RESEARCH ARTICLE

Optimization of ultrasound-assisted cloud point extraction of polyphenols from pomegranate peels

Feilong Sun*, Jingye Ning, Yajing Wang, Yu Shi, Jiamin Li, Hanyu Li, Wanna Li

School of Environmental & Chemical Engineering, Xi'an Polytechnic University, Xi'an, Shaanxi, China

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Polyphenols from plant sources have received increasing attention due to their diverse biological activities, especially in the fields of food and medicine. Pomegranate peel is a potential source of polyphenols and other active substances due to its rich content. Most of the current methods for polyphenols extraction from plants involve flammable and toxic organic solvents or rely on professional extraction equipment. In order to separate polyphenols from pomegranate peel more effectively and environmentally friendly, ultrasonic-assisted cloud point extraction (UACPE) method was employed for the extraction operation, and the process parameters were also optimized in this study. The results showed that, based on single factor experiment and response surface methodology, the optimum extraction was obtained at 1:40 of solid:liquid ratio, 10% (v/v) Triton X-110, 70°C, pH 4.0 with 14% (w/v) of NaCl for 40 minutes. The maximum extraction yield of polyphenols was 82.37mg gallic acid equivalent (GAE)/g, which well matched the predicted value. Therefore, UACPE was an effective method to extract polyphenols from pomegranate peel. This method was green and environmentally friendly, and the extractives could be used in the field of diet or medicine.

Keywords: polyphenols; ultrasound-assisted cloud point extraction (UACPE); extraction optimization; pomegranate peel.

***Corresponding author:** Feilong Sun, School of Environmental & Chemical Engineering, Xi'an Polytechnic University, 58 Shaangu Avenue, Lintong District, Xi'an, Shaanxi, China. Phone: +86 29 6277 9289. E-mail: feilong.sun@hotmail.com.

Introduction

Pomegranate (*Punica granatum L.*) plant from *Punicaceae* family has been widely concerned by food, pharmaceutic, and chemical processing industries due to its multifunctional, high nutritional, and bioactive properties in recent years [1-3]. Pomegranate fruit consists of three parts, the juice, the seeds, and the peels. Pomegranate juice can be used as a beverage with a whitening effect. Pomegranate seeds can be used to extract oil, which is rich in pomegranate acid and has the effects of antioxidation, anti-inflammation, and anti-cancer. Pomegranate peels can be used as a traditional Chinese medicine, which have the effects of antidiarrheal, hemostasis, and disinsectization [4]. However, in the deep processing of pomegranate, the pomegranate peels which account for nearly 40% of the whole fruit are mostly directly discarded as "useless" leftovers, except only small quantities are used in medicine [4]. Pomegranate peels are rich in bioactive components such as polyphenols, flavonoids, and organic acids. Polyphenol compounds have shown a wide range of biological activities including antimicrobial, antioxidant, antitumor, and have important development value in the fields of food and medicine [5, 6]. Lintong District of Xi'an City, Shaanxi province, China is the most famous pomegranate production area in the country. The comprehensive development and utilization of pomegranate peel is of great significance to waste utilization, environmental protection, and local economic development.

The current methods used for polyphenol extraction from pomegranate peel mainly include conventional solvent extraction method, ultrasonic or microwave-assisted extraction method, and supercritical extraction method [7-11]. However, these technologies have many disadvantages such as low extraction yield, use of a large number of flammable and toxic organic solvents, and dependence on professional extraction equipment, *etc*., which are not satisfactory for dietary applications [12, 13]. Cloud point extraction (CPE) is a novel liquid-liquid extraction technology that utilizes the solubilization and cloud point phenomenon of surfactants to enrich the components to be separated by replacing organic solvent with surfactant aqueous solution [14]. It has the characteristics of environmental friendliness, simple operation, safety and efficiency, wide application range, and easy to enlarge the process, and is widely used in the efficient extraction of natural active ingredients [15].

Response surface method (RSM) is the most used statistical method to solve multivariable problems. Through reasonable design and experiments, certain data is obtained. Multiple quadratic regression equations are used to fit the functional relationship between factors and response values, and regression equation analysis is used to find the best experimental conditions [16]. This will significantly reduce the workload of experiments, save time, and effectively reduce the use of solvents and materials. Response surface method has been widely used in the optimization of process parameters [3, 17].

The purpose of this study is to apply a novel ultrasonic-assisted cloud point extraction (UACPE) with aqueous solutions of surfactants to extract polyphenols from pomegranate peel and optimize the extraction process parameters including solid/liquid ratio, Triton X-100, salt concentration, equilibration time, and temperature by single factor experiment and response surface method (RSM), employing a five-variable, three-level Box–Behnken design (BBD). This study would be helpful to further research and application of polyphenols from pomegranate peels.

Materials and methods

Pomegranate peel and reagents

The pomegranate peel used in this study was harvested from a pomegranate fruit forest in Lintong District, Xi'an City, Shaanxi province, China with the edible part and the aril being removed by manual peeling. The collected pomegranate peel was placed in a ventilated and dry place at room temperature, and was then treated by baking to constant weight in an electric blast drying oven (Huyue Instrument Co., Ltd, Shaoxing, Zhejiang, China) at 55°C. The dried pomegranate peel was ground into powder with a plant powder extractor (Taisite Instrument Co., Ltd, Tianjin, China), and then, screened through a sieve with a particle size of 80 mesh and stored in a dryer for future experiments.

Ultrasonic-assisted cloud point extraction

Polyphenols were extracted from pomegranate peel in an ultrasonic water bath (Boxun Medical Biological Instrument Co., Ltd, Shanghai, China) by using modified UACPE method of Pavankumar, *et al*. [18]. Trixton-100 (Shanghai McLean Biotechnology Co., Ltd, Shanghai, China) was used as a surfactant to separate polyphenols from pomegranate peel. The best extraction conditions were first screened by using single factor method followed by the response surface analysis method for further optimization, and finally the validation experiment was carried out. Briefly, 1 g of pomegranate peel powder was added to surfactant Trixton-100 (%, v/v) in different concentrations (Table 1) and mixed on a thermostatic magnetic stirrer for 30 mins. The

Table 1. Conditions and parameters for single factor experiments.

Extractive conditions	Parameters				
solid:liquid ratio(w/v)	1:30	1:40	1:50	1:60	1:70
pH	3	4	5	6	7
Triton X-100(%, v/v)	4	6	8	10	12
salt concentration (%, w/v)	8	10	12	14	16
centrifugation time (min)	5	10	15	20	25
centrifugation speed (rpm)	8,000	9,000	10,000	11,000	12,000
equilibration time (min)	10	20	30	40	50
equilibration temperature (°C)	35	45	55	65	75

Table 2. Independent variables and levels of response surface experimental design.

Independent variables	Level		
	-1	0	1
A: solid:liquid ratio(w/v)	1:30	1:40	1:50
B: Triton X-100 (%, v/v)	6	8	10
C: salt concentration (%, w/v)	12	14	16
D: equilibration time (min)	20	30	40
E: equilibration temperature (°C)	45	55	65

mixtures were then incubated in a constant temperature water bath ultrasonic instrument at different temperatures and times before centrifugation at different speeds (Table 1). The supernatants were collected and the different concentrations of NaCl solutions (Table 1) were then added to the supernatants. After centrifugation at 6,000 rpm for 20 mins, the upper organic phase that contained polyphenols was recovered. Three sets of parallel experiments were conducted in this study.

Determination of total polyphenol content

Folin-cicalteau method described by Torres, *et al.* [19] was applied to determine the total polyphenols content in pomegranate peel. Briefly, 100 µL of sample extract was added into a 10 mL disposable centrifuge tube with the same volume of Folin-cicalteau reagent (Shanghai McLean Biotechnology Co., Ltd, Shanghai, China). After fully mixing, 700 µL of 20% Na_2CO_3 solution was added, and then, supplemented by distilled water to the final volume of 5 mL. The reaction was incubated at room temperature for 30 mins under a dark environment before the absorbance at 750 nm was measured by using a UV-VIS spectrophotometer (Meipuda Instrument Co., Ltd, Shanghai, China). Gallic acid (Shanghai McLean Biotechnology Co., Ltd, Shanghai, China) was employed to make the standard calibration curves. The final yield of total polyphenol content in the sample was expressed in mg of gallic acid equivalent (GAE) per g of pomegranate peel powder (mg GAE/g). The yield of total polyphenol content was calculated by using the following formula.

$$Y = CV \times 1{,}000 \times N/m$$

where Y (mg GAE/g) was the total polyphenol content. C (mg/L) was the polyphenol concentration. V (mL) was the solvent volume. N was the dilution ratio; m (g) is the weight of pomegranate peel powder.

Influence of single factor on extraction yield

Eight (8) factors including solid-liquid ratio (w/v), surfactant concentration (%, v/v), pH, salt concentration (%, w/v), equilibrium temperature (°C), equilibrium time (min), centrifugation time (min), and centrifugation speed (rpm) were selected to investigate the influence of each factor on the extraction rate and determine the appropriate level of each factor. The specific parameters of each single factor were shown in Table 1.

Response surface experiment

In order to further determine the optimal extraction conditions of UACPE, based on the analysis of the single-factor experimental results, five factors that showed the greater impacts on the extraction rate were selected including solid-liquid ratio (A), Trixton-100 (B), NaCl concentration (C), equilibrium time (D), and equilibrium temperature (E) (Table 2). Under the employment of Design Expert 8.0 software (Stat-Ease Inc., Minneapolis, Minnesota, USA), the influence of these five independent variables on the total polyphenol content (TPC) was studied by using Box-Behnken design.

Statistical analysis

All the experiments were performed in triplicate and the data were analyzed by using SPSS (22.0) for Windows (IBM Corp., Armonk, New York, USA). Statistical analyses were conducted by one-way ANOVA. Multiple comparisons versus control were completed by using Dunn's method with significance at the $P < 0.05$ level.

Results and discussion

Optimization of UACPE conditions with single factor test

(1) The solid to liquid ratio

The solid to liquid ratio can be considered as one of the critical variables in CPE system. Lesser the liquid or solvent content than optimum level leads to incomplete extraction of polyphenols, while higher solvent volume would be just an extraordinary waste [20, 21]. Single-factor experiment was performed at varied solid to liquid ratio ranging from 1:30 to 1:70 with 8% (v/v) Triton X-100. The effect of solid:liquid ratio was shown in Figure 1. The responses of TPC were gradually increased as the ratio of solid to liquid increased from 1:30 to 1:50, which might be due to that higher solid to liquid ratio led to an increase in solvent volume, which was necessary for solubilization of bioactive substances before saturation. The maximum TPC was observed in the solid to liquid ratio of 1:50. After that, it showed an apparent decrease in the ratio of 1:60, and 1:70, which might be explained by the lower concentration of solute leading to the lower extraction yield. Considering comprehensively, the solid liquid ratio of 1:30, 1:40, and 1:50 were selected as the conditions for further polyphenol CPE research in the response surface experimental design.

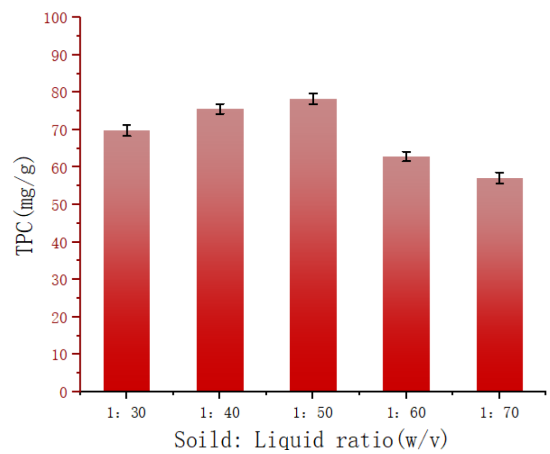

Figure 1. Effects of solid:liquid ratio on total polyphenol content (TPC) extracted from pomegranate peel.

(2) pH values

The most crucial factor during the extraction process in CPE was the system pH. By changing the electrical charge form of ionizing molecules like polyphenols, pH has significantly influence on effective partitioning and surfactant phase separation in CPE system [22]. The effect of pH on TPC yield was studied by varying pH from 3.0 to 7.0 at sodium chloride concentration of 12% (w/w), 100% (40KHz) ultrasonic power for 30 mins at 55°C using 8% (v/v) Triton X-100 at 1:50 solid-liquid ratio. The results were illustrated in Figure 2. It was observed that the extraction yields increased and then slightly decreased with the increasing pH value. At pH 4.0, the yield of TPC reached a maximum of 70.96 mg GAE/g. A similar trend was also reported by Chen, *et al* using Triton X-114 [23]. Therefore, pH 4.0 was selected for the following experiments.

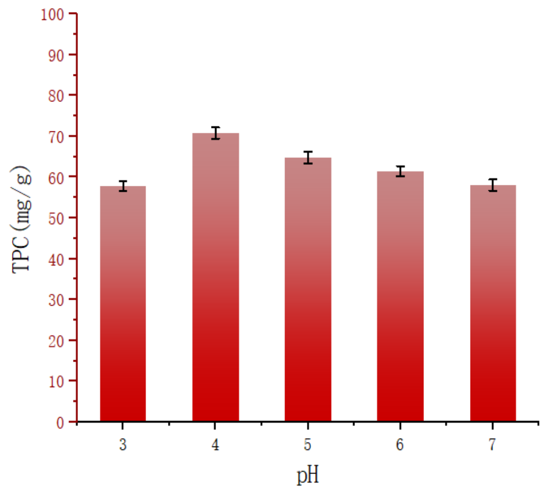

Figure 2. Effects of pH on total polyphenol content extracted from pomegranate peel.

(3) The concentration of Triton X-100

The concentration of surfactant affects the extraction yield and the phase volume ratio during phase separation in the CPE system. In order to obtain effective cloud formation, surfactant concentration higher than the critical micelle concentration (CMC) is required [24]. Generally, the ratio of aqueous phase volume to surfactant rich phase volume increases with the decrease of surfactant concentration [12]. The effect of Triton X-100 concentration from 4 to 12% (v/v) was evaluated for maximum TPC (Figure 3). When the concentration of surfactant increased from 4% to 12%, the extraction yield increased first and then decreased, and reached the peak at 10% (v/v) of Triton X-100 with the TPC of 76.78 mg GAE/g. Therefore, 6%, 8%, and 10% (v/v) Triton X-100 were selected as the surfactant concentrations for further optimization experiment.

(4) Salt concentration

In order to avoid the loss of thermosensitive compounds such as polyphenols due to high temperature during the extraction process, ionic strength plays an important role [25]. It can induce salting out effect in Triton X-100 aqueous solution by reducing the cloud point temperature of surfactant to achieve effective phase separation [26]. The effects of salt (NaCl) concentrations from 8 to 16% was investigated for the maximum TPC yield (Figure 4). The results showed that, when the salt concentration increased from 8% to 14%, the yields of TPC increased rapidly, which might be related to the fact that the electrolyte like NaCl in the micellar system helped to increase the number and size of micelles, thus promoting the phase separation process. When the ion concentration exceeded 14%, the change of extraction efficiency was relatively insignificant. In the response surface experiment design, the salt concentrations of 12%, 14%, and 16% were selected for further study.

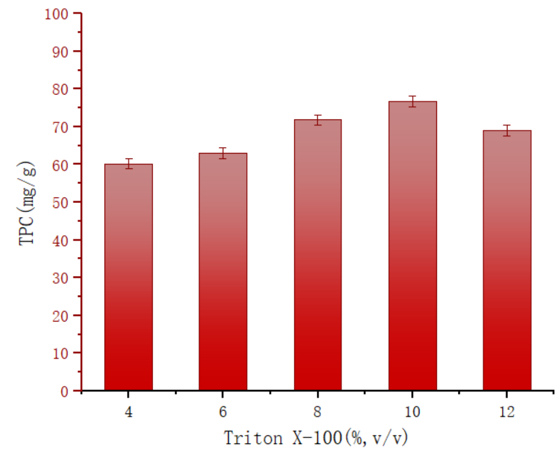

Figure 3. Effects of Triton X-100 on total polyphenol content extracted from pomegranate peel.

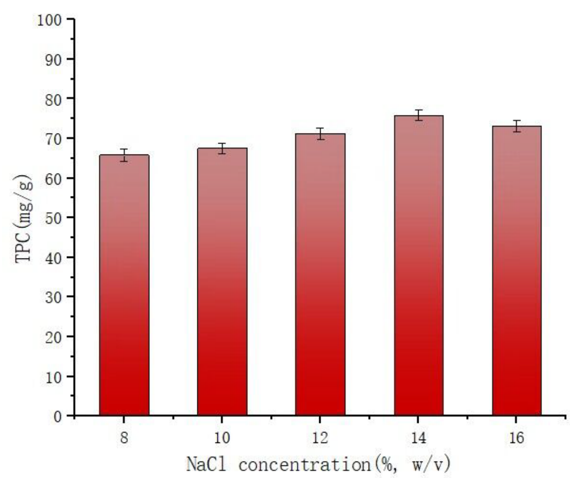

Figure 4. Effects of salt (NaCl) concentration on total polyphenol content extracted from pomegranate peel.

(5) The centrifugation time and speed

Centrifugation increases the equilibration and reduces the time required for CPE. In general, under the condition of insufficient centrifugation speed and time, the separation is incomplete. However, too long centrifugation time or too high centrifugation speed probably leads to partial back extraction to the aqueous phase, which will reduce the polyphenol yield and CPE efficiency [27]. The effects of the centrifugation time and speed on the TPC yield were studied in the range of 8,000 - 12,000 rpm for 5 - 25 mins. The results showed that, within the selected level range, the influence trend was not very obvious (Figure 5). Therefore, these two factors were rarely optimized in the literature.

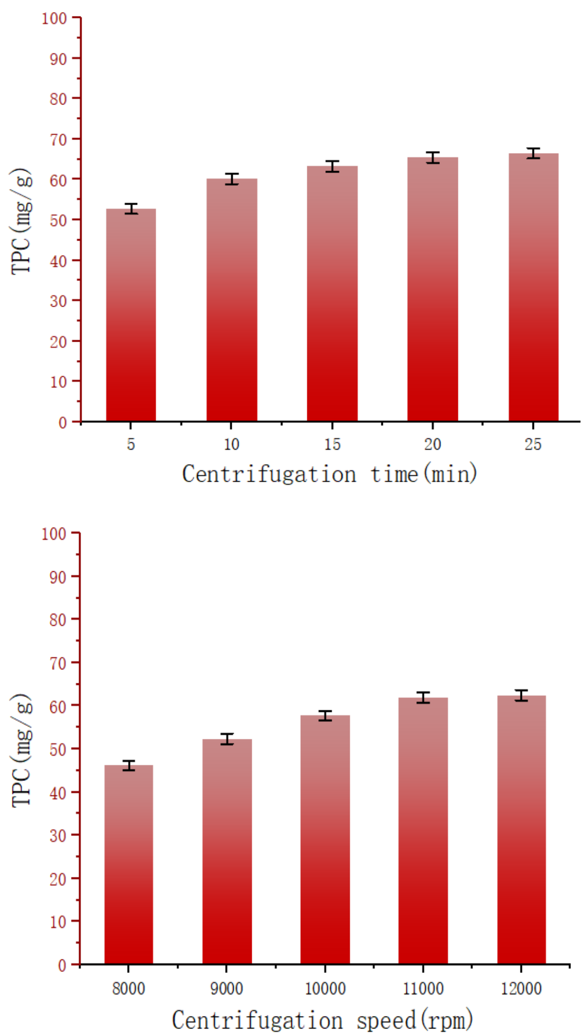

Figure 5. Effects of the centrifugation time and speed on the TPC yield.

(6) Equilibration temperature and time

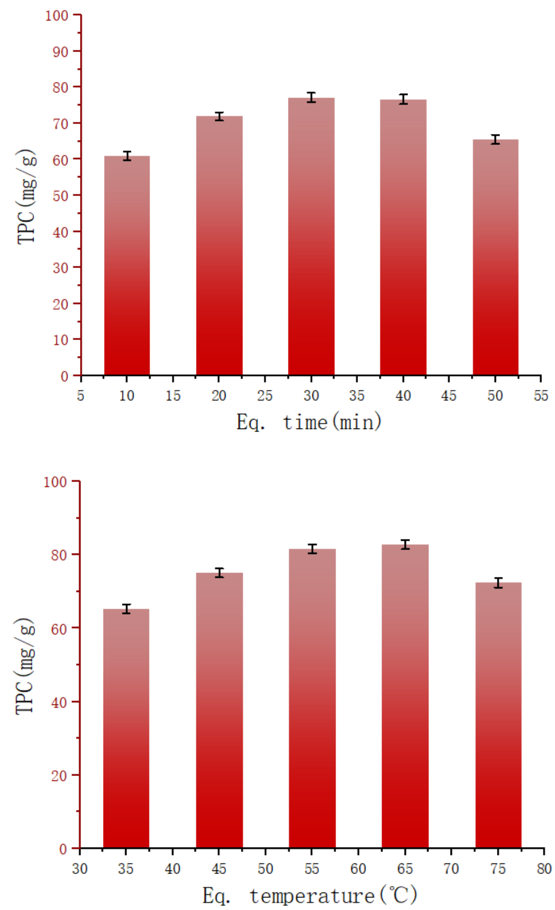

Figure 6. Effects of the equilibration temperature and time on the TPC yield.

Temperature is one of the most important parameters in cloud point extraction system. Theoretically, CPE can only be realized when the equilibrium temperature of the extraction solution is higher than the cloud point temperature of the surfactant. In addition, the equilibrium temperature is closely related to the formation time of organic molecules and surfactant micelles [28]. The influence of equilibration time on the CPE yield was studied in the range of 10 to 50 mins at room temperature. Figure 6 showed that 30 mins equilibration time was well enough to obtain maximum yield of TPC in CPE system, which was about 76.97 mg GAE/g. The effect of equilibration temperature on the yield of TPC was investigated at different temperatures varying from 35°C to 75°C. The

Table 3. Box–Behnken experimental design and results for yield of TPC.

Run No.	A solid:liquid ratio (w/v)	B Triton X-100 (%, v/v)	C salt concentration (%, w/v)	D Eq. time (min)	E Eq. Temperature (°C)	Y TPC (mg GAE/g)
1	50	10	14	50	65	60.01
2	50	10	14	30	65	64.57
3	40	10	14	40	65	85.13
4	30	10	14	40	75	75.01
5	30	10	14	50	65	65.27
6	40	12	14	50	65	67.61
7	40	8	14	40	55	61.03
8	40	10	16	40	55	58.05
9	40	12	14	30	65	71.27
10	40	10	14	50	55	58.81
11	50	12	14	40	65	59.14
12	30	10	16	40	65	62.11
13	50	10	14	40	75	69.35
14	40	10	16	40	75	65.63
15	40	10	14	40	65	85.15
16	40	12	12	40	65	65.27
17	40	10	12	40	55	67.89
18	30	10	12	40	65	59.74
19	30	10	14	40	55	65.17
20	40	10	12	50	65	65.87
21	50	10	12	40	65	60.79
22	40	12	14	40	75	77.58
23	50	8	14	40	65	54.22
24	40	10	14	40	65	85.35
25	40	12	14	40	55	56.79
26	40	10	14	40	65	85.89
27	40	8	14	50	65	44.48
28	40	10	16	50	65	63.69
29	30	12	14	40	65	61.72
30	40	8	12	40	65	63.47
31	40	8	16	40	65	58.98
32	40	10	14	40	65	79.15
33	40	12	16	40	65	76.45
34	50	10	14	40	55	55.13
35	40	10	14	30	75	76.32
36	40	10	12	40	75	71.72
37	40	10	14	40	65	86.16
38	40	10	14	30	55	62.89
39	40	10	12	30	65	63.29
40	40	8	14	40	75	69.62
41	30	10	14	30	65	58.03
42	40	10	16	30	65	61.98
43	40	8	14	30	65	65.79
44	30	8	14	40	65	73.06
45	50	10	16	40	65	58.87
46	40	10	14	50	75	72.48

Table 4. Estimated regression model of relationship between response variable (yield of TPC) and independent variables (A, B, C, D, E).

Source	Sum of squares	df	Mean square	F value	P value	
Model	3390.893	20	169.55	6.95	< 0.0001	**
A	90.39	1	90.39	3.7	0.0657	
B	127.578	1	127.58	5.22	0.0310	*
C	9.42	1	9.42	0.39	0.5399	
D	41.99	1	41.99	1.72	0.2015	
E	528.42	1	528.42	21.66	< 0.0001	**
AB	66.09	1	66.09	2.7	0.1123	
AC	4.6	1	4.6	0.19	0.6678	
AD	34.81	1	34.81	1.43	0.2435	
AE	4.796	1	4.796	0.19	0.6613	
BC	61.39	1	61.39	2.51	0.1253	
BD	77.88	1	77.88	3.19	0.0861	
BE	37.21	1	37.21	1.52	0.2283	
CD	0.18	1	0.18	0.0078	0.9305	
CE	3.51	1	3.51	0.14	0.7074	
DE	0.01	1	0.01	0.00059	0.9808	
A^2	1308.35	1	1308.34	53.63	< 0.0001	**
B^2	911.51	1	911.51	37.36	< 0.0001	**
C^2	951.79	1	951.79	39.01	< 0.0001	**
D^2	973.79	1	973.79	39.91	< 0.0001	**
E^2	444.58	1	444.57	18.22	0.0002	*
Residual	609.95	25	24.39			
Lack of Fit	575.1	20	28.76	4.126	0.0612	not significant
Pure Error	34.85	5	6.97			

*significant ($P < 0.05$). **very significant ($P < 0.01$).

results showed that the maximum yield of TPC (82.12 mg GAE/g) at temperatures 65°C was observed. When the temperature exceeds 65°C, the TPC yield decreased instead of increasing, which might be explained by the degradation of polyphenols caused by high temperature. Similar observations reported by Luo, *et al*. [25]. If it is kept at a high temperature for a longer time, degradation may occur, leading to a decrease in TPC yield. Based on the fact, a temperature of 65°C was selected as the optimum equilibrium extraction temperature for further studies.

Optimization of UACPE conditions with response surface methodology

In order to enhance the yield of the target compounds, response surface methodology (Box–Behnken design) was employed to further optimize the extraction conditions of polyphenols from pomegranate peels through UACPE. Based on the single factor experiments results, the relationships among solid:liquid ratio, Triton X-100, salt concentration, equilibration time and temperature were further optimized by using Design Expert 8.0.5. Three values of solid:liquid ratio (1:30, 1:40, and 1:50 (w/v)), Triton X-100 (6, 8, and 10% (v/v)), salt concentration (12, 14, and 16% (w/v)), equilibration time (20, 30, and 40 mins), and equilibration temperature (45, 55, and 65°C) were applied by using a Box-Behnken design. The independent variables and levels of experimental design (5 factors & 3 levels) were shown in Table 2. The experimental design and results using Design expert 8.0 software were shown in Table 3. The results of multiple regression analysis were shown in Table 4. According to the response surface analysis, the regression equation was induced as follows.

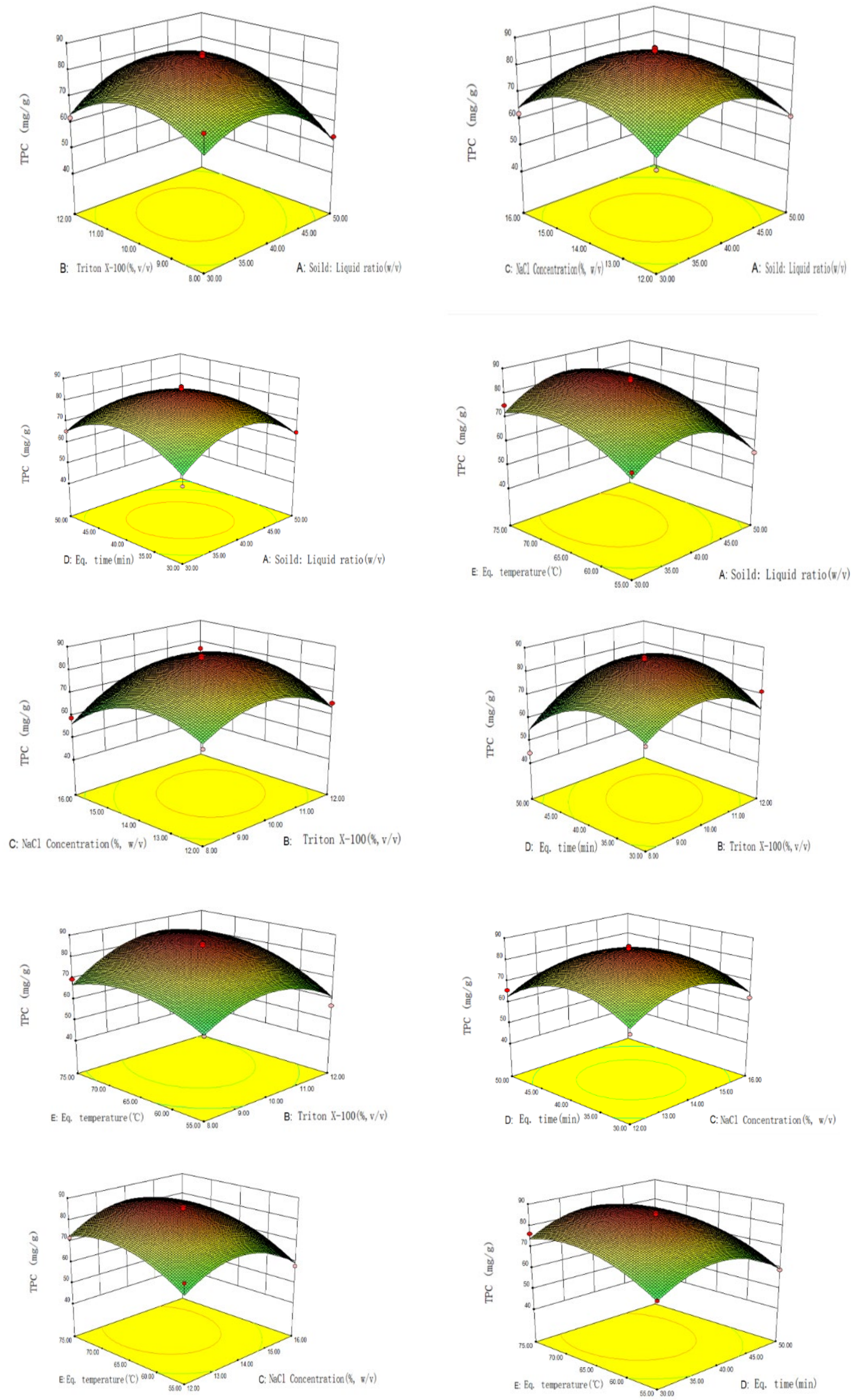

Figure 7. Response surface (3D) showing the effect of different extraction parameters (A: solid:liquid ratio,w/v; B: Triton X-100, % (v/v); C: NaCl concentration, % (w/v); D: Eq. time, min; E: Eq.temperature, °C) on the response yield of TPC.

Y = 84.47 - 2.38A + 2.82B - 0.77C - 1.62D + 5.75E + 4.07AB - 1.07AC - 2.95AD + 1.09AE + 3.92BC + 4.41BD + 3.05BE - 0.22CD + 0.94CE + 0.06DE - 12.24A2 -10.22B2 - 10.44C2 - 10.56D2 - 7.14E2

Regression model *P* value was 0.0001 ($P < 0.01$). The difference was statistically significant. The influence of five factors on extraction rate was as follows: equilibration temperature (E) > Triton X-100 (B) > solid:liquid ratio (A) > equilibration time (D) > salt concentration (C). BD interaction was the most significant while DE interaction was the weakest.

Through the analysis of Design expert 8.0 software, the response surface of each factor to the extraction rate was obtained, and the results were shown in Figure 7. It was predicted that the best extraction process was (A) solid:liquid ratio 1:39.57 g/mL, (B) Triton X-100 10.39% (v/v), (C) salt concentration 14.04% (w/v), (D) Eq. time 39.71 mins, (E) Eq. temperature 69.43°C. According to the optimum extraction process predicted by response surface software Design expert 8.0, in order to facilitate the actual experiment, the prediction process was revised to (A) solid:liquid ratio 1:40 g/mL, (B) Triton X-100 10% (v/v), (C) salt concentration 14% (w/v), (D) Eq. time 40 mins, (E) Eq. temperature 65°C for the experiment, and the extraction rate was 82.37 mg GAE/g, which was close to the predicted value of 86.08 mg GAE/g.

Conclusion

Cloud point extraction is a novel extraction technology that utilizes the cloud point principle and the solubilization of surfactants to enrich the separated components. Using surfactants to form micelles in the aqueous phase can increase the solubility of the components to be separated, and then change the experimental parameters to initiate phase separation to achieve the purpose of separating the desired components. In this study, the cloud point extraction method was employed to extract pomegranate peel polyphenols. Through single factor and response surface methodology optimization, the optimal extraction process was obtained as (A) solid:liquid ratio 1:40 g/mL, (B) Triton X-100 10% (v/v), (C) salt concentration 14% (w/v), (D) Eq. time 40 mins, (E) Eq. temperature 65°C, and the final extraction rate was up to 82.37 mg GAE/g. At the same time, it was found that the extraction rate could be improved by changing the concentration of surfactant or adjusting the ionic strength. The cloud point extraction method, which does not use organic solvents, has the characteristics of green environmental protection and low cost, and is likely to become a new green process for the extraction of effective components of natural products in the future.

Acknowledgement

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References

1. Sood A, Gupta M. 2015. Extraction process optimization for bioactive compounds in pomegranate peel. Food Biosci. 12:100-106.
2. Puneeth HR, Chandra S. 2020. A review on potential therapeutic properties of Pomegranate (*Punica granatum L.*). Plant Sci Today. 7(1):9.
3. Zhu CP, Zhai XC, Li LQ, Wu XX, Li B. 2015. Response surface optimization of ultrasound-assisted polysaccharides extraction from pomegranate peel. Food Chem. 177:139-146.
4. Zhang JH, Li DM, Zhong XR, Liu N. 2022. Extraction preparation and application of pomegranate peel polyphenols: A review. Food Res Dev. 43(14):204-211.
5. Sharayei P, Azarpazhooha E, Zomorodib S, Ramaswamy HS. 2019. Ultrasound assisted extraction of bioactive compounds from pomegranate (*Punica granatum L.*) peel. LWT - Food Sci Technol. 101:342–350.
6. Rashid R, Masoodi FA, Wani SM, Manzoor S, Gull A. 2022. Ultrasound assisted extraction of bioactive compounds from pomegranate peel, their nanoencapsulation and application

for improvement in shelf life extension of edible oils. Food Chem. 385:132608.
7. Azmir J, Zaidul I, Rahman MM, Sharif KM, Mohamed A, Sahena F, *et al*. 2013. Techniques for extraction of bioactive compounds from plant materials: A review. J Food Eng. 117(4):426-436.
8. Wang Z, Zhang CX, Pu B, Zhu QQ, Zhou JY, Jiao SR. 2015. Study on the extraction of total polyphenols and antioxidant activity of pomegranate peel by solvent refluxing method. Food Ind. 36(7):57-62.
9. Zheng X, Liu B, Li L, Zhu X. 2011. Microwave-assisted extraction and antioxidant activity of total phenolic compounds from pomegranate peel. J Med Plant Res. 5(6):1004-1011.
10. Kaderides K, Papaoikonomou L, Serafim M, Goula AM. 2019. Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasounds extraction. Chem Eng Process. 137:1-11.
11. Mushtaq M, Sultana B, Anwar F, Adnan A, Syed SHR. 2015. Enzyme-assisted supercritical fluid extraction of phenolic antioxidants from pomegranate peel. J Supercrit Fluids. 104:122-131.
12. Arya SS, Kaimal AM, Chib M, Sonawane SK, Show PL. 2019. Novel, energy efficient and green cloud point extraction: technology and applications in food processing. J Food Sci Technol. 56(2):524–534.
13. Sherine ES, Mohamed AF. 2021. Novel trends in extraction and optimization methods of bioactives recovery from pomegranate fruit biowastes: Valorization purposes for industrial applications. Food Chem. 365:130465
14. Guo N, Jiang YW, Kou P, Liu ZM, Efferth T, Li YY, *et al*. 2019. Application of integrative cloud point extraction and concentration for the analysis of polyphenols and alkaloids in mulberry leaves. J Pharm Biomed Anal. 167:132-139.
15. Racheva R, Rahlf AF, Wenzel D, Müller C, Kerner M, Luinstra GA, *et al*. 2018. Aqueous food-grade and cosmetic-grade surfactant systems for the continuous countercurrent cloud point extraction. Sep Purif Technol. 202:76–85.
16. Meriem B, Morad C, Francisco JB, Nabil G, Olivier B, Eugène V. 2015. Optimization of microwave-assisted extraction of polyphenols from Quercus bark. Ind Crop Prod. 77:590–601.
17. Chakraborty S, Rao PS, Mishra HN.2015. Response surface optimization of process parameters and fuzzy analysis of sensory data of high pressure temperature treated pineapple puree. J Food Sci. 80:E1763-E1775.
18. More PR, Arya SS. 2019. A novel, green cloud point extraction and separation of phenols and flavonoids from pomegranate peel: an optimization study using RCCD. J Environ Chem Eng. 7:103306.
19. Torres B, Tiwari BK, Patras A, Wijngaard HH, Brunton N, Cullen PJ, *et al*. 2011. Effect of ozone processing on the color, rheological properties and phenolic content of apple juice. Food Chem. 124(3):721-726.
20. Wang WC, Li QY, Liu YH, Chen BB. 2015. Ionic liquid-aqueous solution ultrasonic-assisted extraction of three kinds of alkaloids from *Phellodendron amurense Rupr* and optimize conditions use response surface. Ultrason Sonochem. 24:13-18.
21. Tang XY, Zhu D, Huai WB, Zhang W, Fu CJ, Xie XJ, *et al*. 2017. Simultaneous extraction and separation of flavonoids and alkaloids from *Crotalaria sessiliflora L*. by microwave-assisted cloud-point extraction. Sep Purif Technol. 175:266-273.
22. Wang Y, Han J, Xu XH, Hu SP, Yan YS. 2010. Partition behavior and partition mechanism of antibiotics in ethanol/2-propanol–ammonium sulfate aqueous two-phase systems. Sep Purif Technol. 75(3):352-357.
23. Chen L, Qi Z, Jin H, Zhang XP, Xu Y, Yu AM. 2010. Determination of xanthohumol in beer based on cloud point extraction coupled with high performance liquid chromatography. Talanta, 81:692-697.
24. Chatzilazarou A, Katsoyannos E, Gortzi O, Lalas S, Paraskevopoulos Y, Dourtoglou E, *et al*. 2010. Removal of polyphenols from wine sludge using cloud point extraction. J Air Waste Manage Assoc. 60(4):454-459.
25. Luo XL, Zhang H, Zhang ZH, Wang MX, Yang BY. 2017. Cloud point extraction for simultaneous determination of 12 phenolic compounds by high performance liquid chromatography with fluorescence detection. Microchem J. 137:148-154.
26. Li FF, Li Q, Wu SG, Tan ZJ. 2017. Salting-out extraction of allicin from garlic (*Allium sativum L.*) based on ethanol/ammonium sulfate in laboratory and pilot scale. Food Chem. 217:91-97.
27. Ma HH, Mu FT, Fan SJ, Zhou X, Jia Q. 2015. Development of a cloud point extraction method for the determination of phenolic compounds in environmental water samples coupled with high-performance liquid chromatography. J Sep Sci. 35(18):2484-2490.
28. Wang L, Jiang GB, Cai YQ, He B, Wang YW, Shen DZ. 2007. Cloud point extraction coupled with HPLC-UV for the determination of phthalate esters in environmental water samples. J Environ Sci. 7:874-878.