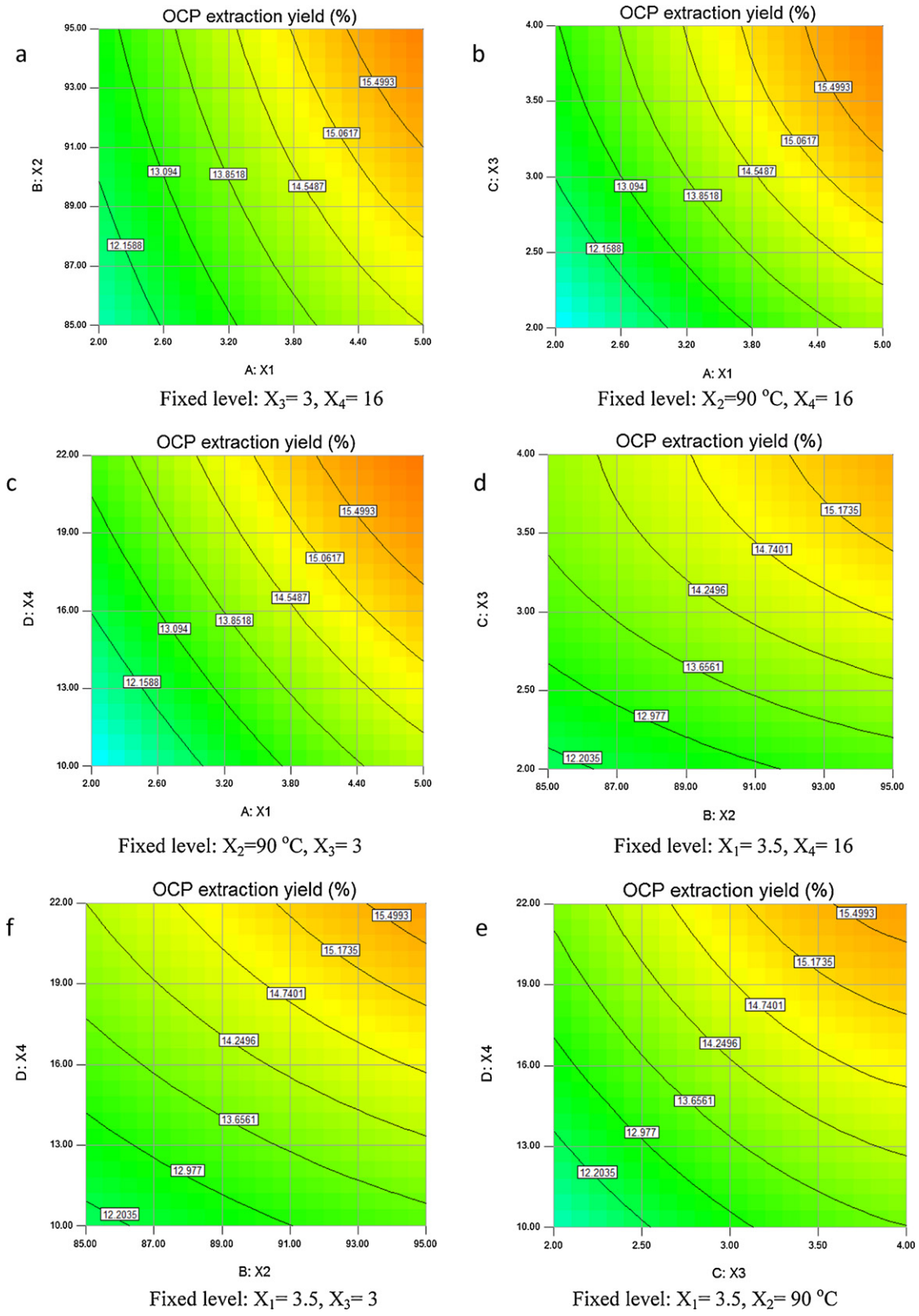

Fig. 4. Contour plots showing the effect of the extraction time (X_1), extraction temperature (X_2), number of extraction cycle (X_3) and water to raw material ratio (X_4) on the OCP extraction yield.

increasing number of extraction cycle from 1 to 5 and reached the maximum value when the extraction temperature at the threshold level of 95 °C. So, extraction yield of OCP increased with simultaneously increasing water to the raw material ratio and temperature. In fact, the aqueous extraction using high water to the raw material ratio at high extraction temperature led to an increase in extraction yield of OCP. There are different mechanisms explaining the significant effect of water to the raw material ratio on the extraction

Table 4
The significance of each response variable effect showed by using *F* ratio and *P* value in the nonlinear second order model.

	Variables	DF[a]	SS[b]	MS[c]	*F*-Value ($F_{cal.}$)	*P*-Value[d] (probability)
Linear effects	X_1	1	97.6067	97.6067	1523.8831	<0.0001
	X_2	1	1.6017	1.6017	25.0060	0.0002
	X_3	1	44.8267	44.8267	699.8559	<0.0001
	X_4	1	55.8150	55.8150	871.4111	<0.0001
Quadratic effects	X_1^2	1	6.7433	6.7433	105.2802	<0.0001
	X_2^2	1	0.1376	0.1376	2.1486	0.1665 ns
	X_3^2	1	3.2805	3.2805	51.2164	<0.0001
	X_4^2	1	1.3376	1.3376	20.8836	0.0005
Interaction effects	X_1X_2	1	0.0025	0.0025	0.9758	0.3413 ns
	X_1X_3	1	0.0225	0.0225	5.6205	0.0339
	X_1X_4	1	0.9025	0.9025	4.7228	0.0488
	X_2X_3	1	0.2025	0.2025	0.0390	0.8464 ns
	X_2X_4	1	1.1025	1.1025	0.6245	0.4436 ns
	X_3X_4	1	0.7225	0.7225	4.7228	0.0488

ns: not significant.
[a] Degrees of freedom.
[b] Sum of squares.
[c] Mean sum of squares.
[d] *P* values <0.05 were considered to be significant.

yield. It seems that the raw materials are swollen after the aqueous extraction by dispersing the okra pods in water. The accumulation of water in the endosperm leads to the binding of the water-soluble components, thus raising the extraction yield. On the other hand, the presence of a higher amount of water makes a less sticky slurry, thus providing a more efficient extraction of the mucilage (Tabatabaee Amid & Mirhosseini, 2012). Koocheki, Kadkhodaee, Mortazavi, Shahidi, and Taherian (2009) reported an exponential increase in the yield caused by increasing water to the raw material ratio. They explained this by the availability of high liquid content which led to an increase in the driving force of mucilage out of the raw materials into the extract. Therefore, the presence of insufficient amount of water or high amount of raw materials may reduce the extraction efficiency.

In Figs. 3e and 4e, when the 3D response surface plot and the contour plot were developed for the extraction yield of OCP with varying extraction temperature and water to the raw material ratio at fixed number of extraction (3) and extraction time (3.5 h). The yield increased exponentially with temperature of extraction. At higher temperatures, the viscosity of mucilage, decreases and makes the slurry less sticky. As a result, the mucilage can be easily released and the extraction yield increases (Koocheki et al., 2008). The extraction yield of OCP increased with the increasing ratio of water to raw material from 4 to 22, and reached the maximum value rapidly at an extraction temperature around 95 °C, but beyond this temperature, extraction yield of OCP reached the plateau region where the yield was maximized and did not further increase the yield.

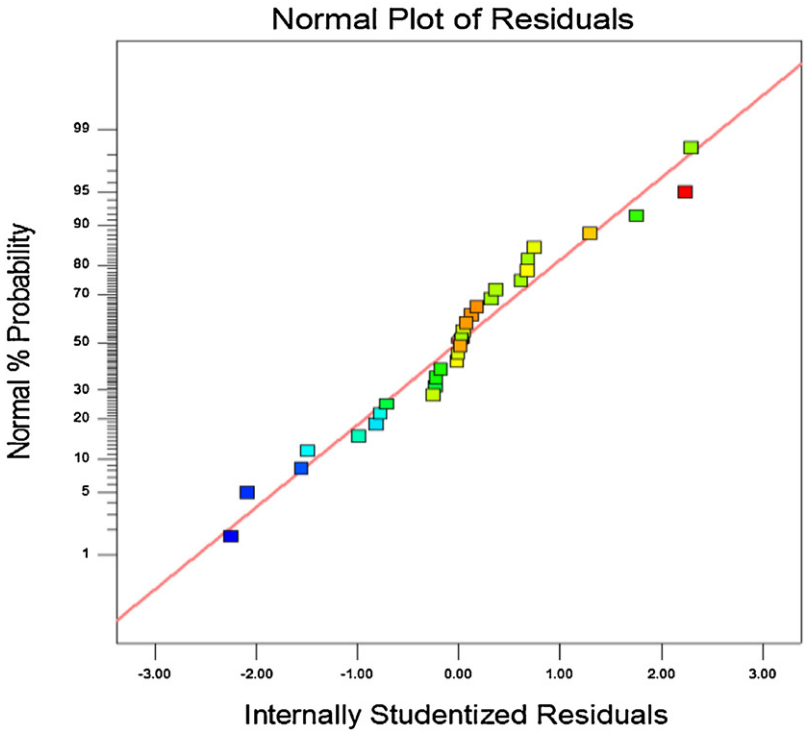

Fig. 5. Normal probability of internally studentized residuals.

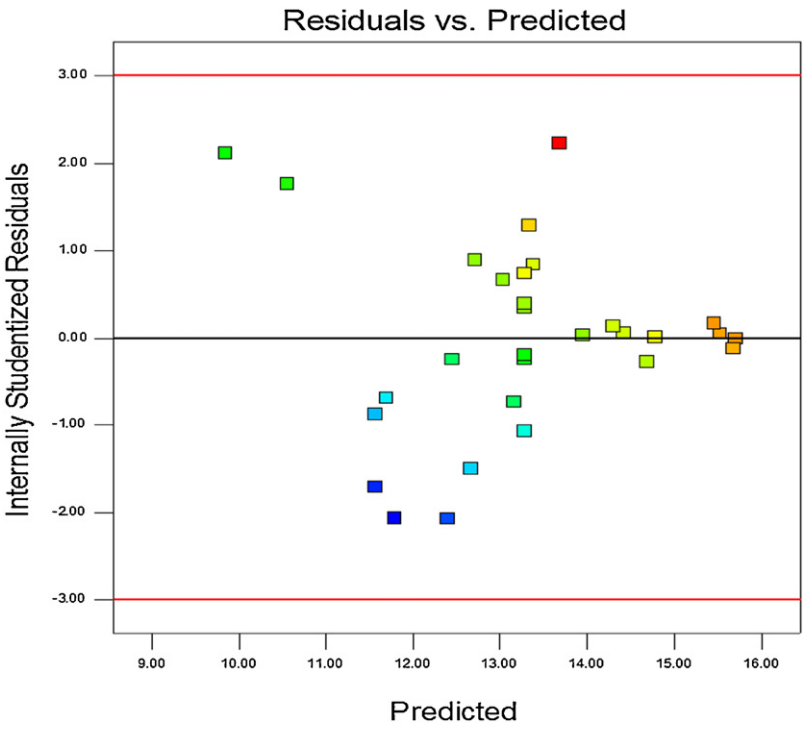

Fig. 6. Plot of internally studentized residuals vs. predicted response.

The 3D response surface plot and the contour plot based on the independent variable ratio of water to the raw material and number of extraction cycle were shown in Figs. 3f and 4f, while the other two independent variables, extraction temperature and extraction time were kept at 90 °C and 3.5 h respectively. An increase in the extraction yield of OCP could be significantly achieved with the increases of the ratio of water to raw material. It is observed that the extraction yield of OCP was increased with the increasing number of extraction cycle from 1 to 4, meaning that further increases of number of extraction cycle would not increase the extraction yield of OCP any longer.

3.4. Model adequacy checking

Usually, it is necessary to check the fitted model to ensure that it provides an adequate approximation to the real system. Unless the model shows an adequate fit, proceeding with the investigation and optimization of the fitted response surface likely give poor or misleading results (Li, Cui, & Kakuda, 2007). The residuals from the least squares fit play an important role in judging model adequacy (Myers & Montgomery, 2002). By constructing a normal probability plot of the residuals, a check was made for the normality assumption, as given in Fig. 5. The normality assumption was satisfied as the residual plot approximated along a straight line. Fig. 6 presents a plot of residuals vs. the predicted response. The general impression is that the residuals scatter randomly on the display, suggesting that he variance of the original observation is constant for all values of Y. Both of the plots (Figs. 5 and 6) are satisfactory, so we conclude that the empirical model is adequate to describe the OCP extraction yield by response surface.

3.5. Verification of predictive model

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material (Ye & Jiang, 2011). In order to validate the adequacy of the model equations, Eq. (8), a verification experiment was carried out under the optimal conditions (within the experimental range): extraction time 4.94 h, extraction temperature 94.97 °C, number of extraction cycle 4 and water to raw material ratio 21.74. Good agreement must exist between the values predicted using the model equations and the experimental values at the points of interest. To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. This set of conditions was determined to be optimal by the RSM optimization approach and was also used to validate experimentally and predict the values of the responses using the model equation. A mean value of 16.985 ± 0.29 (%) ($n = 3$), obtained from real experiments, demonstrated the validation of the RSM model. The validation result revealed that there was no significant difference between experimental and predicted values, suggesting that the response model was adequate for reflecting the expected optimization (Table 5). This result of analysis indicated that the experimental values were in good agreement with the predicted ones, and also suggested that the model of Eq. (8) is satisfactory and accurate.

Table 5
Predicted and experimental values of the responses at optimum conditions.

Optimum condition				Extraction yield of OCP (%)	
Extraction time (h)	Extraction temperature (°C)	Number of extraction cycle	Water to raw material ratio	Experimental	Predicted
4.94	94.97	4	22	16.895 ± 0.29[a]	16.916

[a] Mean ± standard deviation ($n = 3$).

4. Conclusion

The present study revealed that the aqueous extraction condition significantly ($P < 0.05$) influenced the extraction yield of okra curd polysaccharides. In the current work, the extraction temperature and number of extraction exhibited the most and least significant ($P < 0.05$) impact on the extraction yield of okra curd polysaccharides, respectively. The present work revealed that the desirable okra curd polysaccharide was extracted by using water to raw material ratio of 21.74 (w/w), extraction time 4.94 h, at elevated temperature 94.91 °C, and number of extraction cycle 4. The present study showed that the okra pods can be used as a potential, low cost source of hydrocolloid.

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