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Short communication

Polyethylene glycol as a novel solvent for extraction of crude polysaccharides from *pericarpium granati*

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ABSTRACT

Polyethylene glycol aqueous solution (PEGs), an environmentally friendly solvent was used, for the first time, to develop an extraction method for crude polysaccharides from *pericarpium granati* with ultrasonic–microwave-assisted extraction (UMAE). Compared with other extraction solvents, PEGs, especially PEG400, displayed a higher extraction yield. The extraction conditions were an ultrasonic power of 240 W, a microwave power of 365 W, a PEG400 concentration of 30% and a ratio of liquid to raw material of 20 mL/g, optimized by using Box–Behnken experimental design. Under these conditions, an extraction yield of 7.94 ± 0.3% ($n = 3$) was obtained, which is about 25% higher than that with water as a solvent, and the polysaccharides content in *pericarpium granati* was 6.56 ± 0.01 mg/g expressed as glucose.

1. Introduction

Polysaccharides are mainly extracted from plant materials, and widely used in food as thickener and/or emulsifier. The extraction solvent is an important factor that determines the yield of polysaccharides. Polysaccharides are usually extracted with water (Rout & Banerjee, 2007). Aqueous ethanol (Georgiadis et al., 2012), alkaline solutions (Liu, Sun, Liu, & Yu, 2012), and ionic liquids (Abe, Fukaya, & Ohno, 2010) are also used as solvent. Recently, aqueous poly(ethylene glycol) solution (PEGs) has received increased attention as a "green" solvent because of its biodegradability, low flammability, non-volatility and stability in high temperature, strong oxidants and hydrogen reduction systems, especially for PEG with a low degree of polymerization (Bulgariu & Bulgariu, 2008). PEGs was used as solvent for the extraction of flavonoid and coumarin compounds from medicinal plants (Liu, Liu, Zhang, & Zhang, 2012; Zhou, Xiao, Li, & Cai, 2011). However, it has not been reported for extraction of polysaccharides.

Pomegranate, which belongs to the *punicaceae* species, is a native fruit of the Mediterranean and South Asian regions, and has been widely used in the folk medicines of many cultures (Adams et al., 2006). *Pericarpium granati* (PG) is one of the by-products of this production, but much of it has been wasted. In the past few decades, increasing attention has focused on the extraction, anti-glycation and antioxidant potentials of PG extracts (Balasundram, Sundram, & Samman, 2006). But there is little information available regarding the extraction of PG polysaccharides (PGP).

The objective of the present work was to investigate the feasibility of using PEGs as a solvent for polysaccharide extraction from plant materials and to develop a new method for UMAE of crude PGP.

2. Materials and methods

2.1. Materials

The PG was purchased from a Chinese medicinal herbs store in Xi'an, China. The materials were milled and passed through a sieves of 40-mesh size, then defatted with ethanol and pretreated twice with 80% ethanol (Piazza, Bertini, & Milany, 2010). Prepared samples were stored in a container under dry and dark conditions until use.

2.2. Extraction of polysaccharides

An ultrasonic–microwave-assisted reaction system with 1200 W maximum microwave (2450 MHz) and 800 W maximum ultrasonic (25 kHz) power (Xianou Instrument Manufacture Co., Ltd., Nanjing, China) was used for UMAE. Prepared samples were extracted with 30% PEG400 (PEG with a molecular weight of

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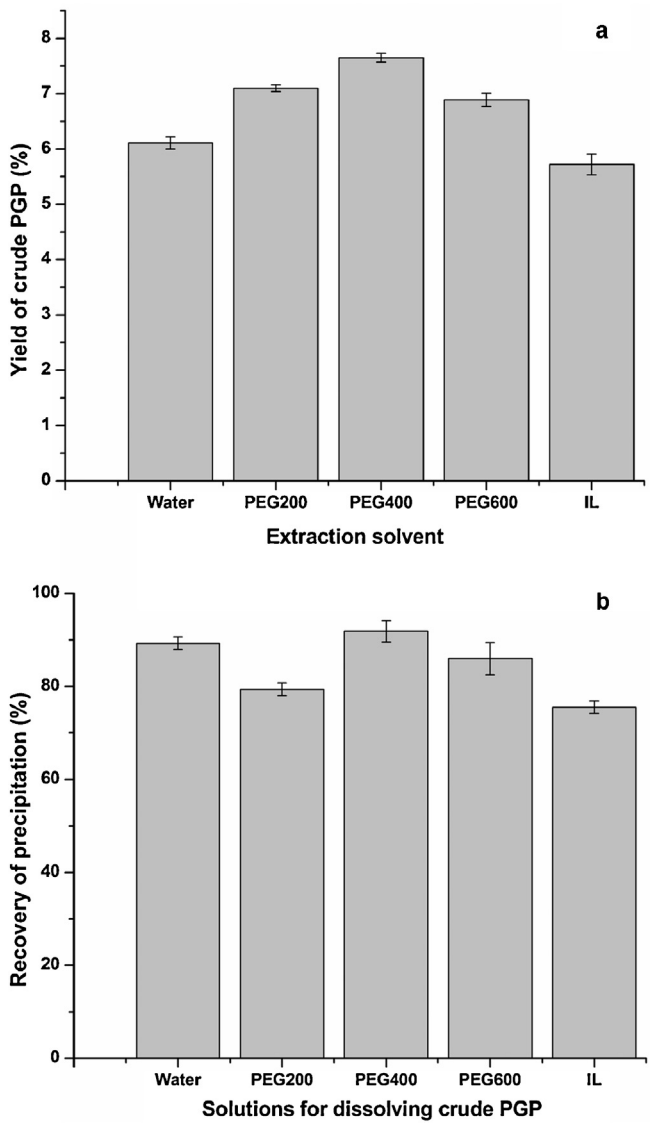

Fig. 1. Effects of various solvents on the yield of extraction (a) and recovery of precipitation (b) for PGP.

400 Da) aqueous solution at a ratio of liquid to raw material of 15 mL/g assisted the irradiations of 240 W ultrasonic and 360 W microwave power at 90 °C for 10 min. The extracts were centrifuged (8000 rpm, 10 min) and the supernatant was deproteinized by mixing with Sevage reagent (chloroform:*n*-butanol = 4:1) three times. PGP was precipitated with 4-fold volumes of anhydrous ethanol at 4 °C overnight, centrifugated, washed with acetone and anhydrous ethanol for three times, and finally dried at 50 °C. The obtained crude PGP was weighed, and the yield was calculated according to the quantities of polysaccharide obtained and the raw material used.

2.3. Precipitation recovery experiment

Crude PGP samples were dissolved in 30% of PEG200, PEG400 and PEG600 aqueous solution (v/v), water, and 10^{-3} M ionic liquid (IL, 1-ethyl-3-methylimidazolium dimethylphosphate), respectively. Then the PGP was precipitated from solutions, centrifugated, washed and dried. The rate of crude PGP quantities before and after precipitation was calculated as recovery.

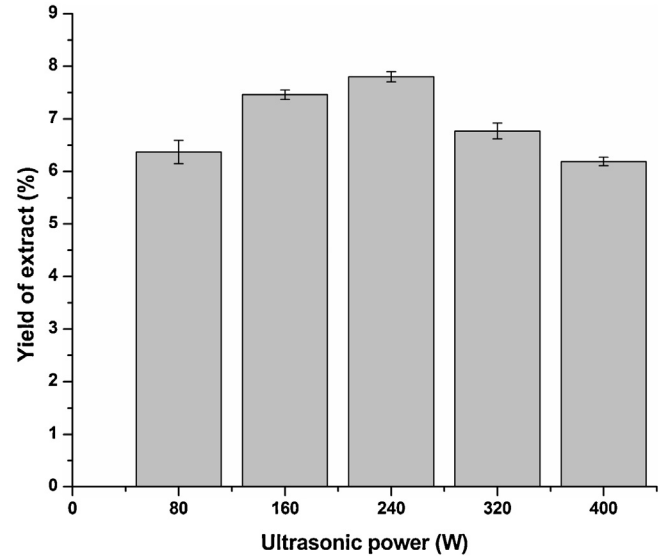

Fig. 2. Effect of ultrasonic power on the yield of crude PGP.

2.4. Optimization of extraction process using the Box–Behnken design for UMAE

A three-variable and three-level Box–Behnken design (BBD) was used to optimize the extraction condition. A trial version of Design-Expert 8.0.5 was used for modeling and regression analysis. The three independent variables were microwave power (X_1, W), concentration of PEG400 solution (X_2, v/v), and the ratio of liquid to raw material (X_3, mL/g) with a fixed reaction time of 10 min and ultrasonic power of 240 W at 90 °C. The extraction yield of crude PGP was taken as the response (Y).

2.5. Determination of polysaccharides content

The polysaccharides were determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) with D-glucose as a standard, and the polysaccharides content (mg/g) of PG was calculated according to the quantities of polysaccharide obtained and the raw material used.

3. Results and discussion

3.1. Effects of extraction solvents on crude PGP yield

To obtain the polysaccharides, two steps are involved. The first step is the extraction and dissolution of polysaccharides from raw material into solvents. The second step is the precipitation of polysaccharides from the extraction solution. PEG200, PEG400, and PEG600 aqueous solutions (30%, v/v), water, and 10^{-3} M IL were used as extraction solvents of PGP with UMAE, respectively. The effect of different extraction solvents on the yield of crude PGP is shown in Fig. 1(a). The maximum yield of crude PGP (7.65 ± 0.08%, $n = 3$) was obtained with a 30% PEG400 aqueous solution as solvent, which is about 25% higher than that with water as a solvent.

PEG could increase the solubility of polysaccharides due to the stronger interaction of —OH groups between polysaccharides and PEG, change the dissipation factor of solution and accelerate the transfer of energy from microwaves to sample (Zhou et al., 2011). These may be the reasons of PEGs having much higher extraction yields compared with water and IL.

The low viscosity of PEGs with low molecular weight is beneficial for the mass transfer during extraction. On the other side, better dissolving capacity resulted in lower recovery of precipitation. It

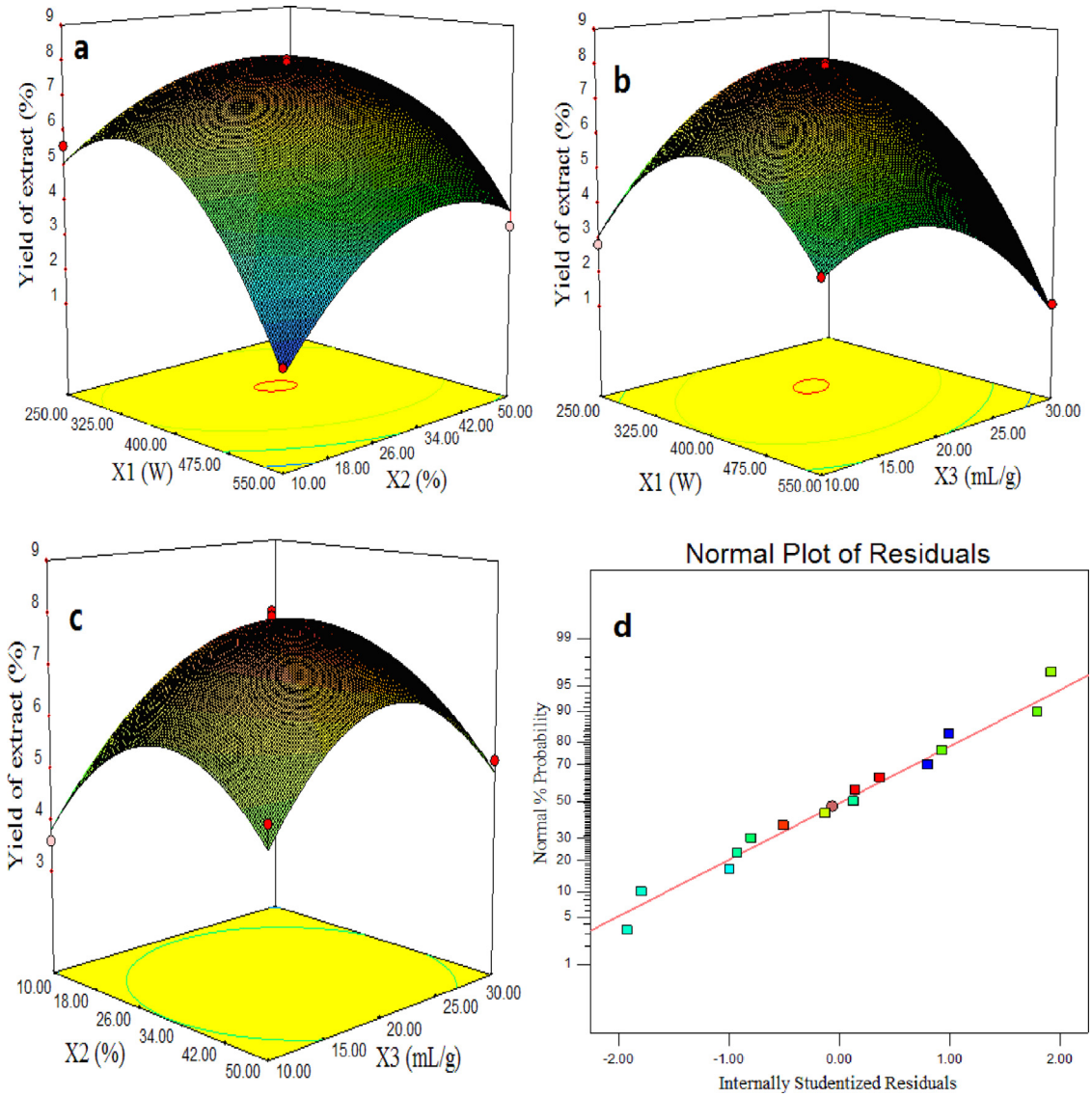

Fig. 3. Response surface plots for the effect of independent variables on the extraction yield of PGP. (a) Microwave power (X_1) and concentration of PEG400 solution (X_2) (ratio of liquid to raw material, 20 mL/g); (b) microwave power (X_1) and ratio of liquid to material (X_3) (concentration of PEG400 solution, 30%); (c) concentration of PEG-400 solution (X_2) and ratio of liquid to raw material (X_3) (microwave power, 400 W); and (d) normal plot of residuals.

could be obviously seen from Fig. 1(b) that PGP had lower recovery of precipitation in PEG200 and IL solutions. As a compromise result of dissolution and precipitation, PEG400 aqueous solution came into the most suitable solvent for the extraction of PGP.

3.2. Selection of the ultrasonic power for UMAE

The ultrasonic power in the UMAE was investigated in the range from 80 to 400 W, while other extraction conditions were held constant. The effect of ultrasonic power on the crude PGP yield is shown in Fig. 2 and the optimal value of ultrasonic power was 240 W.

3.3. Optimization of process parameters with BBD for UMAE

From BBD and experimental results of crude PGP yield (see supplementary Table S1), an empirical second-order polynomial model was established (Eq. (1)).

$$Y = 7.89 - 1.16X_1 + 0.50X_2 + 0.07X_3 + 0.95X_1X_2 - 1.33X_1X_3 + 0.100X_2X_3 - 2.70X_1^2 - 1.71X_2^2 - 1.89X_3^2 \quad (1)$$

From the analysis of variance (ANOVA) results (see supplementary Table S2), a P-value of 0.0007 and an F-value of 31.63 indicate that the model term is significant, and a large value of R^2 (0.9827) indicates the strong relevance of the dependent variables in the model. The effects of the independent variables on the crude PGP yield are displayed in Fig. 3(a–d).

The optimal extraction conditions calculated according to the model equation were a microwave power of 366.22 W, a PEG400 solution of 31.75% and a 21.00 mL/g ratio of liquid to raw material. For convenience, the actual experimental extraction condition was chosen as a microwave power of 365 W, a ratio of liquid to raw material of 20 mL/g and a PEG400 solution concentration of 30%. A crude PGP yield of 7.94 ± 0.03% (n = 3) was obtained from the chosen experimental conditions, which was in close agreement with the value of 8.05% predicted by the model.

3.4. Polysaccharides content in PG

The polysaccharide content in PG was determined according to the quantities of polysaccharide obtained and the raw material used to be 6.56 ± 0.01 mg/g, expressed as D-glucose.

4. Conclusions

The use of PEG as a solvent for the extraction of polysaccharides was comprehensively investigated for the first time, and a PEG-based UMAE was successfully developed to extract polysaccharides from *pericarpium granati*. Compared with classical solvents, polysaccharides could be extracted from *pericarpium granati* and precipitated from the extraction solution at maximum efficiency when the extraction process was performed with PEG400 aqueous solution. The experiment results demonstrated that a PEG400 aqueous solution is feasible for polysaccharide extraction. We believe that PEG solutions have strong potential as green solvents in the extraction of polysaccharides from other materials.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.10.017.

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