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Optimization of polysaccharides from *Lycium ruthenicum* fruit using RSM and its anti-oxidant activity

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ABSTRACT

Dynamic microwave-assisted extraction (DMAE) technique was employed for the extraction of polysaccharides from *Lycium ruthenicum* (LRP). The extracting parameters were optimized by using three-variable-three-level Box–Behnken design and response surface methodology (RSM) based on the single-factor experiments. RSM analysis indicated good correspondence between experimental and predicted values. The optimum extraction parameters for the yield of polysaccharide were ratio of water to raw material 31.5 mL/g, extracting time 25.8 min and microwave power 544.0 W. Polysaccharide was analyzed by chemical methods and Fourier-transform infrared (FT-IR). The antioxidant activities of LRP were investigated including scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide and free radicals of superoxide anion *in vitro*. The results of antioxidant activity exhibited LRP had the potential to be explored as novel natural antioxidant for using in functional foods or medicine.

1. Introduction

Lycium ruthenicum Murr. is a unique nutritional food, which widely distributes in salinized desert of Northwest China. Its special physiological characteristics of drought-resistance and salt-resistance make it an ideal plant for preventing soil desertification and alleviating the degree of soil salinity–alkalinity, which are very important for the ecosystem and agriculture in the remote area [1,2]. In addition to that, *L. ruthenicum* has been recorded in Tibetan medical classic "Jing Zhu Ben Cao" as a traditional valuable herb. Its ripe fruits had been used for treatment of heart disease, abnormal menstruation and menopause. *Lycium* polysaccharide, the main bioactive component of *Lycium* plant, has a large variety of bioactivities, such as enhancing body's immunity, anti-tumor, protecting hepatic function, anti-fatigue, anti-aging and hypoglycemic effects [3–5]. *L. ruthenicum* contains many functional components such as pigments, essential oils and polysaccharides. The antioxidant activity and ability to enhance immunity of pigments have been demonstrated [6], and the composition of pigments and essential oils in *L. ruthenicum* has been well documented [2,7]. Crude *L. ruthenicum* polysaccharides (CLRP) isolated from the fruits of *L. ruthenicum* were shown to have hypoglycemic and anti-fatigue effects [4,8]. And Chemical analysis showed that CLRP was composed of 68.7% neutral sugar and 24.5% acid sugar; monosaccharide composition test indicated that CLRP was composed of arabinose (40.7%), galacturonic acid (26.4%), galactose (18.9%), xylose (5.1%), rhamnose (4.9%), glucose (2.7%), and mannose (1.3%) [9]. Up to now most of the researches were focused on the structural characteristics and genetic diversity [10–12], no comprehensive studies have been conducted to explore the efficient extraction method and antioxidant activity of *L. ruthenicum* polysaccharides. This has restricted the further research and application of *L. ruthenicum*.

Whereas, there have been only few reports on LRP extraction and its antioxidant activity *in vitro*. One of the reasons is the lack of high efficient extraction technology of polysaccharides from *L. ruthenicum*. Conventional hot-water extraction (CHE) is the most commonly used method to extract polysaccharides. Although CHE is simple and safe, high temperature and long extraction time of CHE lead to the degradation of polysaccharides and the decrease of the pharmacological activity of polysaccharides. In recent years, dynamic microwave-assisted extraction (DMAE) has been successfully applied for extraction of numerous biologically active compounds from a wide variety of natural resources [13–15]. This technique consists in the penetration of microwave energy into the material structure, which produces a volumetrically distributed heat source due to molecular friction resulting from dipolar rotation of polar solvents and from the conductive migration of dissolved ions, accelerating the mass transfer of target compounds. In general,

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the compounds are extracted more selectively and quicker by this technique, with similar or better yields in comparison with conventional extraction processes, using less energy and solvent volume, thus being more environmentally friend [16–18]. However, only few researches report the use of microwave-based techniques for extraction of polysaccharides from *L. ruthenicum*. In order to obtain a high yield of LRP, the extraction process must be optimized by mathematics models.

In statistics, response surface methodology (RSM) explores the relationships between several explanatory variables and one or more response variables [19], and it has successfully been applied in order to optimize the conditions in food and pharmaceutical research [20,21]. The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response, and the experiments will be more easily arranged and interpreted using this efficient design [22].

As is known to all, oxidation is imperative to many organisms for the energy production. However, uncontrolled production of oxygen-derived free radicals can damage cellular components such as DNA and lipids [23,24], which brings about some diseases such as atherosclerosis, senescence, and cancer and rheumatoid arthritis [25]. It has been reported that many plant polysaccharides have strong antioxidant abilities and should be paid more attention to exploring them as novel potential antioxidants [26–28].

The objective of this research was to investigate the significant variables (ratio of water to raw material, extraction time and microwave power) and further to optimize the process for extraction of polysaccharides from *L. ruthenicum* fruit using RSM and evaluate its antioxidant activity *in vitro*.

2. Materials and methods

2.1. Materials and chemicals

The fruits of *L. ruthenicum* were collected from Qaidam Basin (Latitude. 36.41° N, Longitude. 96.24° E, Altitude. 2770 m) in August 2010 and dried in the shade at room temperature. The dried fruit samples were ground and passed through 80 mesh screen. D-(+)-Glucose (99.5%), DPPH, ascorbic acid, 2,6-di-tert-butyl-4-methylphenol (BHT), m-hydroxydiphenyl, bovine serum albumin (BSA) and phenol were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of polysaccharides

The powder of *L. ruthenicum* was double extracted with petroleum ether at 85 °C for 3 h each time to remove lipids and some colored materials under reflux in the Soxhlet set. After being vacuum dried at 65 °C for 10 h, the defatted powder was mixed with ultrapure water (18.25 MΩ/cm^2) in a glass flask and then placed in a MG08S-2B microwave extraction apparatus (Nanjing Huiyan Microwave System Engineering Co. Nanjing, China). After the extraction with microwave treatment, the extracted slurry was centrifuged at 5000 rpm/min for 15 min to collect the supernatant, and the insoluble residue was treated again for 2 times as mentioned above. The supernatant was incorporated and concentrated to one-fifth of initial volume using a rotary evaporator (N1000D rotary evaporation, Shanghai Huixi Precision Instrument Co., Shanghai, China) at 56 °C under vacuum. The resulting solution was mixed with five volumes of dehydrated ethanol (ethanol final concentration, 80%) and kept about 12 h at 4 °C. Then the solution was centrifuged at 5000 rpm/min for 15 min, washed four times with dehydrated ethanol, and the precipitate was collected and then redissolved in ultrapure water. The aqueous solution was treated with Sevag reagent to remove proteins, dialyzed against ultrapure water for 48 h, concentrated under reduced pressure, and finally lyophilized [10]. A black crude polysaccharide, CLRP, was then obtained.

2.3. Decoloration of crude polysaccharides

The black crude polysaccharide (CLRP) was dissolved in ultrapure water at 45 °C with continuous stirring and the solution was adjusted to pH 8.8 with $NH_3{\cdot}H_2O$. H_2O_2 (30%) was then added drop-wise until the color faded. After stirring for another 4 h, the color turned primrose yellow. The solution was neutralized with 1 mol/L HCl, followed by dialysis against ultrapure water and lyophilization [10]. The decolored polysaccharide was obtained and weighted with a balance (XS 105, Mettler-Toledo International Inc., Germany).

The LRP content was measured by phenol–sulfuric acid method using D-glucose as a standard [29]. And the method used with slight modification.

2.4. Experimental design and statistical analysis

On the basis of single-factor experimentation, preliminary proper ranges of ratio of water to raw material, extraction time, microwave power and extraction times were determined. A three-variable-three-level Box–Behnken design (software Design-Expert 7.0.1.0, Stat-Ease, Inc., Minneapolis, USA) was used to determine the best combination of extraction variables for the production of LRP. Based on single-factor experiments, the key variables were determined to be ratio of water to raw material (mL/g, X_1), extraction time (min, X_2) and microwave power (W, X_3). Table 1 details the BBD matrix and response values carried out for developing the model. The whole design consisted of 17 experimental points carried out in random order. Five replicates (treatments 13–17) at the center of the design were used to allow for estimation of a pure error sum of squares.

Regression analysis was performed for the experimental data and was fitted into an empirical second-order polynomial model, as shown in the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii}X_i^2 \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j$$

Here, β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients of variables for intercept, linear, quadratic, and interaction terms, respectively, and X_i and X_j are independent variables ($i \neq j$). The coefficients of the second polynomial model and the responses obtained from each set of experimental design were subjected to multiple nonlinear regressions using Design-Expert software. The fitness of the polynomial model equation is expressed by the coefficient of determination R^2, and its statistical significance was confirmed by *F*-test at a probability (*P*) of 0.001, 0.01, or 0.05. The significances of the regression coefficients were also confirmed by *F*-test.

2.5. Chemical analysis of LRP

LRP was obtained by the finally optimum extraction conditions. Total carbohydrate content of LRP was measured by phenol–sulfuric acid method [29]. Protein content was determined by the method of Bradford [30]. The content of uronic acid was determined by m-hydroxydiphenyl [31].

Table 1
BBD matrix and the response values for extraction yield of LRP.

Standard order	Independent variable			Extraction yield (%)	
	X_1 (ratio of water to raw material, mL/g)	X_2 (extraction time, min)	X_3 (microwave power, W)	Experimental	Predicted
1	−1 (25)	−1 (20)	0 (450)	6.95	6.99
2	+1 (35)	−1 (20)	0 (450)	7.20	7.19
3	−1 (25)	+1 (30)	0 (450)	7.13	7.13
4	+1 (35)	+1 (30)	0 (450)	7.84	7.81
5	−1 (25)	0 (25)	−1 (350)	6.89	6.77
6	+1 (35)	0 (25)	−1 (350)	7.19	7.21
7	−1 (25)	0 (25)	+1 (550)	7.48	7.55
8	+1 (35)	0 (25)	+1 (550)	7.97	7.99
9	0 (30)	−1 (20)	−1 (350)	6.81	6.84
10	0 (30)	+1 (30)	−1 (350)	7.42	7.48
11	0 (30)	−1 (20)	+1 (550)	7.93	7.88
12	0 (30)	+1 (30)	+1 (550)	8.02	8.00
13	0 (30)	0 (25)	0 (450)	8.11	8.05
14	0 (30)	0 (25)	0 (450)	8.18	8.05
15	0 (30)	0 (25)	0 (450)	7.93	8.05
16	0 (30)	0 (25)	0 (450)	8.07	8.05
17	0 (30)	0 (25)	0 (450)	7.96	8.05

2.6. Infrared spectroscopy of LRP

LRP was mixed with spectroscopic grade potassium bromide powder, ground and pressed into a 1 mm pellets for Fourier-transform infrared (FT-IR) measurement. A FT-IR spectrum of the LRP was determined using a Bruker TENSOR 27 FT-IR spectrometer in the frequency range of 4000–400 cm^{-1}.

2.7. Determination of antioxidant activity

The scavenging activity of the DPPH free radical was assayed according to the method of Shimada et al. [32]. Hydroxyl radical scavenging activity was determined by Fenton-type reaction [33,34]. The superoxide anion scavenging activity of various concentrations polysaccharide samples were investigated based on the method of Marklund and Marklund [35]. All these three methods were used with some modifications. The experimental data were subjected to an analysis of variance for a completely random design.

3. Results and discussion

3.1. Effect of different ratio of water to raw material on extraction yield of LRP

To investigate the effect of ratio of water to raw material on extraction yield of LRP, the extraction process was carried out using different ratio of water to raw material: 10, 15, 20, 25, 30, 35 and 40 mL/g, while other extracting parameters were fitted as follows: extracting time 10 min, microwave power 350 W and extracting number two times. As shown in Fig. 1A, when extracting ratio of water to raw material varied from 10 to 40 mL/g, the variance of extraction yield was relatively rapid, and LRP production reached a maximum at 30 mL/g, and then no longer changed. This may be due to the increase of the driving force for the mass transfer of the polysaccharides. Therefore, 30 mL/g was selected as the center point of extracting ratio of water to raw material in the RSM experiments.

3.2. Effect of different time on extraction yield of LRP

Extraction time is another factor that would influence the extraction efficiency and selectivity of the fluid. It was reported that a long extraction time favors the production of polysaccharides [36]. On the other hand, excessive lengthening of extraction time may induce the change of polysaccharides molecule structure [37]. In this research, the effect of extraction time on the yield of LRP was investigated using different extracting time (5, 10, 15, 20, 25, 30 and 35 min) as shown in Fig. 1B while other extraction variables were set as follows: ratio of water to raw material 30 mL/g, microwave power 350 W and extracting number two times. The variance of extraction yield was relatively rapid when extraction time varied from 5 to 10 min. The extraction yield of LRP increased slowly with increasing time, and reached a maximum at 25 min, then sparingly decreased as the extraction proceeded. This indicated that 25–30 min was sufficient to obtain the polysaccharides production. Thus, for saving of energy and lowering of cost, 25 min was selected as the center point of extraction time in the RSM experiments.

3.3. Effect of different microwave power on extraction yield of LRP

Fig. 1C listed the effect of microwave power on extraction yield of LRP when other extraction conditions were fixed as follows: ratio of water to raw material of 30 mL/g, extraction time of 25 min, extraction number of two times. The results showed that extraction yield of LRP increased significantly with increasing extraction microwave power, and then decreased when the extraction power was over 550 W. It was well known that the strong thermal effect of extraction microwave power facilitated the disruption of cell walls. A larger yield of polysaccharides occurred with the stronger power at the early period. However, higher microwave power of extraction resulted lower yield of polysaccharides in the studied experimental range and increased of the driving force for the mass transfer of other chemicals. Therefore, 450 W was selected as the center point of extraction microwave power in the RSM experiments as higher microwave power would bring about the energy waste and cost increase and yield reduce for extraction process.

3.4. Effect of different extraction times on extraction yield of LRP

The effect of different extraction times on extraction yield of LRP was shown in Fig. 1D. Extraction was carried out at different times (1–5) of extraction conditions while other extraction parameters were fitted as follows: ratio of water to raw material of 30 mL/g, extraction time of 25 min, and microwave power of 450 W. The results (Fig. 1D) demonstrated that the extraction yield of LRP was improved with an increase in extraction times, and the yield was not obviously increased when the extraction times was more than 3 times. Therefore, a extraction times of 3 times was finally employed in this experiment.

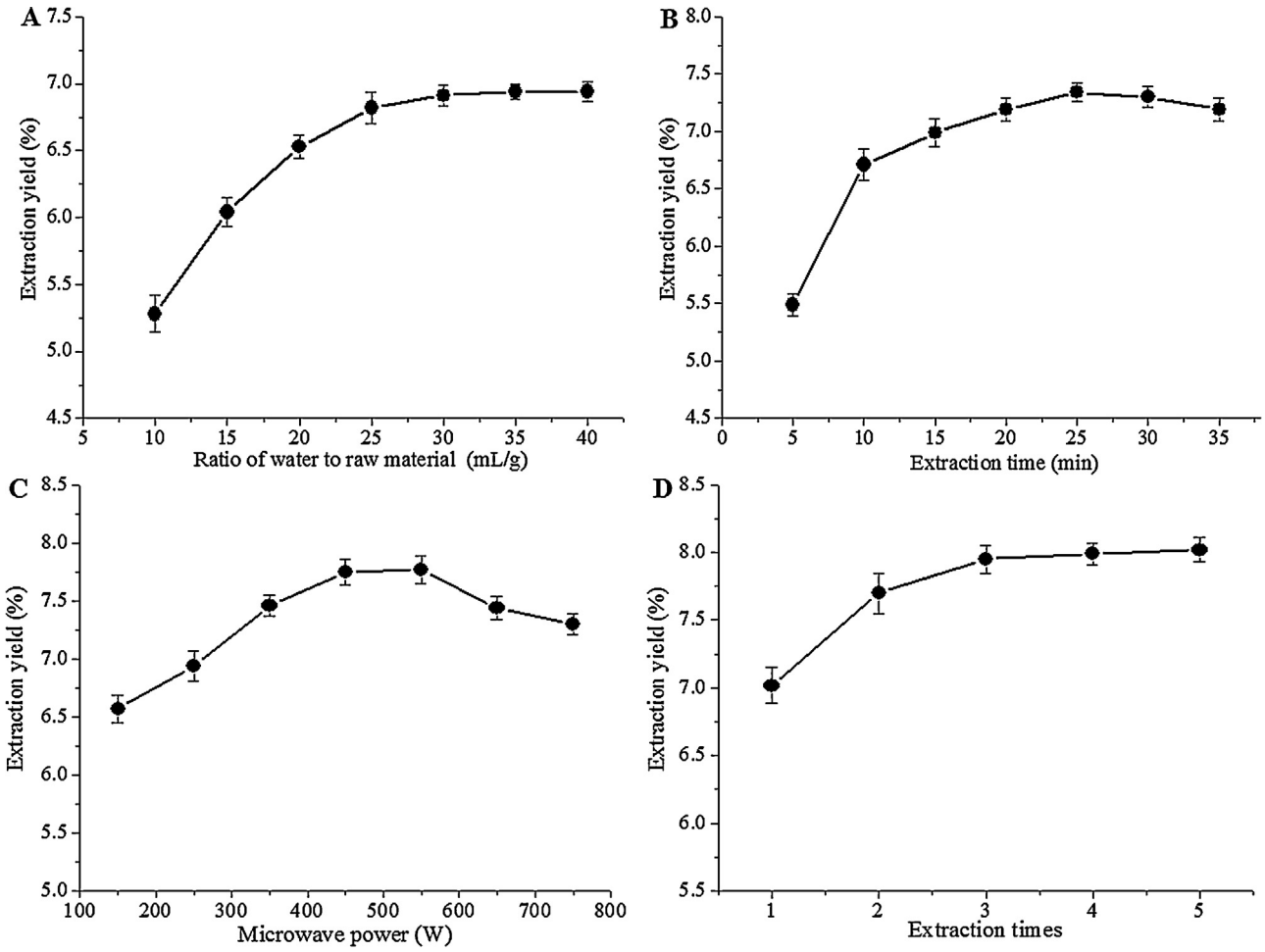

Fig. 1. Effects of (A) ratio of water to raw material, (B) extraction time, (C) microwave power, and (D) extraction times on extraction yield of LRP.

3.5. Response surface optimization of dynamic microwave-assisted extraction condition

Dynamic microwave-assisted extraction parameters were optimized using response surface methodology (RSM). The Box–Behnken design (BBD) was used. The range and center point values of three independent variables were based on the results of the single-factor experiments. The response values (extraction yield of LRP) for different experimental combinations are given in Table 1. It can be seen from Table 1 that there is considerable variation in extraction yield depending upon extraction conditions. The regression coefficients of the intercept, linear, quadratic, and interaction terms of the model were calculated using the least square technique. They are presented in Table 2. The *p*-values are used as a tool to check the significance of each coefficient, which also indicate the interaction strength between each independent variable. Smaller the *p*-value is, more significant the corresponding coefficient is. When value of "Prob > F" is less than 0.05, the model terms is significant. So it was evident that all linear parameters (X_1, X_2, X_3) and quadratic parameters (X_1^2, X_2^2, X_3^2) were significant ($p < 0.05$ or $p < 0.01$). And two interaction parameters (X_1 and X_2, X_2 and X_3) were significant ($p < 0.05$). These results indicated that the effects of extraction conditions (ratio of water to raw material, extraction time and microwave power) were all contributing factors to extraction yield of LRP.

The application of RSM offered, based on parameter estimates, an empirical relationship between the response variable and the test variables. By employing multiple regression analysis on the experimental data, the predicted response Y for extraction yield of LRP can be obtained by the following second-order polynomial equation: $Y = 8.05 + 0.22X_1 + 0.19X_2 + 0.39X_3 + 0.12X_1X_2 - 0.13X_2X_3 - 0.47X_1^2 - 0.30X_2^2 - 0.20X_3^2$, where X_1, X_2, and X_3 are in terms of coded factors of the test variables, ratio of water to raw material, extraction time, and microwave power, respectively.

The analysis of variance for the experimental results of the BBD is also shown in Table 2. The Model *F*-value 43.87, implies that model is significant. The quality of the model can be confirmed by the determination coefficients (R^2) and the multiple correlation coefficients (R). The closer the values of R are to 1, the better the correlation between experimental and predicted values [38]. In this experiment, the coefficient of determination (R^2) of the model was 0.9826, which indicated good agreement between the experimental and predicted values of extraction yield of LRP. Error analysis results indicated that the lack of fit was insignificant ($p > 0.05$). The *F*-value, 0.64, implies that the lack of fit is not significant relative to the pure error. There is a 62.97% chance that a lack of fit *F*-value this large could occur due to noise. So it indicates that the model equation is adequate for predicting extraction yield of LRP under any combination of values of the variables. The coefficient of variation (C.V.) is below 5%. This indicates that the model was reproducible [39]. The model's predicted residual sum of squares (PRESS), a measure of how a particular model fits each point in the design, was 0.40. The value of pred R^2 (0.8915) is in reasonable agreement with the adj R^2 (0.9602). The value of adeq precision measures the signal to noise ratio. A ratio greater than 4 is

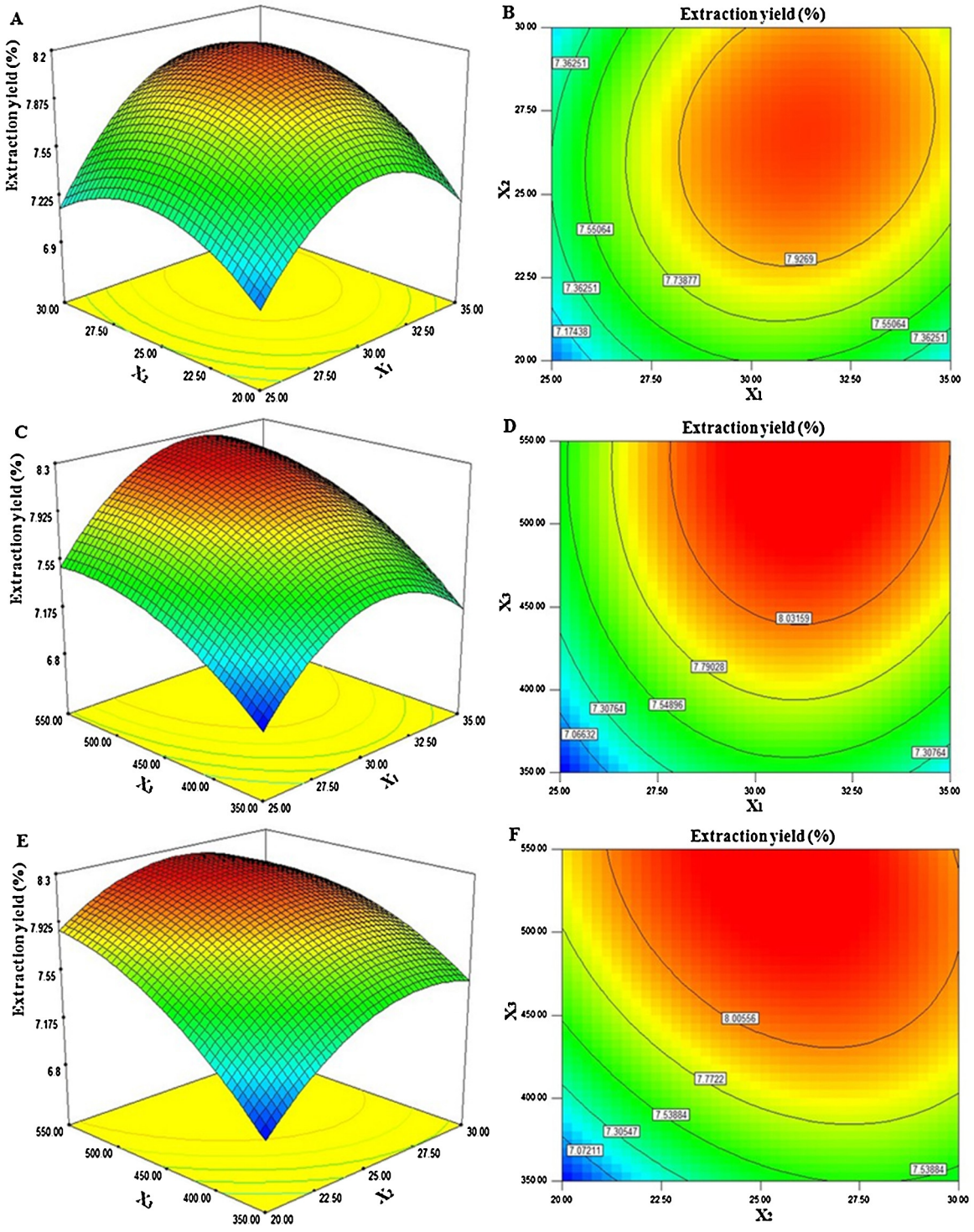

Fig. 2. Response surface plots (A, C, and E) and contour plots (B, D, and F) of extraction yield affected by ratio of water to raw material (X_1), extraction time (X_2), and microwave power (X_3).

desirable. The experimental ratio of 16.688 indicates an adequate signal. In summary, this model can be used to navigate the design space.

The three-dimensional (3D) response surface and two-dimensional (2D) contour plots that are the graphical representations of regression equation obtained from the calculated response surface are indicated in Fig. 2. They provide a means of visualizing the relationship between the responses and experimental levels of each variable and the type of interactions between the two test variables. The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. Circular contour plots indicate that the interactions between the corresponding variables are negligible, while elliptical contour plots indicate that the interactions between the corresponding variables are significant [40]. In the present project, three independent response surface plots and their respective contour plots were generated using Design-Expert as shown in Fig. 2. In Fig. 2, the interactions between

Table 2
Estimated regression coefficients for the quadratic polynomial model and the analysis of variance (ANOVA) for the experimental results.

Parameter	Coefficient estimate	Standard error	Sum of squares	*DF*	Mean square	*F*-value	Prob > *F*
Model			3.62	9	0.40	43.87	<0.0001
Intercept	8.05	0.043		1			
X_1	0.22	0.034	0.38	1	0.38	41.79	0.0003
X_2	0.19	0.034	0.29	1	0.29	31.53	0.0008
X_3	0.39	0.034	1.19	1	1.19	130.29	<0.0001
X_1X_2	0.12	0.048	0.053	1	0.053	5.77	0.0473
X_1X_3	0.047	0.048	9.025E − 003	1	9.025E − 003	0.99	0.3540
X_2X_3	−0.13	0.048	0.068	1	0.068	7.38	0.0299
X_1^2	−0.47	0.047	0.92	1	0.92	99.92	<0.0001
X_2^2	−0.30	0.047	0.39	1	0.39	42.41	0.0003
X_3^2	−0.20	0.047	0.17	1	0.17	18.62	0.0035
Residual			0.064	7	9.161E − 003		
Lack of fit			0.021	3	6.908E − 003	0.64	0.6297
Pure error			0.043	4	0.011		
R^2	0.9826		*Adj* R^2	0.9602			
C.V.%	1.26		*Pred* R^2	0.8915			
PRESS	0.40		Adeq Precision	16.688			

two variables and their optimum ranges can be seen. It is clear that extraction yield of LRP is sensitive to minor alterations of the test variables (ratio of water to raw material, extraction time, and microwave power). In addition, two interactions (ratio of water to raw material and extraction time, extraction time and microwave power) among the tested variables are significant ($p < 0.05$).

Through these 3D response surface and their respective contour plots, it is very easy and convenient to understand the interactions between two variables and to locate their optimum ranges. By analyzing the plots, the predicted extraction yield of LRP was observed as 8.05% and found to lie in the following ranges of the examined variables: ratio of water to raw material 28.54–33.78 mL/g, extraction time 23.80–28.44 min, and microwave power 455–550 W.

By employing the software Design-Expert, the optimum values of the test variables were ratio of water to raw material 31.51 mL/g, extraction time 25.84 min and microwave power, 544.19 W. Under these optimal conditions, the maximum predicted extraction yield of LRP was 8.28%, slightly higher than that obtained from plots analysis. The trial experiments were conducted under optimized conditions. Taking convenience into account, the optimum experimental parameters were determined as follows: ratio of water to raw material, 31.5 mL/g; extraction time, 25.8 min; and microwave power, 544.0 W. To compare the predicted results (8.28%) with practical values, rechecking was performed using deduced optimal conditions. The mean value of 8.25 ± 0.07% ($n = 5$), obtained from real experiments, showed the validity of this RSM model because the differences between 8.28% and 8.25 ± 0.07% ($n = 5$) were found to be insignificant ($p > 0.05$). The strong correlation between real and predicted results confirmed that the response model was accurate and adequate to reflect the expected optimization of the LRP extraction process.

3.6. Chemical analysis

Dynamic microwave-assisted extraction of the fruit of *L. ruthenicum* with hot water yielded a crude polysaccharide sample. The carbohydrate content of CLRP was 67.23% and protein content was 7.18%. After deproteinization and decoloration, the carbohydrate content of LRP was 93.84% and protein content was 4.22%. The total carbohydrate content, protein content and uronic acid content of LRP were shown in Table 3.

3.7. IR spectroscopy

The IR spectroscopy of LRP was displayed a broad and intense peak at around 3410 cm^{-1} as shown in Fig. 3, which was assigned to the hydroxyl groups stretching vibration. The weak peak toward 2935 cm^{-1} was attributed to the C—H antisymmetrical stretching vibration. The bands around 1736 cm^{-1} and 1619 cm^{-1} suggested the presence of the ester carbonyl groups (C=O) and carboxylate (COO^-) stretching band [41]. And at 1736, 1621 and 1406 cm^{-1} in the IR spectroscopy of LRP indicated the presence of uronic acids. The absorption peaks between 1250 cm^{-1} and 950 cm^{-1} indicated that galactose conformation of LRP was of the pyranose type [10,42], and the weak absorbance at 896 cm^{-1} suggested that pyranoses existed in the β-configuration [43].

Table 3
Total carbohydrate content, protein content, uronic acid content of LRP.

Sample	Carbohydrate (%)[a]	Protein (%)[a]	Uronic acid (%)[a]
LRP	93.84	4.22	11.58

[a] Data was shown as mean ± standard deviation, $n = 5$.

3.8. Antioxidant activity

The DPPH free radical, a stable free radical, is widely used to evaluate the free radical scavenging ability of natural compounds, and the DPPH radical-scavenging activity was due to their hydrogen-donating ability. Fig. 4a depicts DPPH scavenging abilities of different concentrations of LRP, and IC_{50} value of the

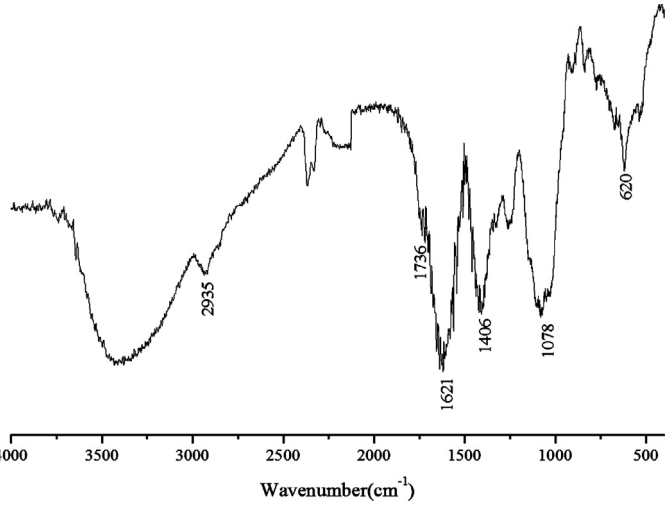

Fig. 3. IR spectroscopy of LRP.

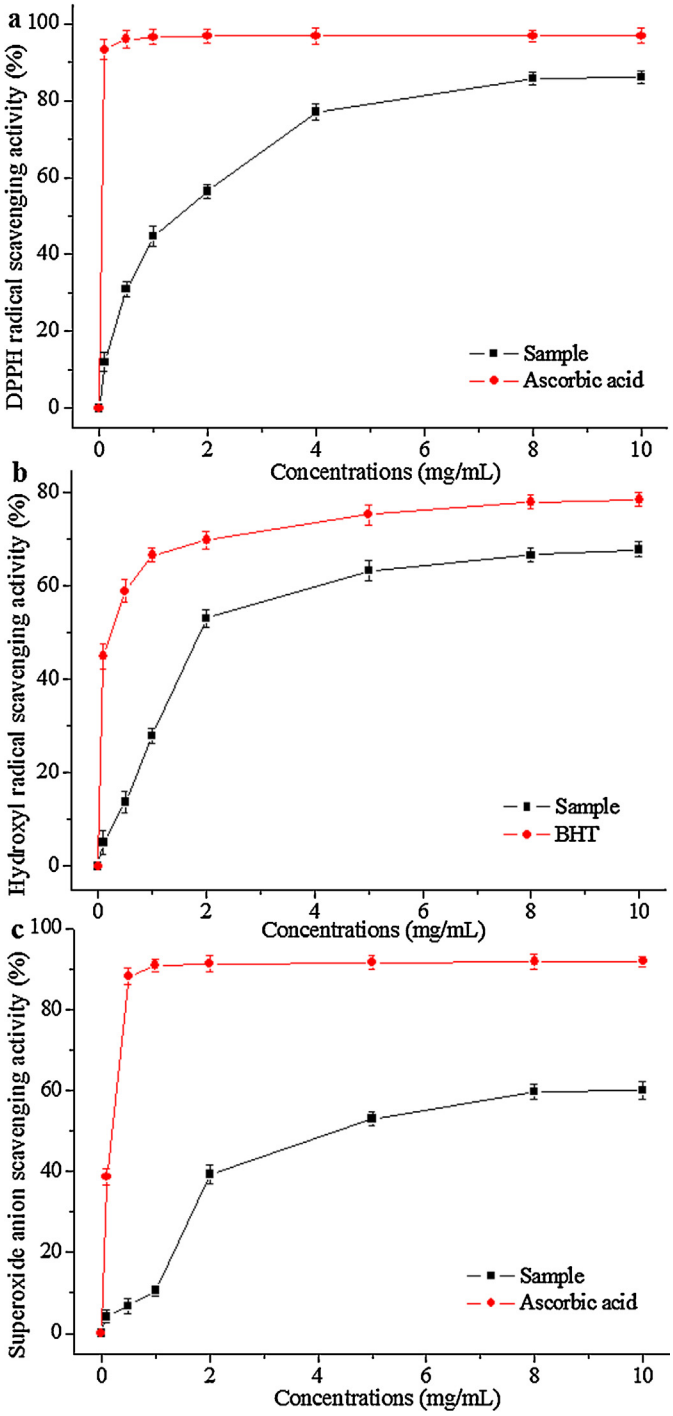

Fig. 4. Antioxidant activity of LRP: (a) scavenging activity to DPPH-radical; (b) scavenging activity to hydroxyl radical; (c) scavenging activity to superoxide anion; values are means ± SD ($n = 5$).

polysaccharides was 1.42 mg/mL, whereas that of ascorbic acid was over 93% at 0.20 mg/mL. Also it shows that scavenging activity increases with the increase of LRP concentration. When the concentration of LRP was 4 mg/mL, the scavenging effects of LRP on DPPH increased at 77.01%. It demonstrated that the effects of scavenging DPPH radicals were concentration related.

For hydroxyl radical, there are two types of antioxidation mechanism, one suppresses the generation of the hydroxyl radical, and the other scavenges the hydroxyl radicals generated [44]. As shown in Fig. 4b, the scavenging effects of LRP on hydroxyl radicals were concentration related well, and the scavenging activity of the polysaccharides and BHT were all above 63% at dose of 5 mg/mL. When the concentration was from 0.1 mg/mL to 2 mg/mL, the increase of scavenging abilities of LRP was faster. It showed a dose-dependent increase in the hydroxyl radical scavenging capacities. The IC_{50} value of tested sample was 1.84 mg/mL suggesting that LRP was good hydroxyl radical-scavenger.

The superoxide radical is a highly toxic species that is generated by numerous biological and photochemical reactions [45]. Also superoxide anion is one of the precursors of the singlet oxygen and hydroxyl radicals, and indirectly initiates lipid peroxidation. 1,2,3-Phentriol rapidly autoxidizes in alkaline solution and formed intermediate products such as $O_2^{\bullet -}$. The antioxidants can interfere with 1,2,3-phentriol autoxidation by acting as scavengers of $O_2^{\bullet -}$. Therefore, the antioxidant ability can be determined by the scavenging activity of self-oxidation of 1,2,3-phentriol. As shown in Fig. 4c, the scavenging capacity rose as the increase of LRP concentration and was 53.01% at the dose of 5 mg/mL. The IC_{50} value of LRP was 4.30 mg/mL, whereas that of ascorbic acid was 0.16 mg/mL. And the scavenging ability had not very obvious changes when the concentration of sample reached 8 mg/mL.

4. Conclusion

Dynamic microwave-assisted extraction (DMAE) was a green, speedy and efficient extraction technique that could be used to improve the extraction yield of LRP. In the present paper, the DMAE of LRP was performed with a three-variable, three-level Box–Behnken design (BBD) based on RSM. Experimental results indicated that the optimized extraction parameters were as follows: ratio of water to raw material, 31.5 mL/g; extraction time, 25.8 min; and microwave power, 544.0 W. The experiment extraction yield of LRP was 8.25 ± 0.07% under the optimal conditions, which was agreed closely with the predicted value.

In the past decades, it has been found that the previous polysaccharides in plants are not only energy resources but they play key biological roles in many life processes as well [46]. The results of antioxidation tests clearly demonstrated water-soluble polysaccharides from *L. ruthenicum*, were found to have antioxidant potential according to the *in vitro* evaluation of their free radicals (DPPH radical, Hydroxyl radical and Superoxide radical) scavenging activities. Therefore, the water-soluble polysaccharides from *L. ruthenicum* have the potential to be explored as novel natural antioxidant for using in functional foods or medicine. Applying to different methods would obtain different effective polysaccharides. One of the reasons is different polysaccharides with different molecular weight and the low molecular weight products are more effective than high molecular weight products [47]. To investigate the main radical scavenging polysaccharides fractions, further researches on purify and functions evaluation are in progress.

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