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

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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%.

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,

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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:

$$X_i = \frac{X_i - X_0}{\Delta X} \qquad (1)$$

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:

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

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).