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Optimization of the polysaccharide extraction process from *Rosa roxburghii* Tratt using Box-Behnken response surface methodology and monosaccharide composition analysis

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Abstract

Rosa roxburghii Tratt polysaccharide (RRTP) has various physiological functions that benefit the human body. Therefore, improving the extraction rate of RRTP is of crucial for its promotion and application. In this study, RRTP was extracted using an intermittent ultrasound-assisted enzymatic method, and the effects of five factors on the extraction rate of RRTP were studied using a single factor test and response surface methodology. The regression equation model was significant and had good reliability, and the enzyme concentration, liquid-solid ratio and ultrasonic temperature exhibited a positive and significant effect on the RRTP content. The optimal extraction conditions were a power of 360 W, an enzyme concentration of 2.0%, a liquid-solid ratio of 40 mL/g, an ultrasonic temperature of 60 °C and an ultrasonic time of 25 min. Under these conditions, the crude polysaccharide of RRT (CRRTP) yield was 15.03%, and the RRTP content was 4.50%. The RRTP comprised mannose, rhamnose, galacturonic acid, glucose, galactose and arabinose with molar percentages of 3.39%, 2.91%, 26.73%, 35.16%, 21.21% and 10.61% by High Performance Liquid Chromatography (HPLC), respectively. These results suggested that the intermittent ultrasonic-assisted enzymatic method can improve the extraction rate of RRTP.

Keywords: *Rosa roxburghii* Tratt; polysaccharide; response surface methodology; intermittent ultrasound-assisted enzymatic extraction.

Practical Application: To provide technical reference for developing RRTP into functional food or health food.

1 Introduction

Rosa roxburghii Tratt (RRT), also known as Roxburgh rose, is a small deciduous perennial shrub of rosaceae used as a medicinal and edible plant with a long history in China. This shrub is also called Cili in China. According to the Compendium of Materia Medica, the flowers, fruits, leaves and seeds of *Rosa roxburghii* Tratt can be used in traditional Chinese medicine (Wang et al., 2021).

Wild RRT grows mainly at altitude of 1000-1600 m in the mountainous areas of southwest, central-south and northwest in China (Xu et al., 2004); however, a large area of commercial cultivation exists in China. RRT is cultivated on at least 30,000 hectares and has developed a range of health products for clinical use (Lu et al., 2016). The fruit yield of RRT in Guizhou ranks first in China; the economic cultivation area of RRT in Guizhou reached 1.76 million acres by 2019, the output of fresh fruit (Figure S1 in the Supplementary document) was 66 000 tons, and the planting scale of RRT industry was the highest in China (Wang et al., 2021). RRT become one of the characteristic agricultural products on which the province focuses for development.

RRT fruit is a highly nutritious and underutilized functional food source with many nutrients and phytochemicals. It contains not only sugar, protein, vitamins, inorganic salts and various essential amino acids but also polysaccharides, phenolic compounds, triterpenoids and organic acids, which are particularly rich in vitamin C and superoxide dismutase (SOD) (Wang et al., 2022a; Wang et al., 2021). Studies have found that RRT fruit has much higher antioxidant activity than other common vegetables and fruits (Yang et al., 2020). Extracts of RRT have been used in traditional Chinese medicine and exhibit a broad range of functional activities against ovarian cancer cell metastasis (Chen et al., 2015), atherosclerosis (Zhang et al., 2001), hyperglycemia (Yan et al., 2022) and hyperlipidemia and can also control intestinal flora disorders caused by diabetes (Wang et al., 2020).

Polysaccharides are macromolecular substances that exist widely in nature, and monosaccharides are their basic units. Monosaccharides form glycosidic bonds with other monosaccharides to form linear or branched polysaccharides (Liu et al., 2020). To date, more than 300 natural polysaccharide compounds have been identified in plants, animals and microorganisms and they are involved in various physiological functions, such as antioxidant function (Li et al., 2022). Plants are crucial sources of natural polysaccharides (Yin et al., 2019).

Many polysaccharides have been found in different plants, and the structural and functional activities of some polysaccharides have been investigated. The monosaccharide composition of various polysaccharides is different. For example, *Cistanche deserticola* comprises glucose (Wu & Tu, 2005), the monosaccharide component of PSPP is dextran (Zhao et al., 2005), and CPE comprises arabinose, galactose, glucose, mannose, rhamnose, and xylose (Kim et al., 2007).

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RRT is also rich in polysaccharides. *Rosa roxburghii* Tratt polysaccharide (RRTP) has many beneficial functions, such as significant antioxidant activity (Chen & Kan, 2018), antitumor activity (Wang et al., 2018a), hypoglycemic activity (Wang et al., 2018b), hypolipidemic effects (Wang et al., 2020) and prebiotic effects (Wang et al., 2019). It can also reduce chronic obesity-induced colitis by improving intestinal flora metabolism (Wang et al., 2022c) and has substantial development and application potential.

Polysaccharides can be extracted from plants using many approaches, such as hot water extraction (Yang et al., 2017), enzyme extraction (Bhotmange et al., 2017), ultrasonic extraction (Xiao et al., 2012), microwave extraction (Ren et al., 2017) and alkali extraction (Yu et al., 2014).

Water alcohol precipitation is a traditional method used to extract plant polysaccharides, but the polysaccharide extraction rate is low (Guo et al., 2010). In recent years, some researchers have used other methods or auxiliary methods to extract polysaccharides more quickly and efficiently, such as auxiliary water enzymes, ultrasonic-assisted extraction, microwave-assisted extraction, vacuum extraction and microwave-assisted enzymatic extraction (Liu et al., 2018; Wang et al., 2018a).

Ultrasound is an effective method to extract plant polysaccharides and primarily uses the cavitation effect of ultrasound to destroy the plant cell wall and improve the extraction rate. It has the advantages of a short extraction time, low temperature and high efficiency (Nuerxiati et al., 2019; Song et al., 2019; Sun et al., 2019). Enzymes are introduced to destroy the plant cell wall, improving the extraction rate (Wu et al., 2019). To improve the RRTP content, this study combined the three methods of hot water extraction, ultrasonic extraction and enzyme extraction.

Response surface methodology (RSM) is a statistical method to determine the optimal process parameters by analyzing the profile of the response surface and reveal the functional relationship between factors and response values by means of multivariate linear fitting or binomial regression analysis (Wang et al., 2018a). RSM is accomplished using sequential experimentation involving factors such as temperature, pressure, duration of reaction, and proportion of reactants, and it can be used to model or optimize any response affected by the levels of one or more quantitative factors (Dean et al., 2017). RSM is also used extensively to extract plant active substances; for example, the optimal extraction conditions of polysaccharides were obtained by optimizing the combination of multiple extraction variables. Additionally, the Box-Behnken design (BBD) of RSM is efficient because of the fewer number of runs (Wu et al., 2020).

In this study, *Rosa roxburghii* Tratt fruit from Guizhou Province (China) was selected as raw materials. Intermittent ultrasound (anterior ultrasound, hot water extraction, and posterior ultrasound) and enzyme-assisted extraction were used to extract polysaccharides from *Rosa roxburghii* Tratt fruits. Single factor test and response surface methodology were combined to explore the optimal extraction conditions of polysaccharides (enzyme concentration, liquid-solid ratio, ultrasonic temperature, ultrasonic time and ultrasonic power). HPLC was used to identify the monosaccharide composition of RRTP, and scanning electron microscopy was used to observe the surface morphological characteristics. The results provide a reference to optimize the extraction process of plant polysaccharides and provide a basis to develop *Rosa roxburghii* Tratt and *Rosa roxburghii* Tratt polysaccharides into health foods or medicines.

2 Materials and methods

2.1 Materials

Rosa roxburghii Tratt was harvested in Liupanshui in Guizhou (China). Petroleum ether and acetonitrile were purchased from Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Citric acid was purchased from Reagent Chemicals Co., Ltd. (Tianjin, China). Cellulase (50,000 U/g) and D101 macroporous resin were purchased from Solarbio Science & Technology Co. Ltd. (Beijing, China). Neutral protease (100,000 U/g) was purchased from Yangshao Biotechnology Co., Ltd. (China). Anhydrous ethanol was purchased from Zhiyuan Chemical Reagent Co. Ltd. (Tianjin, China). Chloroform was purchased from Xilong Science Co., Ltd. (Sichuan, China). Hydrochloric acid was purchased from Jincheng Reagent Co., Ltd. (Kunshan, China). Disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Guangfu Technology Development Co. Ltd. (Tianjin, China). Trifluoroacetic acid was purchased from Cologne Chemicals Co., Ltd. (Chengdu, China). 1-Pheny-3-methyl-5-pyrazolone (PMP) was purchased from Macklin (China). The standard substances glucose (Cas 50-99-7), galacturonic acid (Cas 91510-62-2), glucuronic acid (Cas 6556-12-3), rhamnose (Cas 6155-35-7), fructose (Cas 57-48-7), galactose (Cas 59-23-4), and arabinose (Cas 10323-20-3) were purchased from Nanjing Herb Source Biotechnology Co., Ltd. (Nanjing, China).

An HPLC instrument (12601C) was purchased from Agilent (USA). A selective evaporator (RE-52) was purchased from Shanghai Yarong Biochemical Instrument Factory (Shanghai, China). A high flux microwave digestion instrument (MDS-15) was purchased from Shanghai Xinyi Microwave Chemical Technology Co., Ltd. (Shanghai, China). A constant temperature shaking table (TS-111B) was purchased from Shanghai Tiancheng Test Instrument Manufacturing Co., Ltd. (Shanghai, China). A multipurpose constant temperature ultrasonic extractor (TL-1000CT) was purchased from Jiangsu Tianling Instrument Co., Ltd. (Jiangsu, China). A vacuum drying oven (DZF-6050) was purchased from Shanghai Qixin Scientific Instrument Co., Ltd. (Shanghai, China). A low-speed refrigerated centrifuge (TDL-5000bR) was purchased from Shanghai Anting Scientific Instrument Factory (Shanghai, China). An enzyme standard instrument (1510) was purchased from Shanghai Thermo Fisher Technology Co., Ltd. (Shanghai, China). A scanning electron microscope (GeminiSEM 300) was purchased from German ZEISS.

2.2 Extraction and purification of RRTP

RRT fruit was dried and ground into a powder using a grinder and then passed through a 40 mesh sieve. The powder was heated with petroleum ether at 80 °C for reflux for 4 h to remove lipid substances. After drying, the powder was heated

with 80% ethanol for reflux for 4 h to remove color. Finally, RRT powder samples were obtained by vacuum drying at 60 °C.

Five grams of RRT powder samples were placed into a 500 mL conical flask, a specific concentration of enzyme was added according to the ratio of neutral protease to cellulase (2:1), an appropriate amount of double steam water was added according to a specific ratio of liquid to material, and then an appropriate amount of citric acid was added to adjust the pH to 5.8~6.2. The samples were placed on a shaking table (41 °C; 150 rpm/min) for 4 h for enzymatic hydrolysis. Anterior ultrasound was performed immediately after enzymatic hydrolysis at a preset temperature, power and time. After anterior ultrasound, the extract was placed in 90 °C hot water for 3 h, and the enzyme was removed. Next, posterior ultrasound was performed at the same temperature, power and time. After ultrasonic treatment, the residue solution was centrifuged at 4000 rpm/min for 10 min to obtain the clarified RRT stock solution, which was reduced to 1/5 of the original volume using a rotary evaporator, and anhydrous ethanol was added to 80% of the volume. The samples were stirred evenly and placed in a refrigerator at 4 °C for 24 h. Next, the samples were removed, filtered and washed with anhydrous ethanol to precipitate flocculants and vacuum dried to constant weight to obtain the crude polysaccharide of *Rosa roxburghii* Tratt (CRRTP).

2.3 Single-factor test

According to the above experimental procedure, the effects of five factors (enzyme concentration, liquid-solid ratio, ultrasonic temperature, ultrasonic time and ultrasonic power) on the CRRTP yield were investigated. The specific conditions were set as follows: the enzyme concentrations was set to 1.0%, 1.5%, 2.0%, 2.5% or 3.0%. The liquid-solid ratio was set to 10:1, 20:1, 30:1, 40:1 or 50:1. The ultrasonic temperature was set to 30 °C, 40 °C, 50 °C, 60 °C or 70 °C. The ultrasonic time was set to 10 min, 15 min, 20 min, 25 min or 30 min. The ultrasonic power was set to 120 W, 240 W, 360 W, 480 W or 600 W (Equation 1).

$$CRRTP\ yield\,(\%) = \frac{m}{M} \times 100\% \quad (1)$$

Where m refers to the mass of CRRTP (g) and M refers to the mass of the RRT powder samples (g).

2.4 Response Surface Methodology (RSM)

Considering the single-factor test results, the Box-Behnken design (BBD) of RSM in the present study was a design of four variables, each with three levels. Statistical analysis was performed using Design Expert Software (8.0.6). Considering the RRTP content as the response value, the regression model was optimized by interaction analysis of the response surface factors, and the final extraction parameters of RRTP were obtained.

2.5 RRTP content of RRT fruit powder

The phenol-sulfuric acid colorimetric method (Yi et al., 2020) was used to determine the RRTP content of RRT fruit powder. Glucose was used as a standard reference substance to draw the standard curve. After dilution, 2 mL of CRRTP sample solution was added to 1 mL of 6% phenol solution and 5 mL of concentrated sulfuric acid, and then the mixture was shaken well and heated in a boiling water bath for 15 minutes. After cooling to room temperature, the absorbance was measured at 490 nm. The absorbance was measured three times in parallel, and distilled water was used as a blank control. The ω (polysaccharide content of CRRTP) was calculated according to the concentration, volume and absorbance value of the CRRTP sample. Next, the RRTP content was calculated (Equation 2).

$$RRTP\ content\,(\%) = \frac{m \times \omega}{M} \times 100\% \quad (2)$$

Where m refers to the mass of CRRTP (g), ω refers to the polysaccharide content in CRRTP (%) and M refers to the mass of the RRT powder samples (g).

2.6 Monosaccharide composition analysis

External standards, mannose (Man), rhamnose (Rha), glucuronic acid (GluA), galacturonic acid (GalA), glucose (Glu), galactose (Gal), xylose (Xyl) and arabinose (Ara), were used to identify and quantify the monosaccharides. The RRTP sample was dissolved in trifluoroacetic acid in a microwave digestion tube for microwave digestion and then was derivatized with PMP methanol solution; the standard product was also derivatized. The monosaccharide composition was determined by HPLC using the following chromatographic conditions: the column was Hedera C18 (5 μm, 4.6 mm × 250 mm; H181C; Jiangsu Hanbang Technology Co., Ltd., China), the mobile phase was phosphate buffer : acetonitrile = 83:17, the column temperature was 25 °C, the flow rate was 1 mL/min, the detection wavelength was 254 nm, the injection volume was 20 μL, and the analysis time was 70 min.

2.7 Morphological examination

The surface and morphological features of RRTP were observed by scanning electron microscopy (SEM; ZEISS Gemini 300; Germany). The RRTP sample was directly glued to the conductive adhesive and sprayed with gold using a sputtering coater (Oxford Quorum SC7620; UK). Next, the sample morphology was photographed by SEM. The acceleration voltage was 3 kV. Representative images of each sample were acquired at 100×, 1000× and 10000× magnification.

3 Results and discussion

3.1 Single-factor test results

By observing the effects of five factors (enzyme concentration, liquid-solid ratio, ultrasonic temperature, ultrasonic time and ultrasonic power) on the CRRTP yield, with other parameters being equal, the CRRTP yield reached the highest value (14.63%) when the enzyme concentration reached 2.0% and then gradually decreased with a further increase in the enzyme concentration (Figure 1a). Therefore, an enzyme concentration of 2.0% was considered in the RSM experiments.

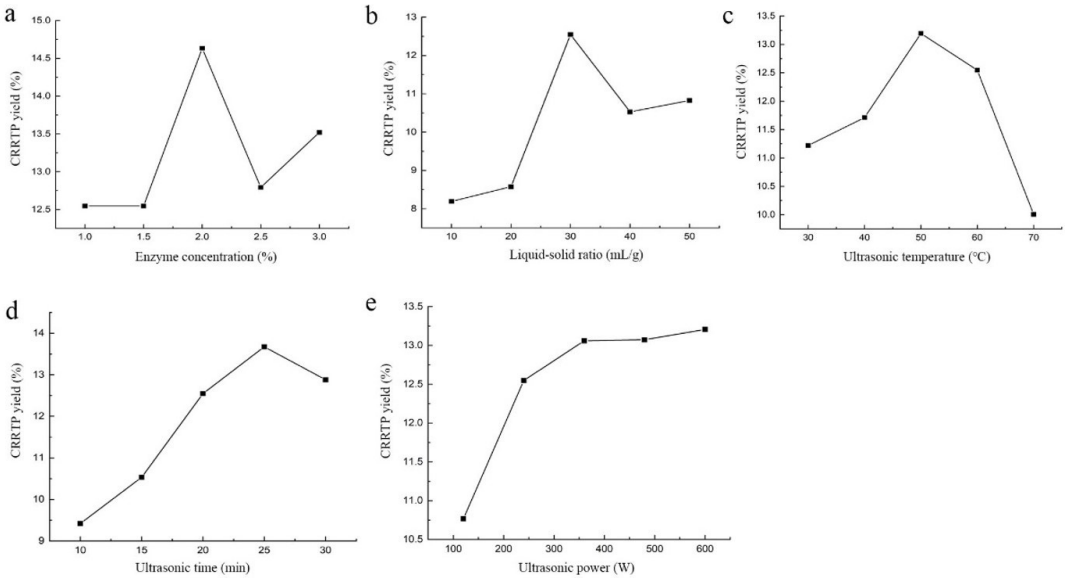

Figure 1. Effects of five factors on the CRRTP yield. Panels a, b, c, d and e represent the effects of enzyme concentration, liquid-solid ratio, ultrasonic temperature, ultrasonic time and ultrasonic power on the CRRTP yield, respectively.

With the increase in the liquid-solid ratio, the CRRTP yield first increased and then decreased and peaked (12.54%) at 30:1 mL/g. At this time, the solid-liquid balance was reached, and polysaccharide precipitation was saturated (Figure 1b). The increase in solvent could accelerate the dissolution of polysaccharides; however, after reaching the peak, excessive liquid could increase the diffusion distance of internal tissues, leading to a decrease in the CRRTP yield (Wang et al., 2018a). Therefore, a liquid-solid ratio of 30:1 was selected in the RSM experiments.

The ultrasonic temperature and time also showed the same trend; the CRRTP yield first increased and then decreased, and the peak values were 13.20% and 13.67%, respectively. The corresponding ultrasonic temperature and ultrasonic time were 50 °C (Figure 1c) and 25 min (Figure 1d), respectively. Therefore, 50 °C and 25 min were selected as the extraction temperature and time, respectively, in the RSM experiments.

However, with increasing ultrasonic power, the CRRTP yield also increased. When the ultrasonic power reached 360 W, the CRRTP yield reached the highest value (13.06%) and then stabilized (Figure 1e). Therefore, 360 W was chosen as the optimum extraction power.

3.2 Regression model and analysis of variance

Box-Behnken RSM was used to optimize the extraction process of RRTP using the intermittent ultrasonic-assisted enzymatic method. Based on the results of the single-factor experiment, the ultrasonic power was fixed at 360 W, the enzyme concentration, liquid-solid ratio, ultrasonic temperature and ultrasonic time were considered independent variables, and the RRTP content was considered the response value. According to the principle of the Box-Behnken central combination experiment (BBD), Design-Expert 8.0.6 software was used to design 4 factors and 3 horizontal RSMs. The factor levels are shown in Table 1, the Box-Behnken RSM results are shown in Table 2, and the variance analysis results are shown in Table 3.

Table 1. Factors and levels of Box-Behnken RSM.

Factors	Levels		
	-1	0	1
Enzyme concentration (%)	1.5	2.0	2.5
Liquid-solid ratio (mL/g)	20:1	30:1	40:1
Ultrasonic temperature (°C)	40	50	60
Ultrasonic time (min)	20	25	30

The quadratic polynomial showed the relationship between the RRTP content (Y) and four extraction parameters as follows (Equation 3):

$$Y = 3.23 + 0.27\mathrm{A} + 0.93\mathrm{B} + 0.61\mathrm{C} + 0.0000\mathrm{D} - 0.025\mathrm{AB} - 0.20\mathrm{AC} + 0.015\mathrm{AD} + 0.14\mathrm{BC} + 0.090\mathrm{BD} - 0.11\mathrm{CD} - 0.35A^2 - 0.061B^2 - 0.20C^2 - 0.23D^2 \quad (3)$$

Where A is the enzyme concentration, B is the liquid-solid ratio, C is the ultrasonic temperature and D is the ultrasonic time.

The *P* value of the model was much lower than 0.05, indicating that the regression equation model was significant, and the P value of the lack of fit was more than 0.05, suggesting that the regression model had good reliability (Table 3). The P values of the linear coefficient (A, B, C), cross-product coefficients (AC), and quadratic term coefficients (A^2, C^2 and D^2) were all lower than 0.05, indicating that they markedly affected the RRTP content. Additionally, the determination coefficient ($R^2 = 0.9803$) indicated that the model fit well, and the experimental value was very close to the predicted value. This model could be used to analyze and predict the technological conditions of the intermittent ultrasound-assisted enzymatic extraction of RRTP. The adjusted coefficient (Adj R^2) was 0.9606, the predicted

Table 2. Box-Behnken design and response data for the RRTP content.

Run	A	B	C	D	The RRTP content (%)
1	1.5	20	50	25	1.72
2	2.5	20	50	25	2.16
3	1.5	40	50	25	3.68
4	2.5	40	50	25	4.02
5	2.0	30	40	20	2.25
6	2.0	30	60	20	3.75
7	2.0	30	40	30	2.22
8	2.0	30	60	30	3.28
9	1.5	30	50	20	2.18
10	2.5	30	50	20	2.78
11	1.5	30	50	30	2.32
12	2.5	30	50	30	2.98
13	2.0	20	40	25	1.54
14	2.0	40	40	25	3.16
15	2.0	20	60	25	2.33
16	2.0	40	60	25	4.53
17	1.5	30	40	25	1.54
18	2.5	30	40	25	2.53
19	1.5	30	60	25	3.22
20	2.5	30	60	25	3.41
21	2.0	20	50	20	2.10
22	2.0	40	50	20	3.68
23	2.0	20	50	30	2.00
24	2.0	40	50	30	3.94
25	2.0	30	50	25	3.18
26	2.0	30	50	25	3.23
27	2.0	30	50	25	3.10
28	2.0	30	50	25	3.17
29	2.0	30	50	25	3.45

A: enzyme concentration; B: liquid-solid ratio; C: ultrasonic temperature; D: ultrasonic time.

coefficient (Pred R^2) was 0.9036, and the difference value was less than 0.10, indicating good consistency. The coefficient of variation (C.V. = 5.44%) was low, suggesting that the fitting model was reproducible. The adeq precision (27.295) was higher than 4, suggesting that the signal-to-noise ratio was adequate (Wu et al., 2020).

The interaction effects of the enzyme concentration, liquid-solid ratio, ultrasonic temperature and ultrasonic time on the RRTP content are shown in Figure 2. The interaction effects can be determined by the curvature of the response surface plot and shape of the contour plot's center circle. For the enzyme concentration and ultrasonic temperature, the shape of the response surface plot was curved, and the shape of the contour plot was elliptical, indicating that the mutual interaction between them was significant. These results were consistent with those in Table 3.

Using Design-Expert 8.0.6, according to Box-Behnken RSM, the optimal process conditions for the intermittent ultrasound-assisted enzymatic extraction of RRTP were predicted as follows: with an enzyme concentration of 2.18%, a liquid-solid ratio of 39.42 mL/g, an ultrasonic temperature of 59.79 °C and an ultrasonic time of 25.10 min, the predicted RRTP content was 4.56%. Considering the operability of the experiment, the final extraction conditions were as follows: an enzyme concentration of 2%, a liquid–solid ratio of 40 mL/g, an ultrasonic temperature of 60 °C and an ultrasonic time of 25 min.

3.3 Validation of extraction conditions

The validation test was performed according to the above predicted extraction conditions of RRTP. The CRRTP yield was 15.03%, and the RRTP content was 4.50%, which was very close to the predicted extraction rate, indicating that the extraction process was stable and feasible.

Wu et al. (2020) used response surface methodology to assess the optimal extraction conditions of polysaccharides from *Rosa roxburghii* Tratt leaves by hot water extraction: a liquid-solid ratio of 21.16 mL/g, an extraction temperature of 81.32 °C, and an extraction time of 90.49 min; the extraction rate was 11.04%. Some scholars have also used the hot water extraction method to extract polysaccharides from Guizhou *Rosa roxburghii* Tratt fruit. One study showed that the optimum extraction conditions were as follows: a liquid-solid ratio of 30 mL/g, an extraction temperature of 95 °C and an extraction time of 3 h; the maximum yield was 3.57 ± 0.26% based on

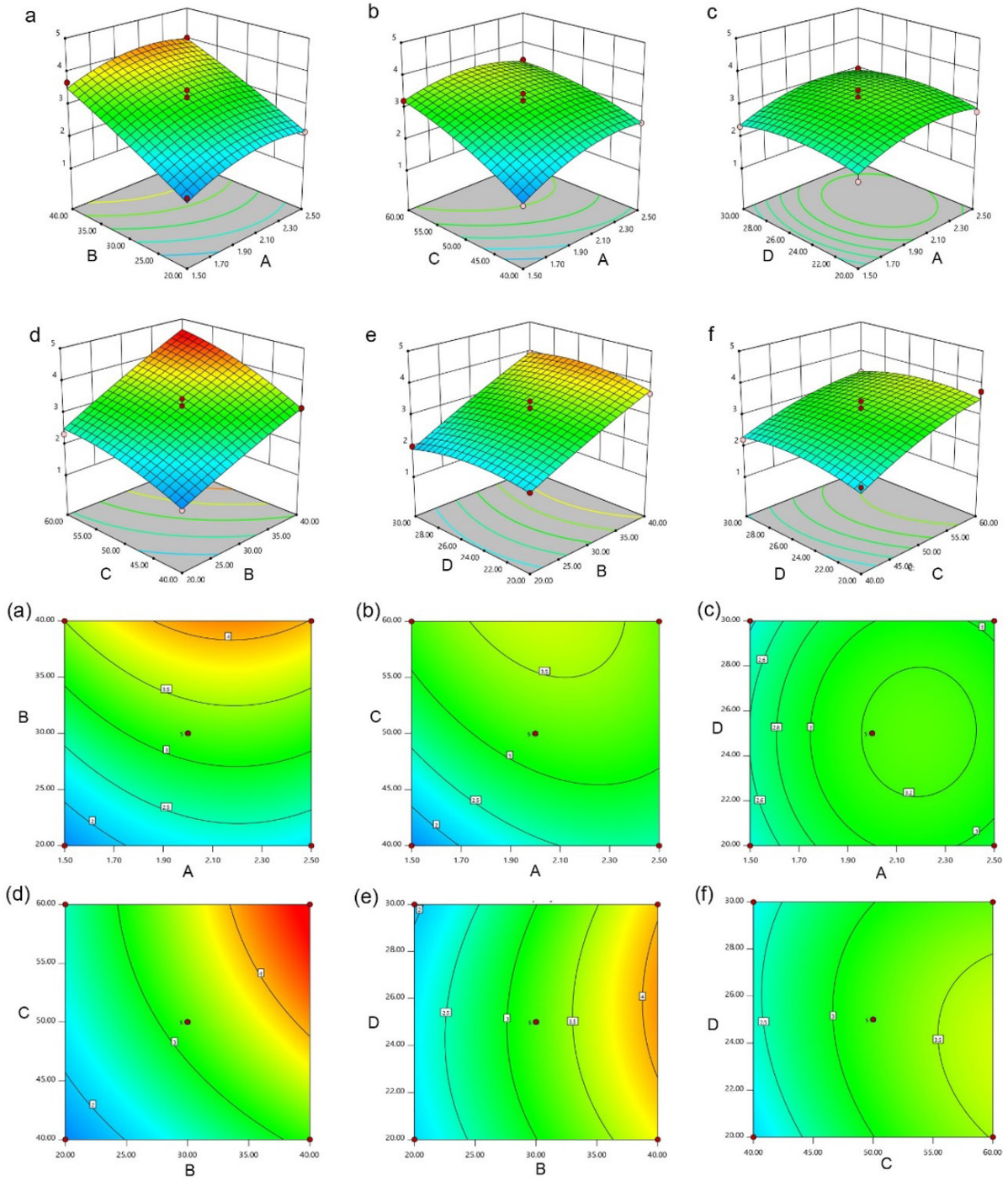

Figure 2. Interaction effects of various factors on the RRTP content. a-f are the response surface plots, and (a)-(f) are their corresponding contour plots. A: enzyme concentration (%); B: liquid-solid ratio (mL/g); C: ultrasonic temperature (°C); D: ultrasonic time (min).

the dry weight (Wang et al., 2018c). In another study, the water alcohol precipitation method was used to obtain RRTP at 95 °C for 3 h and ethanol concentrations of 30%, 50%, and 80% (v/v). Three types of RRTP were obtained with yields of 1.44%, 1.94%, and 1.94% (Wang et al., 2022b). These values were all lower than the values in the present study. Chen & Kan (2018) used ultrasonic-assisted extraction of RRTP, under the conditions of 148 W power and 80 °C for 30 min, the yield of crude RRTP was 6.59%, this value was still lower than those of this study.

3.4 Monosaccharide composition analysis

The HPLC chromatograms of eight standard monosaccharides are shown in Figure 3a, and HPLC chromatograms of RRTP samples are shown in Figure 3b. By comparison, RRTP extracted

Table 3. ANOVA for the response surface quadratic model of the RRTP content.

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*P* value
Model	17.06	14	1.22	49.72	< 0.0001*
A	0.86	1	0.86	35.25	< 0.0001*
B	10.38	1	10.38	423.44	< 0.0001*
C	4.42	1	4.42	180.19	< 0.0001*
D	0.000	1	0	0	1
AB	2.50E-03	1	2.50E-03	0.1	0.7542
AC	0.16	1	0.16	6.53	0.0229*
AD	9.00E-04	1	9.00E-04	0.037	0.8508
BC	0.084	1	0.084	3.43	0.0852
BD	0.032	1	0.032	1.32	0.2695
CD	0.048	1	0.048	1.97	0.1818
A^2	0.80	1	0.80	32.51	< 0.0001*
B^2	0.024	1	0.024	0.97	0.3417
C^2	0.25	1	0.25	10.37	0.0062*
D^2	0.35	1	0.35	14.37	0.0020*
Residual	0.34	14	0.025		
Lack of Fit	0.27	10	0.027	1.52	0.3639
Pure Error	0.071	4	0.018		
Cor Total	17.41	28			
Adj R^2	0.9606				
Pred R^2	0.9036				
C.V.%	5.44				
Adeq precision	27.295				

A: enzyme concentration; B: liquid-solid ratio; C: ultrasonic temperature; D: ultrasonic time. *P values < 0.05 were considered statistically significant.

by the intermittent ultrasound-assisted enzymatic method comprised mannose (Man), rhamnose (Rha), galacturonic acid (GalA), glucose (Glu), galactose (Gal) and arabinose (Ara). Quantitative analysis showed that the molar percentages of the six monosaccharides were 3.39%, 2.91%, 26.73%, 35.16%, 21.21% and 10.61%, respectively (Table 4).

Some scholars also identified the monosaccharide composition of RRTP. Wang et al. (2018c) extracted water-soluble polysaccharides (RTFP) from RRT fruit using hot water as the solvent. The monosaccharide composition of the RTFP included arabinose (33.8%), galactose (37.3%), glucose (20.7%), xylose (3.43%), fucose (2.95%) and mannose (1.74%) (Wang et al., 2018c). Another study used a microwave-assisted enzymatic method to extract RRTP from RRT fruit and determined its monosaccharide composition by HPLC. RRTP contained mannose, ribose, rhamnose, glucosamine hydrochloride, glucuronic acid, galacturonic acid, glucose, galactose, arabinose and caramel at molar ratios of 2.1%, 0.54%, 2.1%, 0.26%, 1.5%, 22.7%, 24.0%, 26.4%, 19.6% and 0.89%, respectively (Wang et al., 2018a). Chen & Kan (2018) extracted a new water-soluble polysaccharide from RRT fruit through the ultrasonic-assisted extraction, and its monosaccharide composition was Man, Rha, GlcA, GalA, Glc, Gal, Ara and Xyl in a molar ratio of 2.88:1.39:2.83:1.00:69.11:3.04:2.52:3.41.

3.5 Morphological examination

The dried RRTP is brownish yellow, flaky and smooth (Figure S2 in the Supplementary document). SEM was used to reveal the surface morphology of RRTP (Figure 4). Some fissures and cracks are visible (Figure 4 f), likely attributed to the rapid evaporation of drops of liquid during the vacuum drying process (Wang et al., 2018c).

Table 4. Monosaccharide composition of RRTP samples.

Monosaccharide	Molar percentages (%)
Man	3.39
Rha	2.91
GalA	26.73
Glu	35.16
Gal	21.21
Ara	10.61

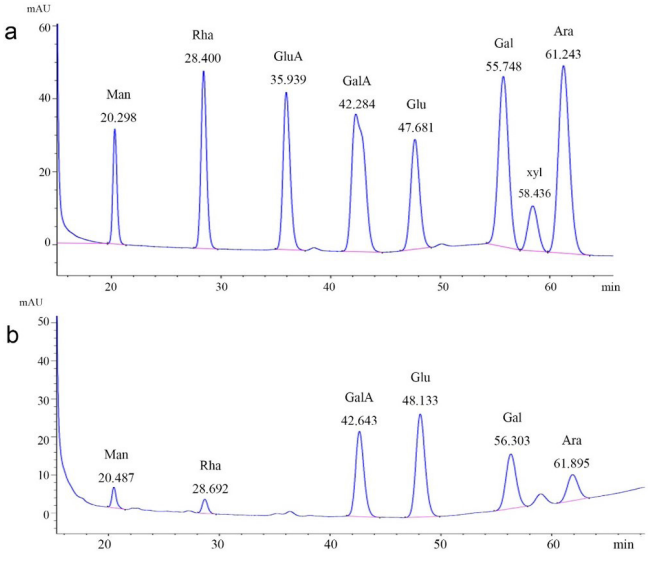

Figure 3. Monosaccharide composition of mixed standard (a) and RRTP (b) by HPLC.

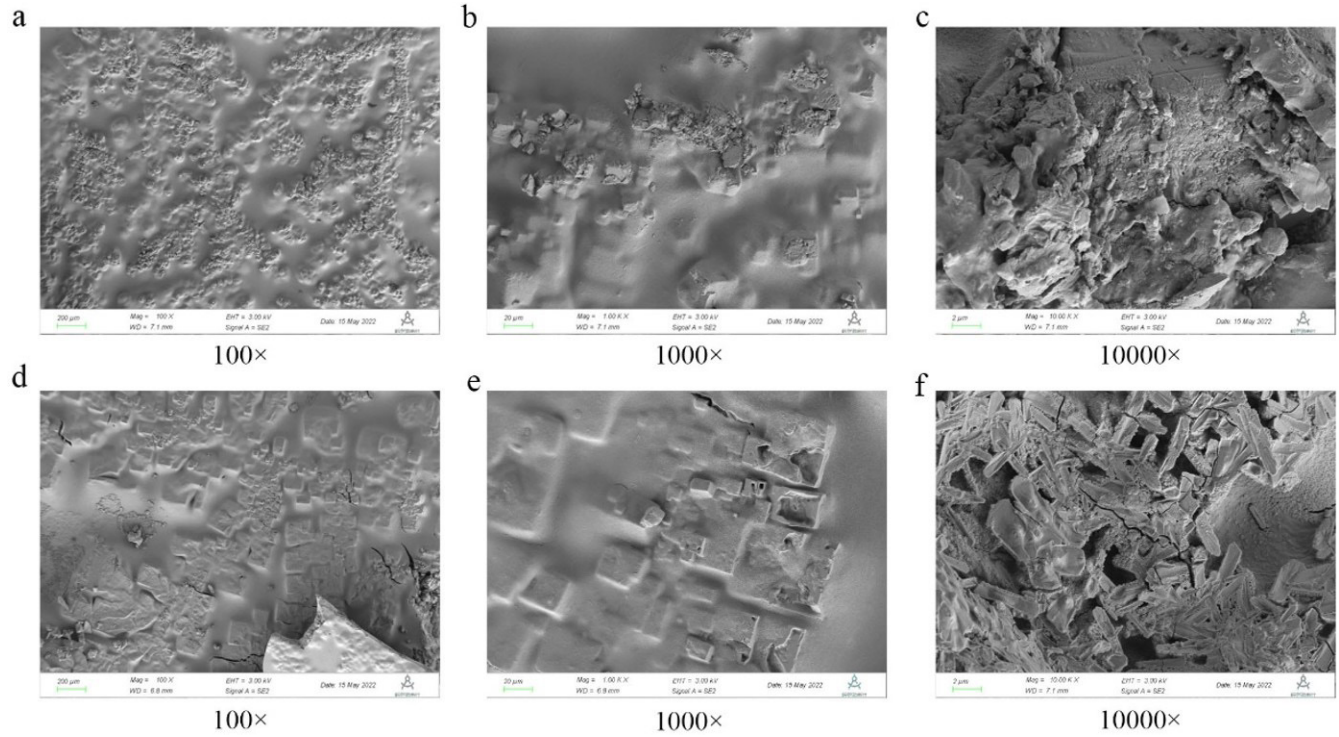

Figure 4. SEM of 100× (a, d), 1000× (b, e) and 10000× magnification (c, f).

4 Conclusion

In this study, the optimized conditions for the intermittent ultrasound-assisted enzymatic method to extract polysaccharides from *Rosa roxburghii* Tratt were achieved using a single factor test and response surface methodology. The results showed that the regression equation model was significant and had good reliability, and the enzyme concentration, liquid-solid ratio and ultrasonic temperature exhibited a positive and significant effect on RRTP content. The optimal extraction conditions for RRTP content were a power of 360 W, an enzyme concentration of 2.0%, a liquid-solid ratio of 40 mL/g, an ultrasonic temperature of 60 °C and an ultrasonic time of 25 min. Under these conditions, the crude polysaccharide of RRT yield was 15.03%, and the RRTP content was 4.50%. The RRTP comprised mannose, rhamnose, galacturonic acid, glucose, galactose and arabinose with molar percentages of 3.39%, 2.91%, 26.73%, 35.16%, 21.21% and 10.61% by HPLC, respectively. Furthermore, the surface and morphological features of RRTP were observed by scanning electron microscopy. The present study suggests that the intermittent ultrasonic-assisted enzymatic method improves the extraction rate of RRTP.

Conflict of interest

Authors declare no competing financial interests or personal relationships with other people or organizations that could inappropriately influence (bias) our work.

Author contributions

Mingyue YIN designed the study. Qingqing ZHAN, Jiangjiang PENG and Ming CHEN optimized the extraction conditions of *Rosa roxburghii* Tratt polysaccharide. Hui ZHONG identified the monosaccharide composition of RRTP. Mingyue YIN wrote the first draft of the manuscript and finished the revision.

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Supplementary Material

Supplementary material accompanies this paper.

Figure S1. Rosa roxburghii Tratt of Guizhou (China).

Figure S2. Dried Rosa roxburghii Tratt polysaccharide.

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