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Optimization of ultrasound-homogenization combined extraction of phenolics in peony roots and leaves

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Abstract

Paeonia ostii is the representative of oil-utilized peony, its roots (PR) and leaves (PL) are discarded as by-products, resulting in a waste of resources. The exploration of extraction process of active ingredients from PR/PL is beneficial to the comprehensive utilization. In this work, the optimum process of Ultrasound-Assisted Extraction (UAEo) of total flavonoids content (TFC) from PR and PL was determined by single factor and response surface methodology. The results showed that UAEo was 80 min, 1:5 g/mL, 250 W, 33.83 mg Rutin/g dw for PR and 60 min, 1:10 g/mL, 250 W, 25.85 mg Rutin/g dw for PL. Then, Homogenization-Assisted Extraction (HAE), Homogenization-Ultrasound-Assisted Extraction (HUAEo) and Ultrasound-Homogenization-Assisted Extraction (UHAEo) were further analyzed. The highest PR(PL)-TFC by HUAEo at homogenization 5 min was 49.58 ± 0.25 mg/g with an increase of 46.6% (33.02 ± 0.04 mg/g with an increase of 27.7%). The highest PR/PL-total phenolic content by the HUAEo reached 77.84 ± 0.52 mg/g dw and 146.62 ± 2.77 mg/g dw for homogenization 3 min. However, there was no significant difference between HUAEo and UHAEo. In conclusion, the TFC increased with the extension of HAE time, and the combined extraction was higher yield than the single extraction.

Keywords: *Paeonia ostii*; response surface methodology; high-speed homogenization; combined extraction.

Practical Application: The combined extraction is conducive to the release of phenolics in peony by-product.

1 Introduction

Peony, a perennial deciduous shrub belonging to the *Paeonia* in the family Paeoniaceae, can be subdivided into ornamental peony and oil-utilized peony according to different purpose. *Paeonia suffruticosa* Andr. is the representative of ornamental peony. Its flowers are large and luxurious and its petals can also be supplemented as food with more than 1500 years of cultivation history (Bai et al., 2021). It is well known that its root is the raw material of Moutan Cortex (Mudanpi, Dan Pi), traditional Chinese medicine (Li et al., 2009; Liu et al., 2017). Kim et al. (2014) demonstrated peony root extract had neuroprotective effects on Parkinson's disease and might be helpful to prevent or treat Parkinson's disease. The oil content of peony seeds (*Paeonia ostii*) is as high as 27% (Li, et al., 2015). The seed oil contains high level unsaturated fatty acids, including α-linolenic acid, linoleic acid and oleic acid, of which the content of α-linolenic acid exceeds 40% (Qiao et al., 2020). α-Linolenic acid is often used as a dietary supplement which reduces the risk of inflammation, diabetes and hypertension (Kaur, et al., 2014, Sergeant et al., 2016). Hence, peony seed oil was approved as a new food resource by the Ministry of Health of the people's Republic of China in 2011 (Chang, et al., 2020). Although peony root in a broad sense has medicinal value as long as paeonol exceeds 1.2% in Chinese Pharmacopoeia (2015), the large-scale utilization of *P. ostii* root (PR) still lacks data support. It has been reported that peony leaves (*P.*'Hexie') is also rich in monoterpene glycosides, phenols and flavonoids, which has strong antioxidant capacity (Tong, et al., 2021). However, almost all peony leaves (PL), including ornamental and oil peony, are discarded as by-products. The phenolic compounds from different sources may play specific role in health by regulating metabolism and cell proliferation, especially phenolics from by-products improve economic benefits (Jiang et al., 2021, Yang, et al., 2020). Therefore, a feasible extraction process is being expected to utilize of these non-medicinal PR and abandoned PL.

The active ingredients of plant natural products are mostly intracellular products, and the cells need to be broken during extraction. Traditional extraction processes cannot achieve the ideal breaking effect, which directly leads to the low extraction rate and waste of resources. With the in-depth research on natural products, ultrasonic-assisted extraction (UAE) and homogenization-assisted extraction (HAE) have replaced traditional processes such as impregnation, hot water diffusion and reflux extraction, and have become common processes for plant extraction. It is generally believed that cavitation, thermal and mechanical effects are the main theoretical basis in ultrasonic extraction. They accelerate the release of active compounds into the solvent by destroying the structure of plant cell walls and cell membranes, so as to improve the extraction rate, shorten the extraction time and achieve high efficiency (Shirsath et al., 2012). For instance, phenolic compounds from turkish propolis were extracted by conventional solvent extraction methods and

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UAE, respectively. The results showed that the extraction rate of chrysin in phenolics extracted increased by 46.73% by UAE optimization (Bakkaloglu et al., 2021). High-speed homogenization is an economic and efficient mechanical crushing process, which can be used for the dispersion of biological tissues. Mechanical shear force can destroy the cell wall and fibrillate, resulting in the decrease of crystallinity and particle size and the increase of porosity (Madison, et al., 2017). Guo et al reported that the extraction yield of pomelo peel pectin was 209 ± 2 g/kg by HAE, which was higher than that by traditional thermal extraction (175 ± 6 g/kg). Moreover, the apparent viscosity of the former was significantly higher than that of the latter (Guo et al., 2017). Additionally, compared with UAE or HAE alone, the lipid extraction rate from microalgae by the combination of UAE+HAE was increased by 8.1 times and 5.3 times, respectively (Park et al., 2015). Although ultrasound and high-speed homogenization work on different principles, both of them can accelerate the rupture of cells and quickly release the effective substances, their combination maybe improve the extraction rate of active ingredients from peony by-products to a greater extent.

Recently, the research and development of oil peony has entered a blossom period, however, due to the lack of extraction process of active ingredients from peony by-products, its comprehensive utilization is limited. In this study, the extraction process of phenolic compounds in PR and PL was deeply explored. The flavonoids content (TFC) is taken as the response value to characterize the release efficiency of phenolic ingredients in the samples. By single factor and response surface experiments, the optimum process of UAE (UAEo) in PR and PL was determined. Then, the effects of single extraction (UAEo, HAE) and combined extraction (UAEo + HAE, UHAEo, HAE + UAEo, HUAEo) on the PR- TFC/PL-TFC were compared. These results will provide feasible process for the phenolic extraction from oil peony by-products.

2 Materials and methods

2.1 Materials and reagents

The PR and PL (*P. ostii*) were collected in August 2020 at the plantation of Nanolattix Biotech Corporation in Changzhi City, Shanxi Province, China. The materials were rinsed with water for 2-3 times, dried to constant weight at 60°C, ground with a small crusher, screened through a 50 mm sieves, then the powder were stored at – 4°C for standby.

Rutin, gallic acid, sodium chloride, aluminium nitrate, sodium nitrite, ethanol, sodium carbonate, sodium hydroxide, Folin-Ciocalteu and all reagents (analytic-grade) were purchased from Macklin Biochemical Co. Ltd. (Shanghai, China).

2.2 The UAE experiment design

Single factor experiment: The effects of ultrasonic time (20, 30, 40, 50, 60 min), ultrasonic temperature (20, 40, 60, 80°C), ultrasonic power (150, 200, 250, 300, 350 W), solid-liquid ratio (1:5, 1:10, 1:15, 1:20 g/mL) and ethanol concentration (0, 20, 40, 60, 80, 100%, v : v) on the TFC in PR and PL were investigated, respectively. The basic process was ultrasonic time of 30 min, ultrasonic temperature of 60°C, ultrasonic power of 250 W, solid-liquid ratio of 1:10 and ethanol concentration of 60% (v : v).

Response surface methodology (RSM): Based on the single factor experiment results, ultrasonic time (A), solid-liquid ratio (B) and ultrasonic power (C) were selected as three factors and three levels to optimize the experiment (Table 1).

2.3 The HAE experiment design

On the basis of the optimum process of UAE (UAEo), the measured samples were homogenized by high-speed homogenizer at 3000 rpm for 0, 0.5, 1, 3 and 5 min to explore and compare

Table 1. The experiment results of RSM.

No.	Factor			PR-TFC (mg Rutin/g dw)	PL-TFC (mg Rutin/g dw)
	A	B	C		
1	60	1:10	250	30.85 ± 0.96	24.71 ± 0.09
2	40	1:10	200	30.55 ± 0.62	21.66 ± 0.51
3	60	1:15	300	29.35 ± 0.37	22.31 ± 1.09
4	60	1:15	200	28.90 ± 1.08	20.63 ± 0.33
5	60	1:10	250	31.10 ± 0.26	24.99 ± 0.72
6	80	1:5	250	33.83 ± 0.49	23.06 ± 0.64
7	80	1:10	200	31.68 ± 1.05	24.41 ± 0.85
8	60	1:5	300	32.02 ± 0.63	22.58 ± 0.37
9	60	1:10	250	31.61 ± 0.59	25.38 ± 0.07
10	40	1:10	300	29.59 ± 1.09	22.59 ± 1.05
11	80	1:15	250	27.11 ± 0.34	20.46 ± 0.59
12	60	1:10	250	30.98 ± 1.12	25.85 ± 0.78
13	60	1:5	200	32.28 ± 0.88	23.68 ± 0.55
14	60	1:10	250	31.16 ± 0.71	24.76 ± 0.29
15	80	1:10	300	30.74 ± 1.08	23.68 ± 0.65
16	40	1:5	250	28.77 ± 0.92	20.70 ± 0.97
17	40	1:15	250	27.95 ± 0.85	18.68 ± 0.51

A, Ultrasonic time (min); B, Solid-liquid ratio (g/mL); C, ultrasonic power (W).

the effects of UAEo, HAE, UHAEo (UAEo + HAE) and HUAEo (HAE + UAEo) on the TFC and total phenolic content (TPC) in PR and PL (Table 2).

2.4 Determination of TFC

According to Yang et al. (2019), the TFC was determined by classical Aluminium salt colorimentry method. The absorbance value of mixture solution was measured at the wavelength of 510 nm, and the TFC in the sample was calculated by the standard curve: Y = 13.34X + 0.0011, R = 0.9996, expressed as mg Rutin/g dry weight (dw).

2.5 Determination of TPC

According to Yang et al. (2019), the TPC was determined by Folin-Ciocalteu method. The absorbance value of mixture solution was determined at the wavelength of 760 nm, and the TPC in the sample was calculated by the standard curve: Y = 1.80X + 0.006, R = 0.998, expressed as mg GAE/g dw.

2.6 Statistical analysis

Each experiment was repeated 3 times, expressed as mean ± standard deviation. All experiment data were analyzed by IBM SPSS statistics 22.0 ($P<0.05$, significant difference). RSM were analyzed using Design-Expert 8.0 software ($P<0.05$, significant difference, marked *, $P<0.01$, very significant difference, marked **). All figures were drawn with Origin software (2018).

3 Results and discussion

3.1 The UAE analysis

The single factor experiment

As shown in Figure 1, five single factor experiments at 4 or 5 levels were investigated under the basic conditions. Firstly, with the extension of ultrasonic time, and the PR-TFC and PL-TFC at 60 min reached the maximum values, 36.16 ± 1.63 mg/g and 27.82 ± 1.07 mg/g, respectively (Figure 1a). The degree of cell disruption is positive correlation with ultrasonic time, which reproduces in some studies (Anticona et al., 2021, Baite et al., 2021). To explore the impact of the interaction between ultrasonic time and other factors on the TFC, 60 min was selected as the central point, and 40 and 80 minutes as the other two levels of the first factor in RSM. Secondly, with the increase of solid-liquid ratio, the TFC decreased continuously. When the solid-liquid ratio was 1:5, the maximum of PR-/PL-TFC were 32.66 ± 0.54 mg/g and 25.88 ± 0.72 mg/g, respectively (Figure 1b). This may be due to the dilution of extraction solution with the increase of solvent, which affects the yield (Liao et al., 2016). However, if the solid-liquid ratio was lower than 1:5, solution concentration is too high to completely soak the material. Therefore, the solid-liquid ratio of 1:5, 1:10 and 1:15 was used as the second factor in RSM. Finally, the PR-TFC and PL-TFC increased first and then decreased in the three single factor experiments of ultrasonic temperature, ultrasonic power and ethanol concentration, and reached the maximum at 60°C, 250 W and 60%, respectively (Figure 1c,1d,1e). If the extraction temperature is too high (> 80°C), it may lead to the structural change and low bioactivity of active compounds (Zhang & Lee, 2021).Both ultrasonic temperature and power have a significant influence on the TFC, and the ultrasonic process was accompanied by heat release, resulting in temperature instability. Therefore, ultrasonic power was selected as the third factor in RSM. When the ethanol concentration was 60% (v:v), the TFC reached the maximum, 33.46 ± 0.71 mg/g (PR) and 22.12 ± 0.69 mg/g (PL). It could be seen that ethanol concentration had a significant impact on the TFC in peony, but higher ethanol concentration directly affect the flavonoids solubility.Considering the safety and price of solvents, and most studies show that 60% ethanol is the best extraction solvent for phenolic compounds (Akbari et al., 2019, Lim et al., 2019), 60% ethanol was selected as one of basic conditions. In short, ultrasonic time, solid-liquid ratio ultrasonic power and were selected three factors in RSM.

Response surface methodology (RSM)

According to the above results, the three factors and three levels of RSM was carried out (Table 1). Taking ultrasonic time (A), solid-liquid ratio (B) and ultrasonic power (C) as independent variables and the TFC as the response value Y, the quadratic multiple regression equations were fitted with Design-Expert software. They were PR-TFC (mg Rutin/g dw) = $31.14 + 0.81 \times A - 1.70 \times B - 0.21 \times C - 1.47 \times AB + 0.00475 \times AC + 0.18 \times BC - 0.86 \times A^2 - 0.86 \times B^2 + 0.37 \times C^2$ and PL-TFC (mg Rutin/g dw) = $25.14 + 1.12 \times A - 0.99 \times B - 0.027 \times C - 0.14 \times AB - 0.67 \times AC + 0.69 \times BC - 1.69 \times A^2 - 2.72 \times B^2 - 0.11 \times C^2$.

The variance analysis of regression equation for PR was shown in Table 3. The data showed that there was a significant multiple regression relationship between dependent variables and independent variables in the regression equation model. The model reached a very significant difference ($P < 0.01$). From the F values of the three influencing factors, it could be concluded that the order of the influence of each factor on the PR-TFC was solid-liquid ratio > ultrasonic time > ultrasonic power, and the first two factors had extremely significant effects on the TFC ($P < 0.01$). There was a reciprocal action between ultrasonic time (A) and solid-liquid ratio (B) ($P < 0.01$). Furthermore, the quadratic term of A^2 and B^2 had significant

Table 2. The HAE experiment design.

Name	Extraction process		HAE Time (min)				
	1st step	2nd step					
HAE	HAE	--	0	0.5	1	3	5
UHAEo	UAEo	HAE	0	0.5	1	3	5
HUAEo	HAE	UAEo	0	0.5	1	3	5

HAE, Homogenization-Assisted Extraction; UHAEo, Ultrasound-Homogenization-Assisted Extraction; HUAEo, Homogenization-Ultrasound-Assisted Extraction; UAEo: 80 min, 1:5 g/mL, 250 W (PR), 60 min, 1:10 g/mL, 250 W (PL).

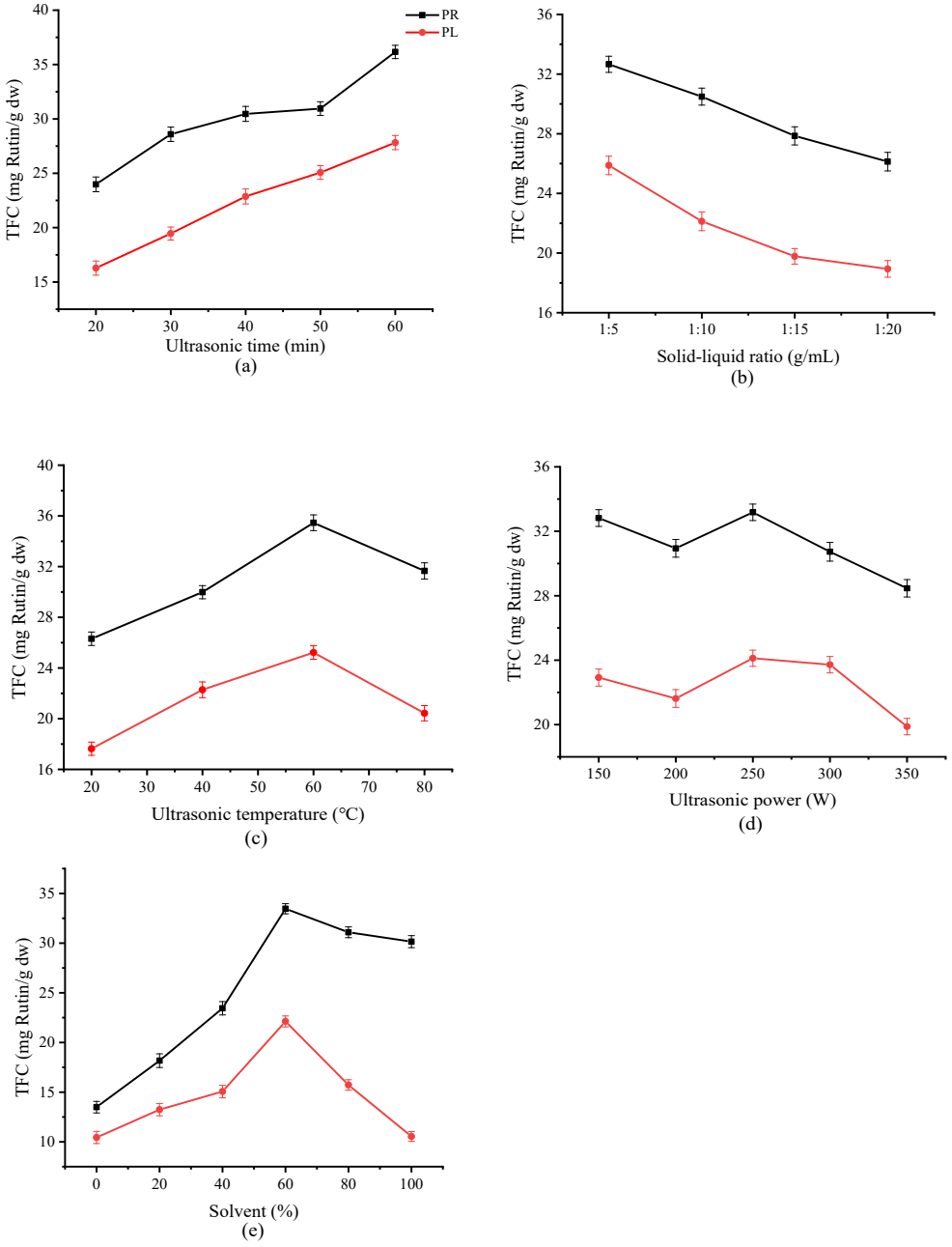

Figure 1. Effects of ultrasonic time (a), solid-liquid ratio (b), ultrasonic temperature (c), ultrasonic power (d) and solvent (e) on TFC. PR, peony roots; PL, peony leaves.

effects on the TFC ($P < 0.01$). The lack of fit was not significant ($P = 0.0733 > 0.05$), indicating good adequacy of the model. The $R_{Adj}^2 = 0.9197$ indicated that the model could reflect 91% of the data, and well fit with the mathematical model. The similar results of PL-TFC were shown in Table 4. The difference was that there was also a reciprocal action between solid-liquid ratio (B) and ultrasonic power (C) besides AB. Both the PR-model and PL-model could analyze and predict the TFC value.

According to the above regression equations, the response surfaces were displayed in Figure 2 and 3, and the effects of various factors on the TFC was investigated. The PR-TFC and PL-TFC increased first and then decreased under the interaction of different factors, and the steepness of the curve reflected the influence of different factors on the response value. For PR, with the change of ultrasonic time and solid-liquid showed a "bell jar" shape and the surface was steep (Figure 2a), indicating that AB had reciprocal action, which was also consistent with the analysis of variance (Table 3). As shown in Figure 2b and 2c, their surfaces were flat, suggesting that there is no interaction between ultrasonic power and the other two factors. Similarly, the curved surfaces were steep for PL (Figure 3a and 3c), indicating that the solid-liquid ratio had significant interaction with ultrasonic time or power. However, there was no reciprocal action between ultrasonic time (A) and ultrasonic power (C) due to a gentle slope in Figure 3b, which was consistent with the analysis of variance (Table 4).

Table 3. Analysis of variance of RSM for the PR-TFC.

Source	Sum of Squares	df	Mean Square	F-value	P-value	Significant
Model	44.48	9	4.94	21.36	0.0003	**
A-Ultrasonic time	5.29	1	5.29	22.85	0.0020	**
B-Solid-liquid ratio	23.09	1	23.09	99.79	<0.0001	**
C-Ultrasonic power	0.36	1	0.36	1.58	0.2496	
AB	8.69	1	8.69	37.57	0.0005	**
AC	0.0009025	1	0.0009025	0.0003900	0.9848	
BC	0.12	1	0.12	0.54	0.4875	
A^2	3.13	1	3.13	13.52	0.0079	**
B^2	3.13	1	3.13	13.54	0.0079	**
C^2	0.57	1	0.57	2.44	0.1620	
Residual	1.62	7	0.23			
Lack of Fit	1.29	3	0.43	5.16	0.0733	
Pure Error	0.33	4	0.083			
Cor Total	46.10	16				

df, degree of freedom; **, very significant difference ($P < 0.01$).

Table 4. Analysis of variance of RSM for the PL-TFC.

Source	Sum of Squares	df	Mean Square	F-value	P-value	Significant
Model	67.70	9	7.52	37.14	<0.0001	**
A- Ultrasonic time	10.08	1	10.08	49.78	0.0002	**
B- Solid-liquid ratio	7.88	1	7.88	38.96	0.0004	**
C- Ultrasonic power	0.00577	1	0.00561	0.028	0.8706	
AB	0.08	1	0.08	0.39	0.0179	*
AC	1.77	1	1.77	8.77	0.5498	
BC	1.92	1	1.91	9.48	0.0211	*
A^2	12.00	1	12.00	59.34	0.0001	**
B^2	31.26	1	31.26	154.53	<0.0001	**
C^2	0.054	1	0.054	0.26	0.6226	
Residual	1.42	7	0.20			
Lack of Fit	0.50	3	0.17	0.74	0.5825	
Pure Error	0.91	4	0.23			
Cor Total	69.12	16				

df, degree of freedom; *, significant difference ($P < 0.05$); **, very significant difference ($P < 0.01$).

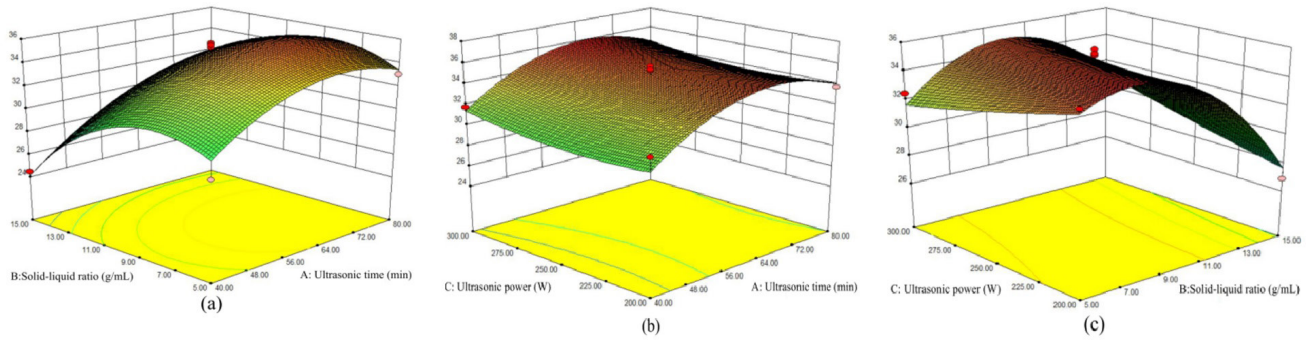

Figure 2. Response surface methodology of various factors on PR-TFC, Solid-liquid ratio and Ultrasonic time (a), Ultrasonic power and Ultrasonic time (b),Ultrasonic temperature and Ultrasonic time (c). PR, peony roots.

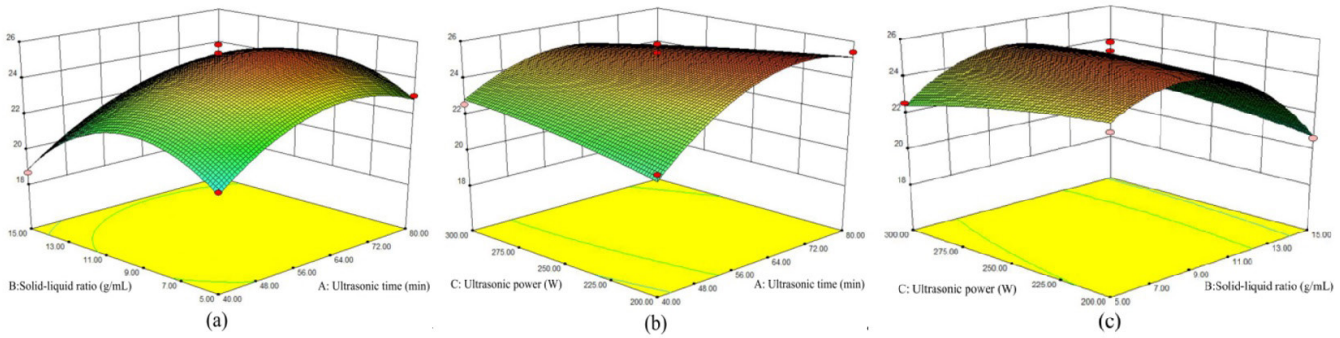

Figure 3. Response surface methodology of various factors on PL-TFC, Solid-liquid ratio and Ultrasonic time (a), Ultrasonic power and Ultrasonic time (b),Ultrasonic temperature and Ultrasonic time (c). PL, peony leaves.

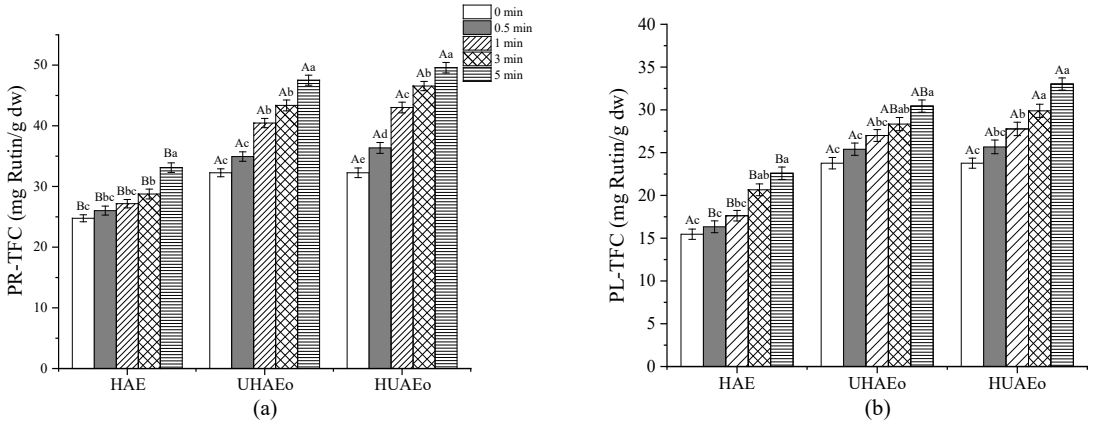

Figure 4. Effects of HAE, UHAEo and HUAEo on PR-TFC and PL-TFC. Different uppercase letters (A-B) indicate significant differences in TFC among different extraction processes of PR and PL ($P < 0.05$); Different lowercase letters (a-e) indicate significant differences in TFC among 0, 0.5, 1, 3, 5 min of PR and PL ($P < 0.05$); PR, peony roots; PL, peony leaves. HAE, Homogenization-Assisted Extraction; UHAEo, Ultrasound-Homogenization-Assisted Extraction; HUAEo, Homogenization-Ultrasound-Assisted Extraction.

Based on the theoretical optimization of RSM and practicability, the PR-UAEo was ultrasonic time of 80 min, solid-liquid ratio of 1:5 g/mL, ultrasonic power of 250 W and the practical maximum of PR-TFC was 33.83 ± 0.49 mg Rutin/g dw, and the PL-UAEo was ultrasonic time of 60 min, solid-liquid ratio of 1:10 g/mL, ultrasonic power of 250 W and the practical maximum of PL-TFC was 25.85 ± 0.78 mg Rutin/g dw. These values were close to the theoretical prediction, 33.40 mg Rutin/g dw (PR) and 25.14 mg Rutin/g dw (PL) by RSM.

3.2 The HAE and combined extraction

The homogenization technology was introduced on the basis of the UAEo to investigate the effects of single extraction (HAE and UAEo), and combined extraction (HUAEo and UHAEo) on the phenolic compounds release with TPC and TFC as response values. Take PR as an example (Figure 4a), with the extension of homogenization time, the TFC increased from 24.76 ± 0.16 mg Rutin/g dw to 33.11 ± 0.21 mg Rutin/g dw, an increase of 33.7% in HAE group. In the combined extraction, the TFC increased from 33.83 ± 0.26 mg Rutin/g dw to 49.58 ± 0.25 mg Rutin/g dw (46.6%) by HUAEo, and 47.52 ± 0.21 mg Rutin/g dw (40.5%) by UHAEo. Similarly, the extension of homogenization time had positive impacts on the PL-TFC in the three processes (Figure 4b). The effects of HUAEo and UHAEo on the PR-TFC/PL-TFC were not statistically different ($P > 0.05$). This may be due to the cavitation, thermal and mechanical effects, which increases mass transfer and significant cell wall destroy (Ardekani et al., 2017, Ghitescu et al., 2015). On the other hand, high-speed homogenization breaks the cells to a greater extent and releases the active compounds into the solvent through mechanical shear, stirring, fluid cutting and crushing (Xia et al., 2017). Therefore, the combined extraction (HUAEo and UHAEo) has synergistic effect, which is more conducive to the extraction of active ingredients. Our previous study on the extraction of polyphenols from coconut mesocarp also showed that the combined extraction improved the yield of total phenols and total flavonoids, especially UHAEo (Yang et al., 2021).

Interestingly, with the extension of homogenization time, the PR-TPC/PL-TPC increased first and then decreased, and at 3 min reached the maximum, 77.84 ± 0.52 mg GAE/g dw and 146.62 ± 2.77 mg GAE/g dw, respectively (Figure 5). This high-speed shear force leads to cell disruption and the release of various active components, including polyphenol oxidase (PPO) in the cytoplasm. The PPO combines with phenols in the solution, resulting in the decomposition of polyphenols (Yang et al., 2021). Coincidentally, the PPO activity and TFC of coconut mesocarp

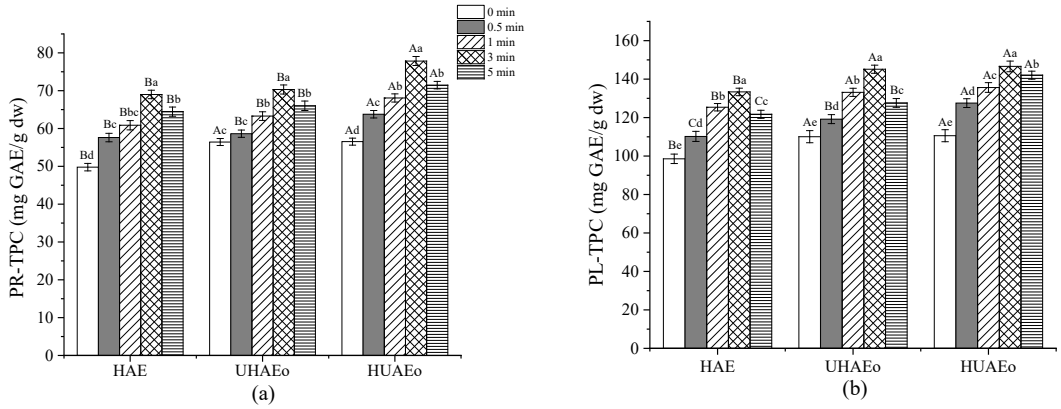

Figure 5. Effects of HAE, UHAEo and HUAEo on PR-TPC and PL-TPC. Different uppercase letters (A-C) indicate significant differences in TFC among different extraction processes of PR and PL ($P < 0.05$); Different lowercase letters (a-e) indicate significant differences in TFC among 0, 0.5, 1, 3, 5 min of PR and PL ($P < 0.05$); PR, peony roots; PL, peony leaves. HAE, Homogenization-Assisted Extraction; UHAEo, Ultrasound-Homogenization-Assisted Extraction; HUAEo. Homogenization-Ultrasound-Assisted Extraction.

gradually increased in HUAE and UHAE during 10 min, however, the TPC reached the maximum at 5 min (Yang et al., 2021). In addition, the PR-TFC was significantly higher than PL-TFC, while the PR-TPC was the opposite. In principle, the TPC and the TFC should be positively correlated in that flavonoids are a subgroup of polyphenols. However, the TPC of the sample was not only related to the concentration of phenols, but also directly related to the electron donating ability of phenols (Tai et al., 2017). The determination of TPC with gallic acid as the standard was based on the SET mechanism (Lafarga et al., 2018), However, the determination of TFC with rutin as the standard is based on the color development mechanism of aluminum salt. The latter is greatly affected by the catechol structure of compounds, thus the correlation of TPC and the TFC is low (Chen et al., 2016). Surprisingly, the PL-TPC was not only almost twice as high as the PR-TPC, but also can be ranked fifth in 54 high polyphenol plants (Chen et al., 2020). This shows that the leaves of oil peony are potential high polyphenol materials, and the leaf extract may be used as a functional food additive. In a word, the combined extraction had obvious advantages, and there is no significant difference between HUAEo and UHAEo in this study.

4 Conclusion

In this study, the UAE was selected to extract phenolic compounds from PR and PL. The single extraction process was further optimized by RSM of three factors and three levels. The PR-UAEo was ultrasonic time of 80 min, solid-liquid ratio of 1:5 g/mL, ultrasonic power of 250 W, and the PR-TFC was 33.83 mg Rutin/g dw. The PL-UAEo was ultrasonic time of 60 min, solid-liquid ratio of 1:10 g/mL, ultrasonic power of 250 W and the PL-TFC was 25.85 mg Rutin/g dw. In the combined extraction, the highest PR-TFC(PL-TFC) by HUAEo at 5 min was 49.58 ± 0.25 mg/g with an increase of 46.6% (33.02 ± 0.04 mg/g with an increase of 27.7%), while the highest PR-TPC(PL-TPC) by the HUAEo at 3 min was 77.84 ± 0.52 mg GAE/g dw (146.62 ± 2.77 mg GAE/g dw). The decrease of polyphenols after 3 min might be due to the decomposition of phenolics. However, there was no significant difference between the HUAEo and UHAEo. The combined extraction (HUAEo and UHAEo) is more conducive to the release of phenolic compounds in peony by-product.

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