

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.

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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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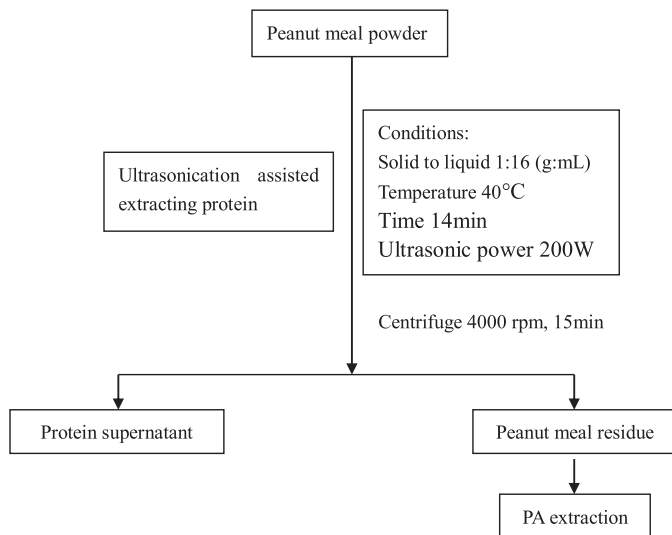


Fig. 1. The protein removal steps from peanut meal.

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 water 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

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

regression equation was obtained as the following equation:

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

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 Table 1. The response variable was fitted by a second-order polynomial as follows:

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

Y: the predicted response; β_0 : the intercept coefficient; β_i : the linear coefficient; β_{ii} : the squared coefficient; β_{ij} : the interaction coefficient; X_i , X_j : the coded independent variables; $X_i X_j$: the interaction terms; X_i^2 : 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:

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

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

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} .

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