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Optimization of extraction process of crude polysaccharides from Pomegranate peel by response surface methodology

Caiping Zhu*, Xiaolin Liu

College of Food Engineering and Nutrition Science, Shaanxi Normal University, Xi'an 710062, PR China

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ABSTRACT

In this study, response surface methodology was employed to optimize the extraction process of crude polysaccharides from Pomegranate peel with water. Three independent and main variables, including extraction time (h), extraction temperature (°C) and ratio of water to raw material (ml/g), which were of significance for the yields of polysaccharides were studied and the Box–Behnken design was based on the results of a single-factors test. The experimental data were fitted to a second-order polynomial equation using multiple regression analysis and also examined using the appropriate statistical methods. The best extraction conditions are as follows: extraction time 1.9 h, extraction temperature 98 °C, ratio of water to raw material 37 ml/g. Under the optimization conditions, the experimental yield was 10.358%, which was well matched with the predictive yield of 10.423%.

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1. Introduction

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and has been used extensively in the folk medicine of many cultures (Li et al., 2006). In the past decade, popularity of Pomegranate has increased tremendously because of anti-microbial, anti-viral, anti-cancer, potent antioxidant, and anti-mutagenic effects of the fruit (George, Singh, Srivastava, Bhui, & Shukla, 2011; Hong, Seeram, & Heber, 2008; Negi, Jayaprakasha, & Jena, 2003; Sundararajan et al., 2010; Tezcan, Gültekin-Özgüven, Diken, Özçelik, & Erim, 2009). Pomegranate peels are one of the most valuable by-products of the food industry.

In the past several years, many reports focus on the extraction, chemical structure and biological activities of the antioxidants extracted from the Pomegranate peels (Negi et al., 2003; Pan, Qu, Ma, Atungulu, & McHugh, 2012; Saad et al., 2012). Whereas, little attention was devoted to the extraction of the crude polysaccharides of Pomegranate peels. Therefore, we reported the optimization of extracting parameters for the production of Pomegranate peels polysaccharides (PPP).

Response surface methodology (RSM) is an effective statistical technique for optimizing complex processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process (Zhong & Wang, 2010). It was widely used in optimizing the natural active ingredient extraction process variables (Kaur, Wani, Oberoi, & Sogi, 2008; Oliveira, Kamimura, & Rabi, 2009; Pierozan et al., 2009; Qiu et al., 2010; Ruan, Zhou, Deng, & Yin, 2008). Box–Behnken design (BBD) is a type of response surface design. It is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design the treatment combinations are at the midpoints of edges of the process space and at the center. These designs are rotatable (or near rotatable) and require 3 levels of each factor. It is more efficient and easier to arrange and interpret experiment in comparison with others. It is widely used in many researches (Khajeh, 2011; Sun, Li, Yan, & Liu, 2010; Sun, Liu, & Kennedy, 2010; Zhao, Wang, & Lu, 2009).

In this paper, RSM was firstly employed for the extraction process of crude polysaccharides from Pomegranate peel. The aim of this research was to develop an approach that would bring a better understanding of the combined effects of the key processing variables (extraction time, extraction temperature and ratio of water to raw material) on the desired response (extraction yield of PPP), as well as to look for optimum conditions of the crude polysaccharides extraction from Pomegranate peel.

2. Materials and methods

2.1. Materials

Pomegranate peels were obtained from fruit purchased from a local market. The peels were separated manually from the fruit,

* Corresponding author. Tel.: +86 85310517; fax: +86 85310517.
E-mail address: zcaiping@snnu.edu.cn (C. Zhu).

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

Independent variables	Levels		
	−1	0	+1
Extraction time (X_1) (h)	1.0	1.5	2.0
Extraction temperature (X_2) (°C)	90	95	100
Ratio of water to raw material (X_3) (ml/g)	20	30	40

sun-dried and powdered, and then kept at room temperature for further study.

All other chemicals and solvents used were of analytical grade and obtained from Xi'an, Shaan Xi province, China.

2.2. Extraction of PPP

Dried ground Pomegranate peel samples (10 g) were extracted with distilled water (ratio of water to raw material (ml/g) ranging from 10:1 to 60:1) at pH 6.5–7.5 (adjusting the suspension pH by 0.1 mol/L NaOH or HCl), while the temperature of the water bath was kept steady for a given temperature (within ±1.0 °C, extraction temperature ranging from 70 to 100 °C). The water-material slurry in a 2.0 L stainless steel boiler in the water bath was stirred with an electric mixing paddle for a given time (extraction time ranging from 0.5 to 3.0 h) during the entire extraction process. The extracted slurry was centrifuged at 2000 × g for 10 min to collect the supernatant, and the insoluble residue was treated again as mentioned above.

The supernatant was incorporated and concentrated to one-fifth of initial volume using a rotary evaporator (RE-52AA, Yarong Technology and Science Inc., Shanghai, China) at 55 °C under vacuum. The resulting solution was mixed with four volumes of dehydrated ethanol (ethanol final concentration, 80%) and kept overnight at 4 °C. Then the solution was centrifuged at 2000 × g for 10 min, washed three times with dehydrated ethanol, and the precipitate was collected as PPP. The extract was air-dried at 50 °C until its weight was constant, and then was weighted with a balance (JA2003N, Tole Metrical Scientific and Technical Co., Shanghai, China). The percentage PPP yield (%) is calculated as follows:

$$\text{PPP yield}\,(\%) = \frac{m_0}{m} \times 100$$

m_0 (g) is the dried PPP weight; m (g) is the dried raw material weight.

2.3. Experimental design

After determining the preliminary range of extraction variables through single-factor test, a three-level-three-factor, Box–Behnken factorial design (BBD) was employed in this optimization study. Extraction time (X_1), extraction temperature (X_2) and ratio of water to raw material (X_3) were the independent variables selected to be optimized for the extraction of PPP. The range of independent variables and their levels were presented in Table 1. PPP yield (Y) was taken as the response for the combination of the independent variables given in Table 2. All the experiments were carried out at random in order to minimize the effect of unexplained variability in the observed responses due to systematic errors.

The variables were coded according to the equation:

$$X_i = \frac{X_i - X_0}{\Delta X} \tag{1}$$

where X_i is the (dimensionless) coded value of the variable X_i, X_0 is the value of X_i at the center point, and ΔX is the step change. The

Table 2
Box–Behnken experimental design and results for yield of PPP.

No.	X_1/extraction time (h)	X_2/extraction temperature (°C)	X_3/ratio of water to raw material (ml/g)	Yield of PPP (%)
1	−1	−1	0	8.69
2	−1	1	0	10.06
3	−1	0	−1	9.25
4	−1	0	1	9.75
5	1	−1	0	9.58
6	1	1	0	10.38
7	1	0	−1	9.72
8	1	0	1	10.34
9	0	−1	−1	8.83
10	0	−1	1	9.52
11	0	1	−1	9.92
12	0	1	1	10.37
13	0	0	0	9.87
14	0	0	0	9.76
15	0	0	0	9.85
16	0	0	0	9.94
17	0	0	0	9.89

behavior of the system was explained by the following quadratic equation:

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

where Y is the dependent variable, A_0 is constant, and A_i, A_{ii}, and A_{ij} are coefficients estimated by the model. X_i and X_j are levels of the independent variables. They represent the linear, quadratic, and cross-product effects of the X_1, X_2, and X_3 factors on the response, respectively. The model evaluated the effect of each independent variable to a response. Analysis of the experimental design and calculation of predicted data were carried out by using Design-Expert 8.0.5 (Trial Version, State-Ease Inc., Minneapolis, MN, USA) software to estimate the response of the independent variables. Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

3. Results and discussion

3.1. Effect of extraction time on yield of PPP

Extraction time is a factor that would influence the extraction efficiency and selectivity of the fluid. This might be due to the time requirement of the exposure of the PPP to the release medium where the liquid penetrated into the dried powdered material, dissolved the PPP and subsequently diffused out from the material (Ye & Jiang, 2011). The effect of extraction time on yield of PPP was shown in Fig. 1. Firstly, the extraction time was set at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h while other extraction parameters were given as the followings: extraction temperature 90 °C, the ratio of water to raw material 30:1. It could be found that the extraction yield increased as extraction time ascended from 0.5 to 1.5 h, and then increased slowly when the extraction time exceeded 1.5 h (Fig. 1a). This indicated that extraction time of 1.5–2 h was sufficient to obtain the PPP production. Thus, extraction of 1.5–2 h was favorable for producing the PPP.

3.2. Effect of extraction temperature on yield of PPP

To study the effect of different temperature on the yield of PPP, extraction process was carried out using the different temperatures of 70, 75, 80, 85, 90, 95 and 100 °C. The extraction time was fixed at 1 h, the ratio of water to raw material was fixed at 30:1 (ml/g).

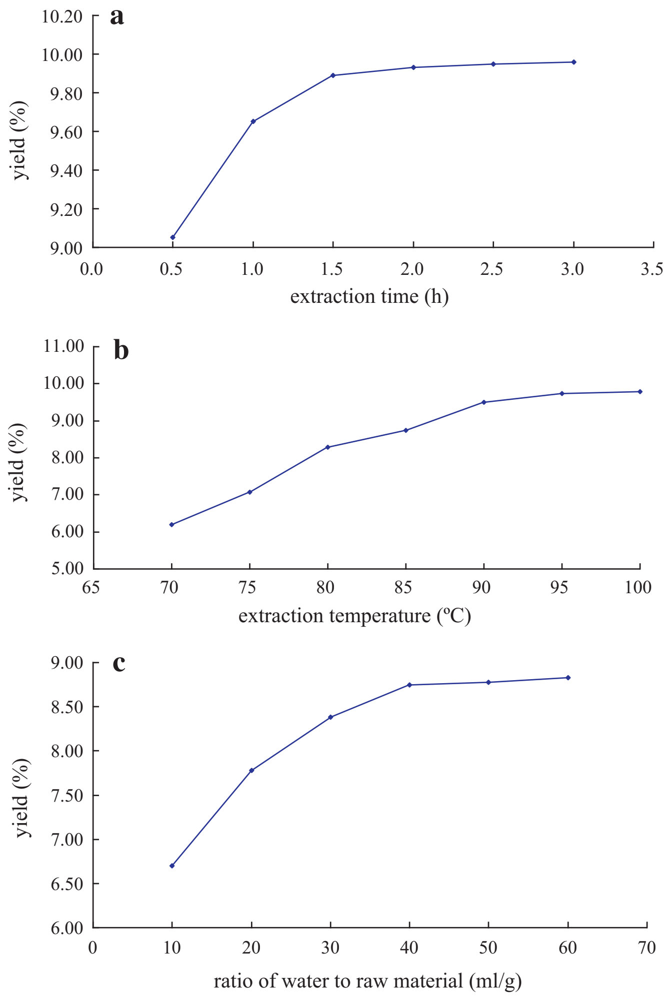

Fig. 1. Effect of extraction parameters on yield of PPP (extraction time, h; extraction temperature, °C; ratio of water to raw material, ml/g).

The extraction yield of PPP had been increasing when extraction temperature increased from 70 to 95 °C. As shown in Fig. 1b, the maximum yield of PPP was observed when extraction temperature was 95 °C. This tendency is in agreement with reports of other authors in extracting polysaccharides (Sun, Li, et al., 2010; Sun, Liu, et al., 2010; Yin & Dang, 2008). Although the yield of PPP 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. Therefore, extraction temperature range of 95–100 °C was considered to be optimal in the present experiment.

3.3. *Effect of the ratio of water to raw material on yield of PPP*

Different ratio of water to raw material will significantly affect extract yield. If ratio of water to raw material is too small, PPP in raw material cannot be completely extracted up. If ratio of water to raw material is too big, this will cause high process cost (Zhu, Gao, Li, Zhao, & Deng, 2010). Therefore, it is necessary to select a suitable ratio of water to raw material for extraction of targeted PPP. In this study, ratio of water to raw material as an important extraction parameter was set at 10, 20, 30, 40, 50 and 60 to investigate the effect of different ratio of water to raw material on the yield of PPP while other extraction parameters were given as the followings: extraction time 1 h, extraction temperature 85 °C. It could be founded that the yield of PPP continued to increase evidently with the increasing ratio of water to raw material. A possible explanation is that increase in ratio of water to raw material may increase diffusivity of the solvent into cells and enhance desorption of the PPP from the cells. But the yield of PPP started to increase slowly after the ratio of water to raw material exceeded 40 (Fig. 1c).

Table 3
Analysis of variance for the fitted quadratic polynomial model of extraction of PPP.

Source	SS	DF	MS	*F*-value	Prob > *F*
Model	3.61	9	0.40	104.17	<0.0001
Residual	0.027	7	3.851×10^{-3}		
Lack of fit	9.475×10^{-3}	3	3.158×10^{-3}	0.72	0.5889
Pure error	0.017	4	4.37×10^{-3}		
Cor total	3.64	16			
	$R^2 = 0.9926$	$R^2_{adj} = 0.9831$	CV = 0.64		

3.4. *Optimization of the extraction parameters of PPP*

3.4.1. *Statistical analysis and the model fitting*

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. There were a total of 17 runs for optimizing the four individual parameters in the current Box–Behnken design. The current design was applied to the production of PPP by heat water extraction. The data were analyzed by multiple regression analysis using the Design-Expert 8.0.5 and the following polynomial equation was derived to represent PPP yield as a function of the independent variables tested. Where Y is the predicted PPP yield and X_1, X_2, and X_3 are the coded values for extraction time, extraction temperature and ratio of water to raw material, respectively. Table 2 shows the process variables and experimental data. The results of the analysis of variance, goodness-of-fit and the adequacy of the models were summarized. The percentage yield range from 8.69% to 10.38%. The maximum yield of PPP (10.38%) was recorded extraction time 2 h, extraction temperature 100 °C, ratio of water to raw material 30 ml/g. The application of RSM suggested, based on parameter estimates, an empirical relationship between the response variable (extraction yield of PPP) and the test variable under consideration. By applying multiple regression analysis on the experimental data, the response variable and the test variables are related by the following second-order polynomial equation:

$$Y = 9.86 + 0.28X_1 + 0.51X_2 + 0.28X_3 - 0.14X_1X_2 + 0.03X_1X_3 - 0.06X_2X_3 - 0.04X_1^2 - 0.14X_2^2 - 0.057X_3^2 \quad (3)$$

The fit statistics of extraction yield (Y) for the selected quadratic predictive model is shown in Table 3. For the model fitted, the coefficient of determination (R^2) was 0.9926, indicating that only 0.74% of the total variations was not explained by the model. *F*-value for the lack of fit was insignificant ($P > 0.05$) thereby confirming the validity of the model. The value of the adjusted determination coefficient (adjusted $R^2 = 0.9831$) also confirmed that the model was highly significant. At the same time, a very low value 0.64 of coefficient of the variation (CV) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values. The model *P*-value (Prob > *F*) was very low (<0.0001), which implied that the model was significant.

The model was found to be adequate for prediction within the range of experimental variables. The regression coefficient values of Eq. (3) were listed in Table 4. The *P*-values were used as a tool to check the significance of each coefficient, which in turn might indicate the pattern of the interaction between the variables. The smaller the value of *P* was, the more significant the corresponding coefficient was (Guo, Zou, & Sun, 2010). It can be seen from this table that the linear coefficients (X_1, X_2, and X_3), a quadratic term coefficients (X_2^2) and cross product coefficients (X_1X_2) were signifi-

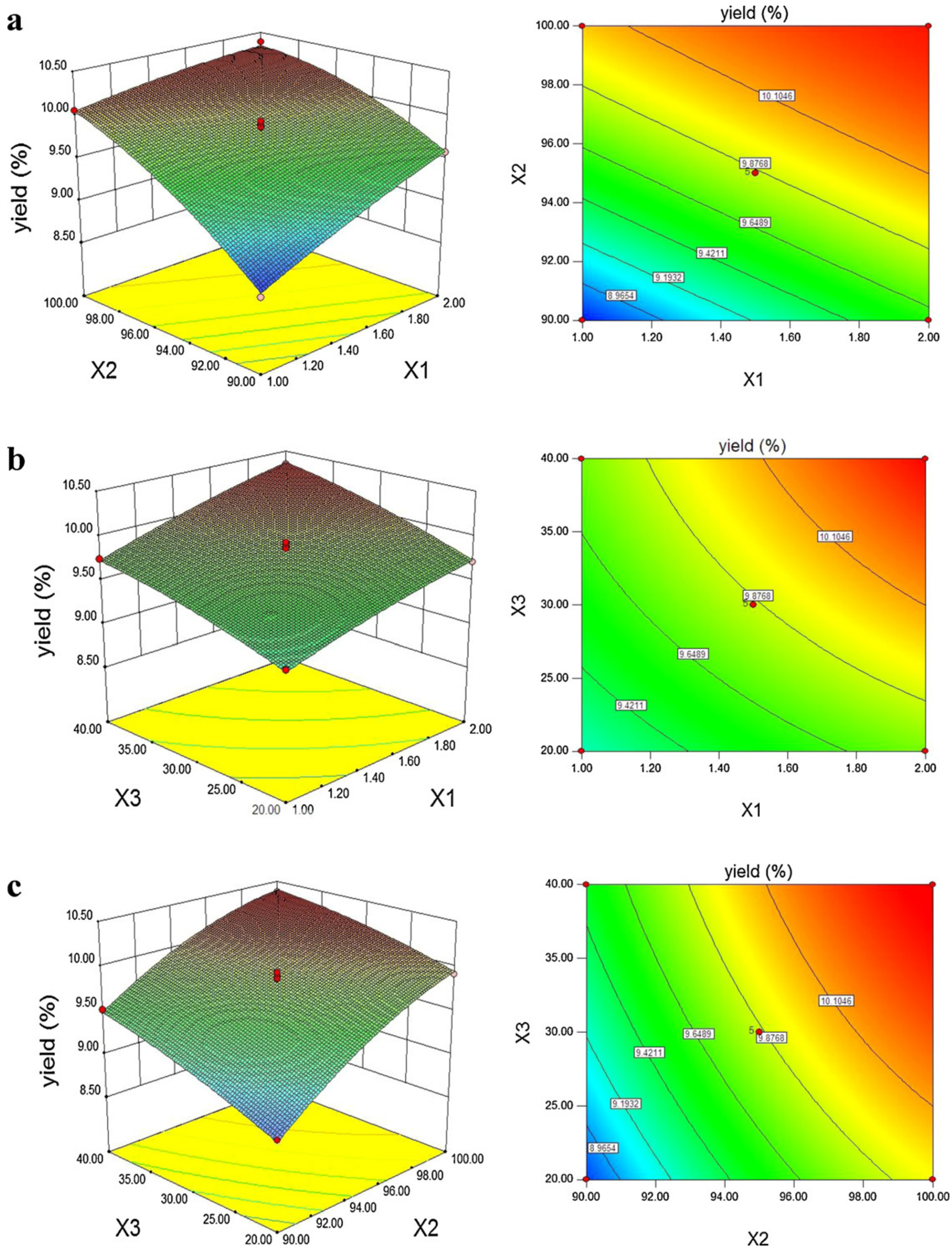

Fig. 2. Response surface plots and contour plots showing the effect of the extraction time, extraction temperature and ratio of water to raw material on the response yield.

cant, with very small P-values ($P < 0.05$). The other term coefficients were not significant ($P > 0.05$). The full model filled Eq. (3) was made three-dimensional and contour plots to predict the relationships between the independent variables and the dependent variables.

3.4.2. Optimization of extraction conditions

The graphical representations of the regression Eq. (3), called the response surfaces and the contour plots were obtained using Design-Expert, and the results of extraction yield of PPP affected by extraction time, extraction temperature and ratio of water to raw material are presented in Fig. 2. Response surface methodology plays a key role in identifying the optimum values of the independent variables efficiently, under which dependent variable could get the maximum response. In the response surface plot and contour plot, the extraction yield of PPP was obtained along with two continuous variables, while the other one variable was fixed constant at its zero level (center value of the testing ranges). In the three figures, the maximum predicted value indicated by the surface was confined in the smallest ellipse in the contour diagram. Elliptical contours are obtained when there is a perfect interaction between the independent variables. The independent variables and maximum predicted values from the figures corresponded with the

Table 4
Estimated regression model of relationship between response variable (yield of PPP) and independent variables (X_1, X_2, and X_3).

Variables	DF	SS	MS	F-value	P-value
X_1	0.64	1	0.64	167.27	<0.0001
X_2	2.11	1	2.11	548.34	<0.0001
X_3	0.64	1	0.64	165.8	<0.0001
X_1X_2	0.081	1	0.081	21.09	0.0025
X_1X_3	3.6×10^{-3}	1	3.6×10^{-3}	0.93	0.3658
X_2X_3	0.014	1	0.014	3.74	0.0944
X_1^2	6.653×10^{-3}	1	6.653×10^{-3}	1.73	0.2301
X_2^2	0.088	1	0.088	22.91	0.0020
X_3^2	0.014	1	0.014	3.58	0.1002

optimum values of the dependent variables (responses) obtained by the equations.

The 3-D plot and the contour plot in Fig. 2a, which gives the ratio of water to raw material (0 level), shows that extraction yield of PPP increased evidently with increasing of extraction temperature from 90 to 96 °C, but beyond 96 °C, the extraction yield of PPP increased slowly as the temperature ascended. The extraction yield of PPP increased very slowly with the extraction time. Fig. 2b shows that the 3-D plot and the contour plot at varying extraction time and ratio of water to raw material at fixed extraction temperature (0 level). From Fig. 2, it can be seen that the extraction yield of PPP increased evidently with increasing of ratio of water to raw material from 20 to 35 ml/g, but beyond 35 ml/g, extraction yield of PPP increased slowly as the ratio of water to raw material ascended. The 3-D plot and the contour plot based on independent variables ratio of water to raw material and extraction temperature were shown in Fig. 2c, while the extraction time was kept at a zero level. An increase in the extraction yield of PPP could be significantly achieved with the increase of ratio of water to raw material. It was obvious that the extraction yield of PPP was increased rapidly with the increasing extraction temperature from 90 to 96 °C, but beyond 96 °C, the extraction yield of PPP increased slowly as the temperature ascended.

According to Fig. 2, and above single parameter study, it can be conclude that optimal extraction condition of PPP were extraction time 1.86 h, extraction temperature 98.51 °C, ratio of water to raw material 37.2. Among the three extraction parameters studied, the extraction temperature was the most significant factor to affect the extraction yield of PPP, followed by extraction time and ratio of water to raw material according to the regression coefficients significance of the quadratic polynomial model (Table 4) and gradient of slope in the 3-D response surface plot (Fig. 2).

3.5. Verification of the models

The suitability of the model equation for predicting the optimum response values was tested by using the selected optimal conditions. The maximum predicted yield and experimental yield of PPP were given in Table 5. Additional experiments by using the predicted optimum conditions for PPP extraction were carried out: extraction time of 1.86 h, extraction temperature 98.51 °C, ratio of water to raw material 37.2 ml/g, and the model predicted a maximum response of 10.423%. To ensure the predicted result was not biased toward the practical value, experiment rechecking was performed by using these modified optimal conditions: extraction time of 1.9 h, extraction temperature 98 °C, ratio of water to raw material 37 ml/g. A mean value of 10.358 ± 0.082% (n = 3) was gained, obtained from real experiments, demonstrated the validation of the RSM model. The results of analysis confirmed that the response model was adequate for reflecting the expected optimization (Table 5), and the model of Eq. (3) was satisfactory and accurate.

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

	Extraction time (h)	Extraction temperature (°C)	Ratio of water to material (ml/g)	Yield of PPP (%)
Optimum conditions	1.86	98.51	37.2	10.423 (predicted)
Modified conditions	1.9	98	37	10.358 ± 0.082% (actual)

4. Conclusion

The response surface method proved to be useful for optimization of technology of PPP extraction. Statistical analysis proved to be a useful and powerful tool in developing optimum extraction conditions. The statistical analysis based on a Box–Behnken design showed that extraction time of 1.9 h, extraction temperature of 98 °C and ratio of water to raw material of 37 ml/g were the best conditions to produce PPP. Under the most suitable conditions, the experimental yield of PPP was 10.358 ± 0.082%, which was closed with the predicted yield value 10.423%.

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