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Research paper

Optimization of extraction condition for phytic acid from peanut meal by response surface methodology

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ABSTRACT

Phytic acid (PA), a molecule with high commercial value, is one of the important component in peanut meal. However, PA has not yet been isolated from peanut meal and played its role. This paper reported the extraction conditions of PA from peanut meal after removed protein. The independent variables were hydrochloric acid (HCl) concentration, solid to liquid ratio, extraction time and extraction temperature. Response surface methodology (RSM) was used to optimize the extraction conditions based on the extraction yield of PA. The results show that the second-order polynomial models derived from responses well with the experimental (R^2 = 0.9783). The optimal extraction condition was obtained with solid to liquid ratio of 1:16 (g:mL), HCl concentration of 0.02 mol/L, extraction time of 105 min, and extraction temperature of 30 °C. At this condition, PA with higher purity were obtained. the extraction ratio was 6.12%, and the content of PA was 182.7 mg/g dry PA extract. The experimental values under optimal condition were in good consistent with the predicted values. The PA extracted from peanut meal was verified qualitatively by IR spectra. The extraction technology of PA from peanut meal has a strong potential for realized high-value utilization of peanut meal.

1. Introduction

Myo-inositol hexakisphosphate, also called phytic acid (PA), is considered as an anti-nutrient component because of its strong ability to combine with multi-charged metal ions, specially Zn^{2+}, Ca^{2+} and Fe^{3+} [1,2]. Recently, a variety of pharmacological activity of PA have been reported. S. Norazalina found the potential value of PA extracted from rice bran in reducing colon cancer risk in rats [3]. The finding showed treatment with 0.2% (w/v) PA extract gave the greatest reduction in the formation of aberrant crypt foci. Yukako Okazaki's study showed that PA may improve the composition of cecal organic acids, microflora, and mucins, and it may decrease the levels of serum proinflammatory cytokines in rats fed a high-fat, mineral-sufficient diet [4]. PA is a potential absorption enhancer of flavonoid components on tight junction integrity in Caco-2 cell monolayers [5]. PA is a naturally occurring constituent which exhibits protective action in Parkinson's disease and it has been shown to lower blood glucose levels [6,7]. Moreover, PA has been widely used in metallic material field as a new generation of green corrosion inhibitor for copper [8–11]. cupronickel B30 [12], brass [13] and Mg-Li alloys [14]. PA is widely used in food and light industry.

PA mainly distributes in seeds of plants, such as cereal grains, legumes, nuts, oilseeds, and so on, acting as a main source of phosphorus. Many cereal grains and oilseeds contain about 1–3% PA [15]. Peanuts is one of the most widely grown oil seeds in the world with 29 million tons produced every year [16]. In China, peanuts are the most important oil seeds, ranking first among the world's peanuts producing countries [17]. Nowadays, a majority of peanuts are crushed to produce oil and this process generates a large amount of peanut meal as the by-product, which is about 9 million tons every year in China [18–20]. A large number of studies showed that peanut meal is a good source of active ingredients, which contains about 47–55% protein, 20–30% carbohydrates, 8–10% crude fiber, 2–3% fat, and 1.0–1.2% PA [21]. However, most of the available peanut meals are used as feed ingredient for animals, and only a small portion was used to recover proteins, causing great waste [22]. Therefore, obtaining the active ingredients and high-value utilization of peanut meal has become increasingly important task for effectively reducing peanut resources waste.

Compared with other plant materials, peanut meal is rich in PA, but there are no good process to isolate PA from peanut meal re-

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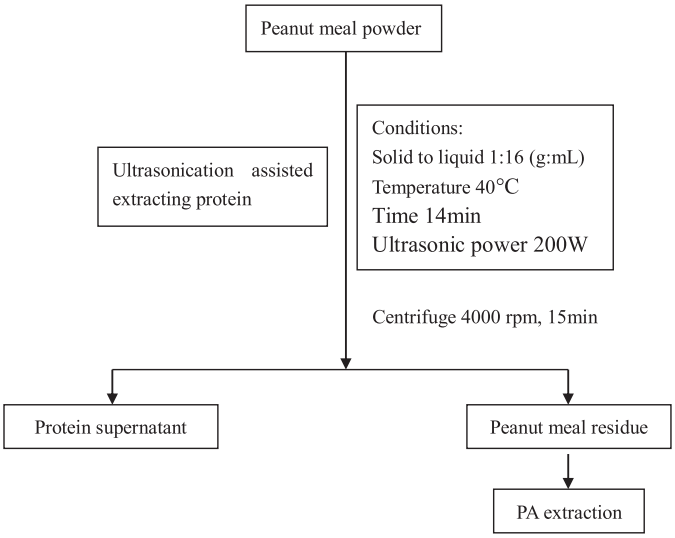

Fig. 1. The protein removal steps from peanut meal.

ported so far. The effective extraction procedure and method to separate PA from peanut meal are very necessary. In this study, we firstly removed proteins from peanut meal, and use response surface methodology (RSM) to optimize the extraction conditions. The objective of present study was to find the optimum conditions for the extraction of PA, including hydrochloric acid concentration, extraction temperature, extraction time and ratio of liquid to material, to maximize the extraction rate of PA from the peanut meal after removing proteins.

2. Materials and methods

2.1. Materials

Peanut meal was provided by Qinglonghu Wanchunyuan Vegetable Center in Beijing. The peanut meal was dried at 50 °C in an oven after removing water-soluble proteins. The processing steps of removing proteins from peanut meal is shown in Fig. 1. The dried peanut meal after removing proteins was ground using a mill.

Phytic acid sodium salt hydrate was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Ferric trichloride was purchased from Tianjin Samtec Chemical Reagent Co., LTD. 5-Sulfosalicylic acid dehydrate was purchased from West Gansu Chemical Plant in Shantou city. HCl was purchased from China National Pharmaceutical Group Chemical Reagent Co., LTD.

2.2. Determination of PA content

The determination of PA content was evaluated by the Ferric trichloride combined 5-Sulfosalicylic acid method according to the procedure described previously [22]. 0.15 g Ferric trichloride and 1.5 g 5-sulfosalicylic acid was dissolved in 500 mL waters to be the ferric trichloride and 5-sulfosalicylic acid reaction solution. Inhale 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 mL of 0.1 mol/L phytic acid sodium salt hydrate solution in 6 colorimetric cylinders respectively, and trickle water into the colorimetric cylinders to 5 mL. A 5 mL Phytic acid sodium salt hydrate solution was combined with 4 mL ferric trichloride and 5-sulfosalicylic acid reaction solution. The absorbance of the solution was measured using a UV–vis spectrophotometer (754 UV–vis Spectrophotometer, Shanghai Jinghua Co, LTD) at a wavelength of 500 nm. Standard curve was draw with the absorbance as the ordinate and PA content as the ordinate. The regression equation was obtained as the following equation:

$$Y = -4.126x + 0.878 \quad (R^2 = 0.9991) \tag{1}$$

Table 1
Independent variables and their levels used for Box-Behnken rotatable design.

Independent variable	Level		
	−1	0	1
HCl concentration (X_1)	0.01	0.02	0.03
Time (X_2)	80	100	120
Temperature (X_3)	25	30	40

2.3. Preparation of solid-liquid extracts

The influence of the solid-to-liquid ratio on the extraction of PA was investigated by using the following six ratios (1:10, 1:12, 1:14, 1:16, 1:18, 1:20; g:mL; sample powder: solvent). The hydrochloric acid concentration was fixed at the concentration of 0.01 mol/L. The mixtures were extracted at 32 °C for 83 min. The extraction solution was centrifuged at 4000 rpm for 10 min to obtain the supernatant. The content of PA (mg PA/g dry peanut meal) was then determined. The ratio that gave the highest value of yield was chosen for RSM.

2.4. Experimental designs

A d-optimal RSM experiment was designed using Design Expert (8.0.6, Stat-Ease Inc., USA). The independent variables were hydrochloric acid concentration (mol/L, X_1: 0–0.04), extraction time (min, X_2: 20–140), extraction temperature (°C, X_3: 25–60). The operating conditions were selected after single factor experiment and shown at Table1. The response variable was fitted by a second-order polynomial as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=0}^{2} \sum_{j=i+2}^{3} \beta_{ij} X_i X_j \tag{2}$$

Y: the predicted response; $\beta 0$: the intercept coefficient; βi: the linear coefficient; βii: the squared coefficient; βij: the interaction coefficient; Xi, Xj: the coded independent variables; XiXj: the interaction terms; Xi2: the quadratic terms.

The optimal conditions for the extraction of PA from peanut meal were then carried out using the equations of RSM.

2.5. The extraction rate and purity of PA

The extraction rate of PA is the ratio of the weight of PA crude extract to the weight of peanut meal, and the purity of PA is the ratio of the pure PA to the PA crude extract. The calculation formulas of the extraction rate and purity are as follows:

$$\text{The extraction rate}(\%) = (\text{The weight of PA extract} / \text{The weight of peanut meal}) \times 100\% \tag{3}$$

$$\text{The PA purity}(\%) = (\text{The weight of PA} / \text{The weight of PA extract}) \times 100\% \tag{4}$$

2.6. Fourier infrared spectrum analysis the PA of peanut meal

FT-IR measurement was recorded by a AVATAR 370 (Thermo Nicolet). 1 mg dried PA sample was mixed with 200 mg KBr. The mixture was milled 5 min in the agate mortar and tablet. The spectra of each sample were used to analysis in the range of 400–4000 cm^{-1}.

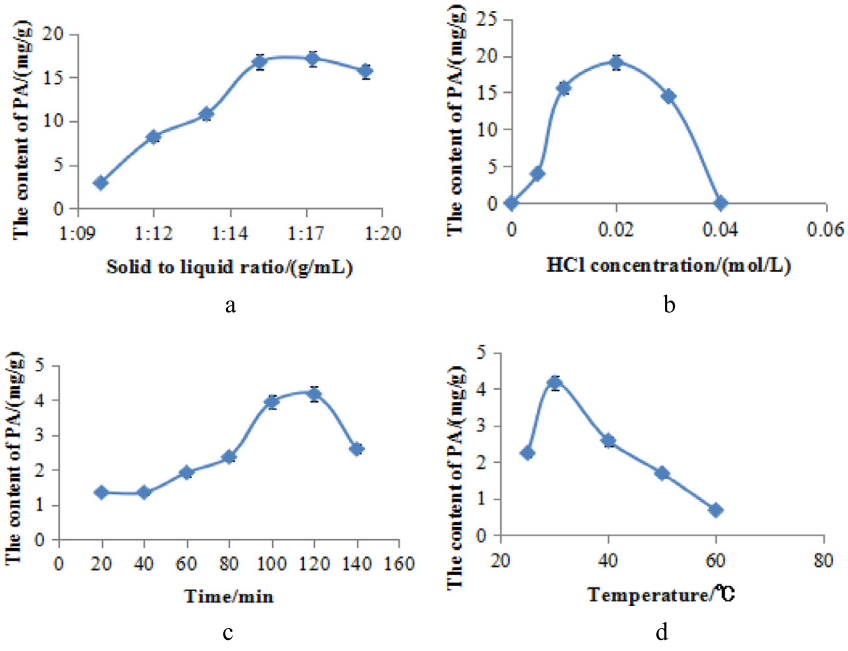

Fig. 2. Single factor experiment. Effect of solid to liquid ratio (a), HCl concentration (b), extraction time (c) and Temperature (d) of PA from peanut meal. Different letters in the same sample indicate significant differences ($p < 0.05$) between treatments.

Table 2
Coded levels, conditions runs and measured responses used in experimental design for response surface methodology.

Run	X_1	X_2	X_3	Yield (mg PA/g peanut meal)
1	0.02	100.00	32.50	13.48
2	0.02	80.00	40.00	9.30
3	0.02	120.00	40.00	10.27
4	0.03	100.00	25.00	1.85
5	0.02	100.00	32.50	13.36
6	0.01	100.00	40.00	4.26
7	0.02	100.00	32.50	11.92
8	0.02	80.00	25.00	9.09
9	0.03	120.00	32.50	0.90
10	0.02	100.00	32.50	12.59
11	0.01	100.00	25.00	9.87
12	0.01	120.00	32.50	8.52
13	0.03	100.00	40.00	6.45
14	0.03	80.00	32.50	1.80
15	0.02	120.00	25.00	10.56
16	0.01	80.00	32.50	5.87
17	0.02	100.00	32.50	13.22

3. Results and discussion

3.1. Determination of single factor experiment

3.1.1. Effect of solid/liquid ratio

In order to optimize the extraction of PA from peanut meal, solid to liquid had to be selected to study [23]. The ratio of solid to liquid varied from 1:5 to 1:20 (g:mL) while other extraction variables were fixed. In Fig. 2a, as the decrease of the solid to liquid ratio, the content of PA increased. The highest yield of PA was found at 1:16 (g:mL). This indicated that more volume of solvent was required to sufficiently solubilize the target PA when the higher driving force of molecules was supposed to be higher in a high solid to liquid ratio. However, there was no significant differences in extraction yield from 1:16 to 1:20 (g:mL) ($p > 0.05$). Therefore, 1:16 (g:mL) was chosen as a compromise and fixed in the RSM experiment.

Table 3
Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of extraction parameters.

Source	Sum of square	Degree of freedom	Mean square	F value	p-value p>F	Significance[a]
Model	279.20	9	31.02	35.12	<0.0001	**
X_1	38.36	1	38.36	43.43	0.0003	**
X_2	2.20	1	2.20	2.49	0.1586	
X_3	0.15	1	0.15	0.17	0.6930	
X_1X_2	3.15	1	3.15	3.56	0.1010	
X_1X_3	26.05	1	26.05	29.50	0.0010	**
X_2X_3	0.062	1	0.062	0.071	0.7982	
X_1^2	173.67	1	173.67	196.60	<0.0001	**
X_2^2	20.78	1	20.78	23.52	0.0019	**
X_3^2	3.30	1	3.30	3.74	0.0945	
Residual	6.18	7	0.88			
Lack of fit	4.47	3	1.49	3.47	0.1301	Not significant
Pure error	1.72	4	0.43			
Cor total	285.38	16				

$R^2 = 0.9783$, Adj $R^2 = 0.9505$, Pred $R^2 = 0.7401$, adeq precision= 16.526.
[a] *significant ($p < 0.05$), **extremely significant ($p < 0.01$).

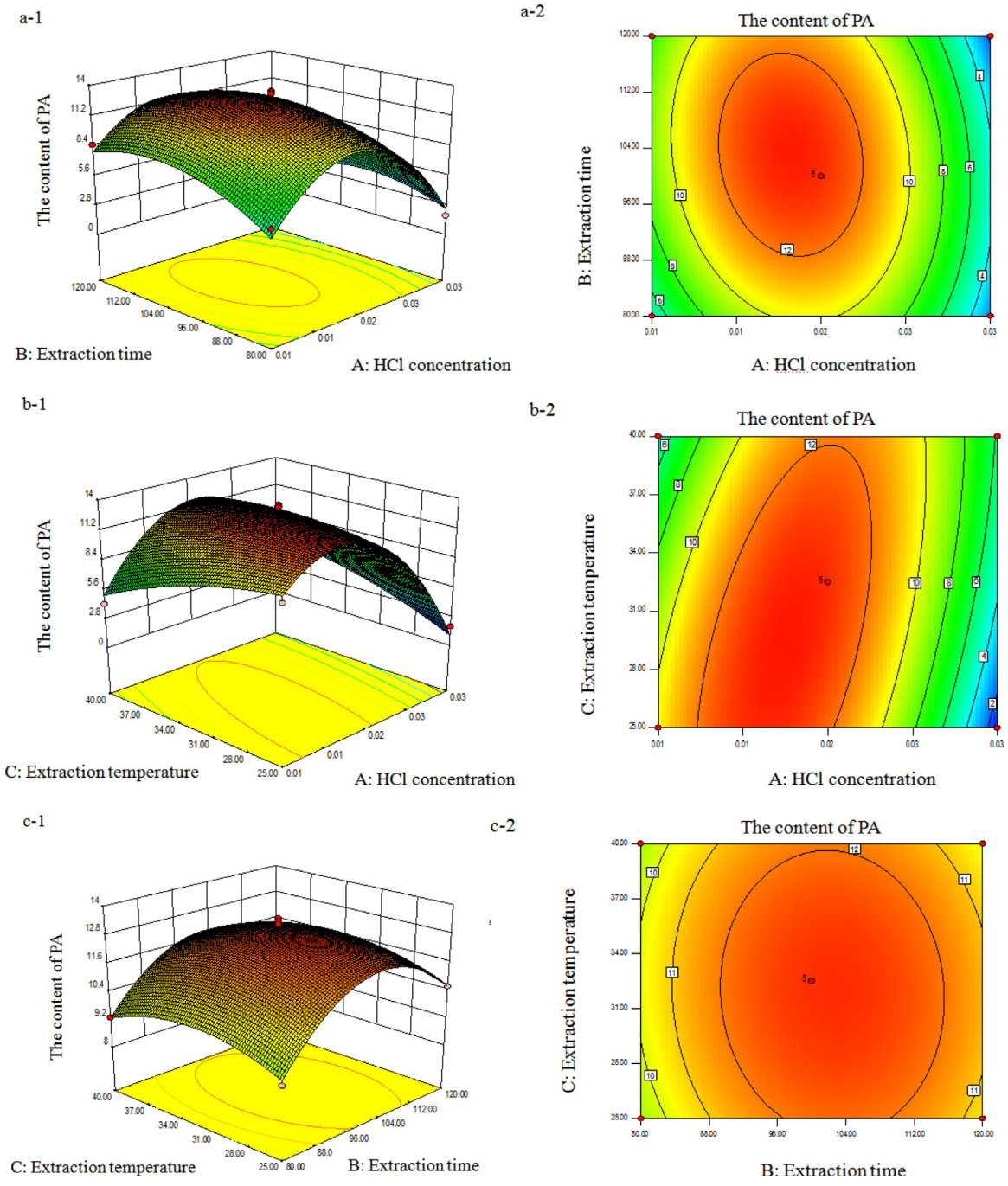

Fig. 3. Response surface plot and contour plot showing the effects of independent variables on the content of PA. a. HCl concentration and extraction time, b. HCl concentration and extraction temperature, c. extraction time and extraction temperature.

3.1.2. Effect of HCl concentration

In acid solutions, the complexing power of PA is declined, and the solubility of PA is increased. The effect of HCl concentration on the content of PA is shown in Fig. 2b. The highest extraction yield of PA was observed at HCl concentration of 0.02 mol/L. From 0 to 0.02 mol/L, the content of PA increased. While from 0.02 to 0.04 mol/L, the content of PA decreased. As increased of HCl concentration, the PA was dissolved in the HCl solution because of the decline of complexing capacity of PA. When the HCl concentration was beyond the optimized concentration, other acid soluble components, such as acid soluble saccharide, amino acids, could be dissolved, may recombine with PA and form complexes, resulting in the decrease of the content of PA.

3.1.3. Effect of extraction time

The effect of extraction time on the content of PA was studied varying time from 20 to 140 min. The content of PA was substantially increased with the time rose from 20 to 100 min (Fig. 2c). It had a decrease at longer time. It could be that other acid soluble components were dissolved and recombined with PA. The optimized extraction time was 100 min.

3.1.4. Effect of extraction temperature

As shown in Fig. 2d, the yield of PA was increased with increased of extraction temperature from 25°C to 30°C. The maximum value of extraction temperature was at 30 °C, having an obviously decrease with a further increase of extraction temperature.

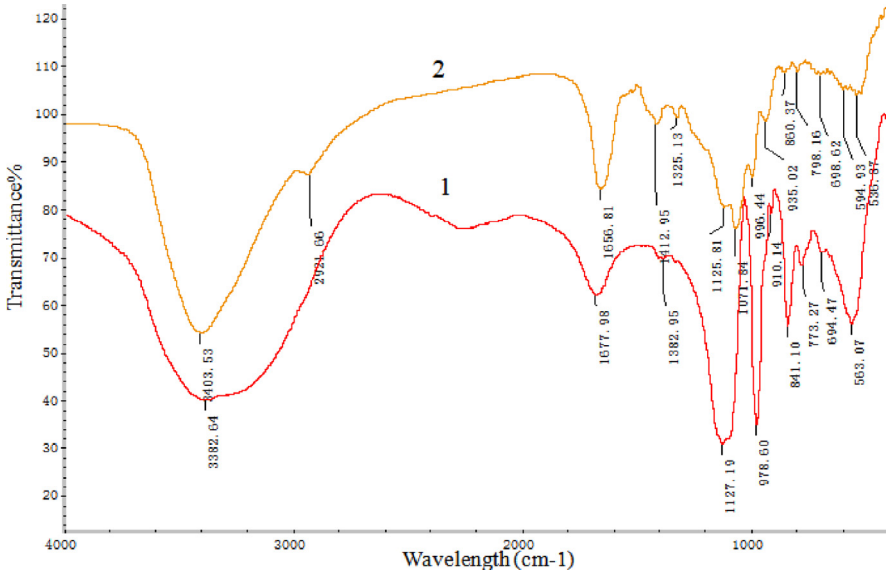

Fig. 4. FT-IR spectra of PA extract. 1: reference material phytic acid sodium salt hydrate from Sigma; 2: PA extract from peanut meal.

As the temperature increased, other ingredients were dissolved and combined with PA, causing the decreased of extraction yield.

According to the findings of above studies, the HCl concentration of 0.02 mol/L, extraction time of 100 min, extraction temperature of 30 °C were selected as a central point for RSM. The ratio of solid to liquid was fixed at 1:16 (g:mL) in the following experiment.

3.2. Response surface analysis

3.2.1. Analysis of the adequacy of the fitted model

RSM was applied to determine the effect of the HCl concentration (X_1), extraction time (X_2), and temperature (X_3) on the content of PA (Y). The responses of each independent variable are listed in Table 2. The mathematical model for describing the content of PA was given by the following second-order polynomial equation:

$$Y = 12.91 - 2.19X_1 + 0.52X_2 - 0.14X_3 - 0.89X_1X_2 + 2.55X_1X_3 - 0.12X_2X_3 - 6.42X_1^2 - 2.22X_2^2 - 0.89X_3^2$$

The response surface quadratic model was drawn up, and the analysis of variance for the fitted quadratic polynomial model for optimization of extraction parameters are presented in Table 3. The p-value for the model was less than 0.0001, which indicated the model was extremely significant and could be used to monitor the optimization. X_1, X_1X_3, and quadratic terms (X_1^2 and X_2^2) were extremely significant on the PA extraction value.

As Table 3 shown that the "Lack of Fit" is not significant relative to the pure error. The coefficient of multiple determinations (R^2) for the quadratic regression model was 0.9783. The R^2 was higher than 0.7, indicated that the model was suitable for use in the experiment [24]. The value of adjusted determination coefficients (R^2_{adj}) was 0.9505, closed to the R^2. Therefore, this model can be used to navigate the design space [25].

3.2.2. Response surface and contour plots

The response surface plots and contour plots shown in Fig. 3. It demonstrated the effect and interaction of independent variables on the yield of PA. Fig. 3a-1 and a-2 shows the effect of HCl concentration and extraction time on the content of PA. when the extraction time was fixed, the content of PA gradually increased with the increase of HCl concentration, up to the HCl concentration of 0.02 mol/L , a large increment of PA content was observed. This due to the increase of HCl concentration improved the solubility of the acid soluble components. When the HCl concentration was fixed, an obvious quadratic effect of extraction time was observed. Fig. 3b-1, b-2, and Table 3 demonstrates the interaction of HCl concentration and extraction temperature is significant ($p < 0.05$). The content of PA as affect by HCl concentration and extraction temperature, exhibiting a clear increase in content with the raise of HCl concentration at a fixed extraction temperature. While an obvious quadratic effect of extraction temperature was observed. In Fig. 3c-1 and c-2, a similar linear increase in content with the increase of extraction time ranged from 80 min to 104 min at a fixed temperature. While a decrease in temperature from 104 min to 120 min led to marked decrease in content of PA. This indicated that the extraction yield of PA depended on the medium level of time. These occurrences might be due to the recombination of PA and acid soluble proteins.

3.2.3. Optimization of extraction craft parameters and the model validation

The optimal extraction conditions for PA were determined in order to simultaneously maximize the extraction. It was found that the optimal conditions were an HCl concentration of 0.02 mol/L, extraction time of 105 min, extraction temperature of 30°C. The predicted and experimental values for the extraction of PA from peanut meal were 13.31 mg/g and 11.18 mg/g, respectively. In the optimal conditions, the experimental value was close to the predicted value, and the extraction ratio and content of PA was $6.12 \pm 0.51\%$ and 182.70 ± 2.35 mg/g, respectively. Removing the protein firstly and using the optimum extraction conditions can improve the extraction yield and purity. These results confirmed the predictability of the model to give the maximum yield of PA in the experimental process used.

3.3. Fourier infrared spectrum analysis the PA of peanut meal

Each FT-IR spectra site of a compound provides qualitative information. PA contains phosphoester bond (P=O, P-O, C–O, the region of 1330–900 cm^{-1}; -OH, the region of 3650–3200 cm^{-1}). Fig. 4 was shown that reference material phytic acid sodium salt hydrate and PA extract from peanut meal have same peaks. Phytic acid sodium salt hydrate has the peaks at the wavelength of

3382 cm^{-1} (-OH), 1127 cm^{-1}, 978 cm^{-1} (P=O, P-O, C–O). PA extract has peaks at the wavelength of 3403 cm^{-1} (-OH), 2921 cm^{-1} (-CH), 1125 cm^{-1}, 1071 cm^{-1}, 996 cm^{-1} (P=O, P-O, C–O). Therefore, the PA extract from peanut meal was verified qualitatively by comparing with phytic acid sodium salt hydrate in the IR spectra.

4. Conclusion

In this study, we first removed proteins from peanut meal, and RSM was used to optimize the experimental variables including HCl concentration (mol/L), extraction time (min), and extraction temperature (°C). Solid to liquid ratio had significant effect on the extraction yield at the ratio of 1:16 (g:mL). This was chosen as the optimum parameter. The optimal conditions to obtain the highest extraction of PA were determined as follows: 0.02 mol/L of HCl concentration, 105 min of extraction time, 30 °C of extraction temperature. At this condition, the extraction ratio was $6.12 \pm 0.51\%$, which is far higher than the extraction ratio of traditional method, and the content of PA was 182.70 ± 2.35 mg/g dry PA extract. The PA extract derived from peanut meal could be verified qualitatively by IR spectra.

PA is the most important anti-nutrient ingredient in peanut meal. Peanut nutrient ingredients removing PA is one of the most attractive and promising sources of plant proteins or plant polysaccharides. On the other hand, peanut meal is the important renewable source of PA, PA extracted from peanut meal will be better applied in medicine and light industry. This study could not only be useful in science research and industry production of PA from peanut meal, but also conducive to high value utilization of peanut meal and reduce waste of peanut resources.

Acknowledgments

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