Extraction and Comparison of *Ophiopogon japonicus* Polysaccharide Using a Novel IH Technology

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Abstract [**Objectives**] To apply the novel induction heating (IH) technology for the extraction of *Ophiopogon japonicus* polysaccharide (OJPS), fully utilize the advantages of IH technology to improve the extraction yield of OJPS, and compare it with traditional Chinese medicine polysaccharide extraction methods. [**Methods**] Using the polysaccharide extraction yield as the evaluation index, the IH extraction process for OJPS was optimized through an $L_9(3^4)$ orthogonal test, and systematic methodological validation was conducted. Finally, the process parameters and procedure for extracting OJPS using IH technology were optimized. [**Results**] The optimal extraction process was as follows: solid-liquid ratio of 1∶55, extraction time of 75 min, and alcohol precipitation ratio of 1∶4. The methodological investigation showed that OJPS had a good linear relationship in the range of 20 - 140 μg/mL, with $R^2 = 0.999\ 3$. The average recovery rate was 99.94% (*RSD* = 1.32%). The *RSD*s for precision, repeatability, and stability were all less than 2%, indicating that the measurement method for OJPS extraction yield was excellent. The extraction yields of OJPS by reflux extraction, ultrasonic extraction, and IH extraction were 26.67%, 40.70%, and 51.74%, respectively. [**Conclusions**] The novel IH extraction technology is stable and reliable, has a significant impact on the extraction yield of OJPS, and also improves the conversion rate of OJPS. It is expected to become an emerging technology and research direction for the extraction of Chinese medicine polysaccharides.

Key words IH extraction, *Ophiopogon japonicus* polysaccharide (OJPS), Polysaccharide conversion rate, Content determination

1 Introduction

Polysaccharides, recognized as one of the four fundamental biomolecules of the human body, have been studied later than proteins, nucleic acids, and lipids. The foundation of modern polysaccharide research is often traced to a seminal 1971 paper *Lentinan, a New Immuno-accelerator of Cell-mediated Responses* in *Nature* by Maeda YY and Chihara G, which identified lentinan as a new immuno-accelerator of cell-mediated responses[1]. This finding first directed scientific attention to the immunopotentiating potential of polysaccharides. Extensive research in recent years has further revealed that numerous traditional Chinese medicine (TCM) polysaccharides exhibit diverse and promising biological activities, such as regulating cell division and differentiation[2], antioxidant[3], immunomodulation[4], antiviral[5], antitumor[6], and anti-inflammatory effects[7]. These compelling findings have established TCM polysaccharides as a prominent hotspot in natural product research. Despite this interest, the common extraction techniques for TCM polysaccharides, including reflux extraction[8], enzymatic extraction[9], acid-base extraction[10], and ultrasonic extraction[11], are plagued by persistent drawbacks. These methods frequently result in low extraction efficiency, risk damaging the native polysaccharide structure, and can diminish their pharmacological activity. Therefore, there is an urgent need for novel extraction technologies to overcome these shortcomings. As an emerging technology, the Induction Heating (IH) utilizes Faraday's principle of electromagnetic induction. When an alternating current passes through a coil, it generates an alternating magnetic field. This magnetic field, when rapidly interacting with a unit area of a magnetic and electrically conductive reaction vessel, induces an electromotive force. This induced electromotive force forms a closed loop with the impedance of the workpiece, leading to electric discharge and generating alternating current—known as eddy currents. As a result, the reaction vessel produces a significant amount of Joule heat, while avoiding energy losses typically associated with heat conduction or radiation. Moreover, the electrified coil can be arranged to surround the object from all directions (Fig. 1), enabling uniform and efficient multi-directional heating of the material. In recent years, IH technology has been widely adopted in various fields such as the electronics and electrical industry, machinery manufacturing, and light industry. Through ongoing verification and continuous optimization, IH technology has become increasingly mature, stable, and reliable.

In this study, the OJPS extraction using IH technology was optimized via an $L_9(3^4)$ orthogonal experiment, with the polysaccharide extraction yield as the evaluation index. A systematic methodological validation was conducted to refine the technical parameters and process for OJPS extraction by IH. In addition, the extraction efficiencies of polysaccharides obtained by reflux extraction, ultrasonic extraction, and IH extraction were compared. The

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aim of this work is to address the limitations of traditional extraction methods for Chinese herbal polysaccharides, enhance the extraction rate, and provide an experimental reference and basis for the development of polysaccharide-based formulations in traditional Chinese medicine.

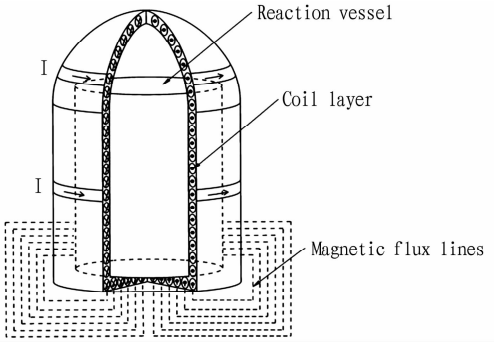

Fig. 1 Three-dimensional configuration of coil

2 Materials and methods

2.1 Instruments YJDJ08 IH Extractor (Changsha Zhuocheng Medical Equipment Co., Ltd.), RE-52 Rotary Evaporator (Shanghai Yarong Biochemical Instrument Factory), SQP Analytical Balance (Sartorius Scientific Instrument Co., Ltd., readability 0.01 mg), HH-2 Digital Constant Temperature Water Bath (Changzhou Langyue Instrument Manufacturing Co., Ltd.), JP-040S Ultrasonic Cleaner (Shenzhen Jiemeng Cleaning Equipment Co., Ltd.), ZNHW Heating Mantle (Gongyi Yuhua Instrument Co., Ltd.), SHB-3 Circulating Water Multi-Use Vacuum Pump (Zhengzhou Dufu Instrument Factory), UV-1800 UV-Vis Spectrophotometer (Shanghai Mapada Instrument Co., Ltd.), GZX-9023MB Electric Blast Drying Oven (Shanghai Boxun Industrial Co., Ltd. Medical Equipment Factory).

2.2 Reagents Glucose (Analytical Reagent, Xilong Scientific Co., Ltd., HY250429), concentrated sulfuric acid (Analytical Reagent, Nanchang Xingguang Fine Chemical Reagent Co., Ltd., G286250526), anthrone (Analytical Reagent, Shanghai Macklin Biochemical Technology Co., Ltd., C11882592), dichloromethane (Analytical Reagent, Xilong Scientific Co., Ltd., SJ250323), petroleum ether (Analytical Reagent, Xilong Scientific Co., Ltd., SJ250716), 95% ethanol (Analytical Reagent, Xilong Scientific Co., Ltd., SJ250521).

2.3 Medicinal materials *Ophiopogon japonicus* (Sichuan Maidong), produced by Anhui Tongtai Tuozutang Pharmaceutical Co., Ltd., with production batch number: YP25020118. The quality standard for the processed slices complies with the *Beijing Processing Standards for Chinese Herbal Medicinal Slices*. It has been authenticated by Professor Zhang Jinlian from the Chinese Herbal Processing Teaching and Research Office, School of Pharmacy, Jiangxi University of Traditional Chinese Medicine, as processed slices of Sichuan Maidong.

2.4 Plotting of glucose standard curve

2.4.1 Experimental principle. Under the action of concentrated sulfuric acid, glucose is rapidly dehydrated to form furfural, and furfural can be dehydrated and condensed with anthrone to form blue-green furfural derivatives. The color of the derivative solution is proportional to the mass concentration of glucose at the maximum absorption wavelength in the visible region. The reaction equation is shown in Fig. 2.

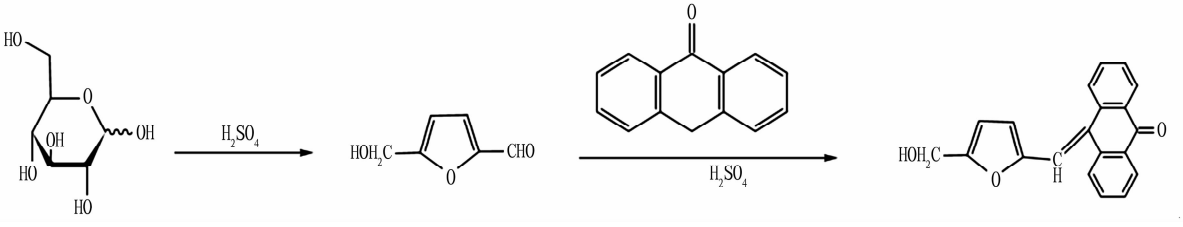

Fig. 2 Principle of sulfuric acid-anthrone reaction

2.4.2 Determination of the maximum absorption wavelength. The sample solution, after color development via the sulfuric acid-anthrone reaction, was scanned using a UV-Vis spectrophotometer within the wavelength range of 400 – 800 nm to determine the maximum absorption wavelength of glucose. Preparation of 80% H_2SO_4-Anthrone: 0.20 g of anthrone was weighed using an analytical balance and dissolved in 25 mL of 80% H_2SO_4. The solution was stored in a brown bottle, protected from light, and was freshly prepared before use. A stopper-equipped tube was taken, and 1 mL of a 0.1 mg/mL glucose solution along with 4 mL of the 80% H_2SO_4-anthrone reagent were added to it. Immediately after addition, the tube was cooled in an ice-water bath to terminate the reaction. Subsequently, the tube was placed in a 100 ℃ water bath to react for 20 min. Upon completion of the reaction, it was immediately cooled in an ice-water bath again to stop the reaction, thus yielding the sample solution for scanning the maximum absorption wavelength of glucose. Another stopper-equipped tube was taken, and 1.0 mL of distilled water along with 4 mL of the 80% H_2SO_4-anthrone reagent were added to it. The remaining procedures were identical to those used in the preparation of the sample solution.

2.4.3 Plotting of the glucose standard curve. (i) Preparation of the standard glucose solution: 0.10 g of glucose standard was accurately weighed using an analytical balance, dissolved in a 100 mL volumetric flask, and diluted to volume to prepare a 1 mg/mL stock solution. Then, 4, 6, 8, 10, 12, 14, and 16 mL aliquots of this stock solution were separately pipetted into 100 mL volumetric flasks and diluted to volume again to obtain glucose solutions with concentrations of 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, and

0.16 mg/mL, respectively. (ii) Procedure for Plotting the standard curve: Eight stopper-equipped tubes were taken and sequentially numbered 0 to 7. Tube 0 (the blank) received 1 mL of distilled water, while tubes 1 through 7 received 1 mL of the corresponding standard glucose solutions of different concentrations. The sulfuric acid-anthrone reaction was carried out in all tubes according to the method described above. The absorbance at the maximum absorption wavelength was measured for each solution. Linear regression was then performed on the glucose mass concentration against the corresponding absorbance values to obtain the equation for the glucose standard curve.

2.5 Determination of extraction yield of OJPS

2.5.1 Polysaccharide extraction process. Dried *Ophiopogon japonicus* was stored in a sealed bag within a desiccator to prevent moisture absorption until use. A 25 g sample of the dried material was weighed, mixed with 10 times its volume of water, and extracted twice in an instant hot water (IH) extractor, with each extraction lasting for 1.5 h. The combined extract was filtered twice through medical gauze, followed by two rounds of hot filtration using fine filter paper to remove residual dregs and silt, yielding a clear filtrate. This filtrate was concentrated using a rotary evaporator to a ratio of 1∶2 (filtrate volume∶raw material mass). The concentrated solution was then treated with dichloromethane and petroleum ether to remove lipid-soluble impurities. After extraction, an appropriate amount of distilled water was added, and the solution was concentrated again until the ratio of the solution to the raw medicinal materials reached 1∶1, ensuring the removal of any residual extraction solvents. Later, a measured volume of 95% ethanol was added to the concentrate with stirring. The mixture was stored in a refrigerator at 5 ℃ for overnight alcohol precipitation. Following precipitation, the supernatant was discarded, and the precipitate was dried until no alcohol odor remained, resulting in the final IH-extracted polysaccharide product.

2.5.2 Determination principle. The polysaccharide was hydrolyzed into monosaccharides under the action of concentrated sulfuric acid. The color development principle for these monosaccharides is the same as that described in the previous section. The concentration of polysaccharide in the sample solution was calculated by applying the measured absorbance to the regression equation of the glucose standard curve. The extraction yield of the polysaccharide was then determined accordingly.

2.5.3 Preparation of the polysaccharide test sample and measurement of its absorbance. The entire quantity of dried polysaccharide was dissolved in an appropriate amount of distilled water and transferred into a 1 000 mL volumetric flask, which was then diluted to volume to obtain the polysaccharide stock solution. Then, a 1 mL aliquot was taken from this stock solution and diluted to volume in a 100 mL volumetric flask to prepare the polysaccharide test sample. Two stopper-equipped tubes, labeled 0 and 8, were prepared, and their absorbance was measured according to the method described in Section **2.4.2**.

2.6 Single factor experiment of OJPS extraction by IH The extraction was performed at 100 ℃ with a solid-to-liquid ratio of 1∶40, repeated twice for 1 h each time. The concentrate was then mixed with dichloromethane at a ratio of 1∶1, and the resulting extract was treated with petroleum ether at a ratio of 2∶3. Finally, alcohol precipitation was carried out at a ratio of 1∶3. Using the OJPS extraction yield as the reference indicators, single-factor experiments were conducted to investigate the effects of the following indicators: solid-to-liquid ratio (1∶30, 1∶40, 1∶50, 1∶60, 1∶70 g/mL), extraction time (40, 50, 60, 70, 80 min), petroleum ether extraction ratio (2∶1, 1∶1, 1∶2, 1∶3, 1∶4), and alcohol precipitation ratio (1∶3, 1∶3.5, 1∶4, 1∶4.5, 1∶5).

2.7 Orthogonal experiment of OJPS extraction by IH

2.7.1 Orthogonal experiment. Based on the results of the single-factor experiments, three key factors were selected for an $L_9(3^4)$ orthogonal experimental design. Then, range analysis and analysis of variance (ANOVA) were performed on the orthogonal experimental data to determine the optimal extraction conditions and refine the process parameters.

2.7.2 Validation of the optimal process. Three separate 25 g batches of *O. japonicus* were weighed. Replicate extractions were performed on these batches under the determined optimal conditions to validate the robustness and reproducibility of the optimal process.

2.8 Methodological validation Polysaccharides often exhibit limited solubility and require extended periods for complete dissolution and homogenization. To address this, ultrasonic treatment was employed. The mechanical and thermal effects of ultrasound accelerate the movement of polysaccharide and water molecules, thereby promoting rapid dissolution and mixing. After ultrasonication, the mechanical effects cease, but the residual thermal energy persists. To prevent this residual heat from affecting the solution's stability, the sample was cooled to allow the molecular motion within the polysaccharide solution to stabilize.

2.8.1 Precision test. The 0.1 mg/mL glucose standard solution was prepared according to the method described in Section **2.4.3**. The glucose standard solution was then ultrasonicated for 20 min and allowed to stand for 1.5 h. Nine portions of the 0.1 mg/mL reference solution were precisely pipetted, and the absorbance value A was determined following the method in Section **2.4.2**. The *RSD* value was calculated to verify whether the precision of the instrument met the requirements.

2.8.2 Stability test. The OJPS obtained under the optimal process conditions was used to prepare the polysaccharide test sample according to the method described in Section **2.4.3**. The test sample was ultrasonicated for 20 min and allowed to stand for 1.5 h. Then, eight 1 mL portions of the polysaccharide test sample were precisely pipetted, and the absorbance value A was determined at 0, 2, 4, 6, 8, 10, 12, and 24 h following the method in Section **2.4.2**. The *RSD* value was calculated to evaluate whether the stability of the sample solution was excellent.

2.8.3 Reproducibility test. The polysaccharide test sample was obtained according to the method described in Section **2.5.3**. Six 1 mL portions of the polysaccharide test sample were precisely pipetted, and the absorbance value A was determined following the method in Section **2.4.2**. The *RSD* value was calculated to verify whether the reproducibility of this method for determining polysac-

charide content was excellent.

2.8.4 Spiked recovery test: First, three 0.5 mL portions of the polysaccharide test sample solution prepared according to the method in Section **2.4.3** were precisely pipetted, and each was then supplemented with 0.5 mL of distilled water to a total volume of 1 mL. The absorbance value A was determined following the method in Section **2.4.2**. Next, nine 0.5 mL portions of the same polysaccharide test sample solution mentioned above were precisely pipetted and divided into three groups. Each group was supplemented with 0.5 mL of the 0.1 mg/mL glucose standard reference solution at concentrations of 80%, 100%, and 120%, respectively. The absorbance value A was determined following the method in Section **2.4.2**, and the average recovery rate and *RSD* were calculated.

2.9 Comparison of different OJPS extraction methods The optimal process conditions from the orthogonal experiment were selected, and using the extraction yield as the evaluation indicator, reflux extraction, ultrasonic extraction, and the novel IH extraction were compared in parallel.

2.9.1 Reflux extraction method. 25 g of dried *Ophiopogon japonicus* was weighed and placed in a 1 000 mL round-bottom flask with 10 times the amount of water. The mixture was subjected to condensing reflux extraction twice, each time for 1.5 h. The resulting extract was filtered twice with medical gauze, followed by two rounds of hot suction filtration with fine filter paper to remove residual herb residues and sediment, yielding a clear filtrate. The obtained filtrate was then concentrated using a rotary evaporator to a ratio of 1∶2 relative to the raw herb material. The concentrated solution was subsequently extracted with dichloromethane and petroleum ether to remove liposoluble impurities. After extraction, an appropriate amount of distilled water was added to the extract for secondary concentration to a ratio of 1∶1 relative to the raw herb material, ensuring the removal of residual extraction solvents. A certain amount of 95% ethanol was then added to the extract under continuous stirring, and the mixture was placed in a refrigerator at 5 ℃ for overnight ethanol precipitation. After ethanol precipitation, the supernatant was discarded, and the precipitate was dried until no alcohol odor remained, yielding the reflux-extracted polysaccharide product.

2.9.2 Ultrasonic Extraction Method. 25 g of dried *Ophiopogon japonicus* was weighed and placed in a 1 000 mL beaker with 10 times the amount of water. The mixture was extracted twice under 40 kHz ultrasonic conditions (extraction mode: 30 min ultrasonication, 20 min interval, 5 cycles per extraction), with each extraction lasting 1.5 h. The resulting extract was filtered twice with medical gauze, followed by two rounds of hot suction filtration with fine filter paper to remove residual herb residues and sediment, yielding a clear filtrate. The obtained filtrate was then concentrated using a rotary evaporator to a ratio of 1∶2 relative to the raw herb material. The concentrated solution was subsequently extracted with dichloromethane and petroleum ether to remove liposoluble impurities. After extraction, an appropriate amount of distilled water was added to the extract for secondary concentration to a ratio of 1∶1 relative to the raw herb material, ensuring the removal of residual extraction solvents. A certain amount of 95% ethanol was then added to the extract under continuous stirring, and the mixture was placed in a refrigerator at 5 ℃ for overnight ethanol precipitation. After ethanol precipitation, the supernatant was discarded, and the precipitate was dried until no alcohol odor remained, yielding the ultrasonically extracted polysaccharide product.

3 Results and analysis

3.1 Plotting of glucose standard curve The maximum wavelength scan of glucose is shown in Fig. 3. The graph indicates three absorption peaks at 450, 490, and 628 nm. Among these, the maximum absorption wavelength was 628 nm, and interference from end absorption can be avoided at this wavelength. Therefore, 628 nm was selected as the most suitable detection wavelength. At this wavelength, the absorbance *A* of various mass concentrations of glucose was measured, yielding the regression equation for the glucose standard curve: $y = 4.857\,5x + 0.003\,1$, as shown in Fig. 4.

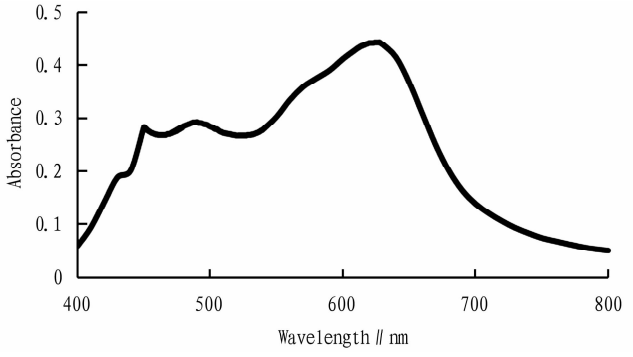

Fig. 3 Maximum wavelength scanning spectrum of glucose

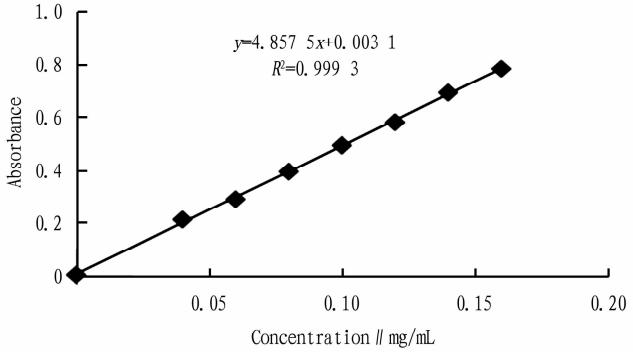

Fig. 4 Standard curve of glucose

3.2 Results of single factor experiment

3.2.1 Solid-to-liquid ratio. As shown in Fig. 5, the polysaccharide content increased with the solid-to-liquid ratio and peaked at 49.67% at a ratio of 1∶60, beyond which it decreased. This trend can be attributed to the fact that at lower solid-to-liquid ratios, the insufficient extraction solvent prevented the complete dissolution of polysaccharides from *O. japonicus*, leading to a lower yield. On the contrary, while higher ratios provided ample solvent, which facilitated dissolution, they resulted in a prolonged concentration process that caused the degradation of some polysac-

charides, thereby reducing the extraction yield[13]. Therefore, the optimal solid-to-liquid ratio was determined to be 1 ∶ 60.

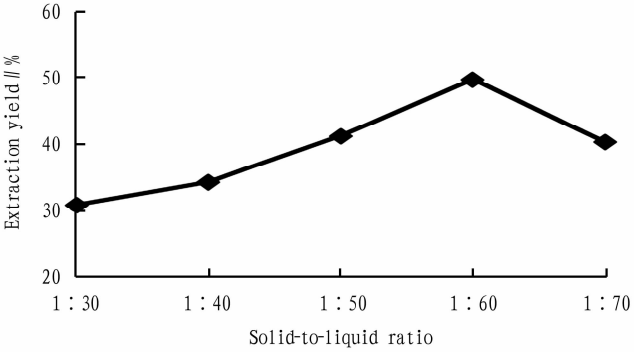

Fig. 5 Effects of different solid-to-liquid ratios on the extraction yield of OPJS

3.2.2 Extraction time. As shown in Fig. 6, when the extraction time was within the range of 40 – 70 min, the polysaccharide extraction yield increased with prolonged time, reaching a maximum value of 36.39% at 70 min. Further extending the extraction time resulted in a downward trend in the polysaccharide extraction yield. This might be due to the fact that a short extraction time did not allow sufficient dissolution of polysaccharides, leading to a low extraction yield, while appropriately prolonging the extraction time could indirectly increase the dissolution time of polysaccharides. However, excessively long extraction times might cause boiling of polysaccharide molecules inside the medicinal material, blocking the diffusion channels and making it difficult for polysaccharides to dissolve, thereby reducing the polysaccharide extraction yield[14]. Therefore, the optimal extraction time should be around 70 min.

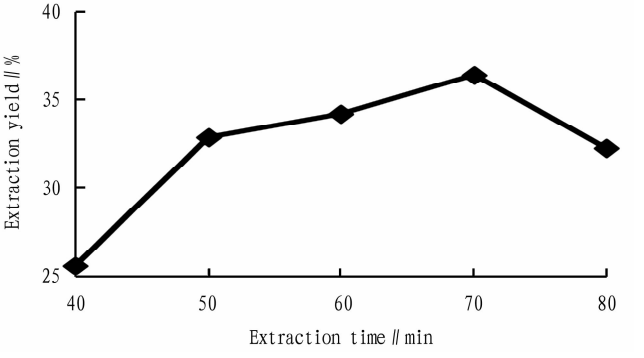

Fig. 6 Effects of different extraction time on OJPS extraction yield

3.2.3 Effect of extraction ratio. As shown in Fig. 7, the polysaccharide extraction yield decreased as the ratio of the extraction liquid to petroleum ether increased. The yield stabilized when the ratio reached 3 ∶ 1. This phenomenon can be attributed to the dependence of the extraction yield on the volume of the organic phase. This correlation is consistent with the formula $E = \frac{D}{D + V_w/V_0}$ discussed by Kumar SP *et al.*[15]. The formula describes the relationship between the recovery rate and phase volumes. In the formula, D is partition coefficient; V_w is aqueous phase volume; V_0 is organic phase volume; E is recovery rate). When the organic phase volume increases, and when the organic phase volume increases to a certain extent, the ratio of V_w/V_0 is small, and the change of E is also small and tends to be stable. Therefore, the best extraction ratio should be about 1 ∶ 2.

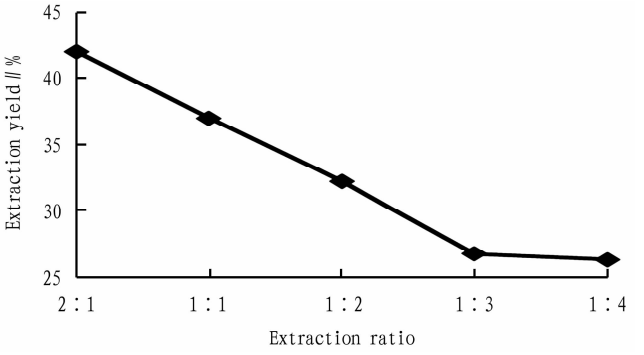

Fig. 7 Effects of different extraction ratio on OJPS extraction yield

3.2.4 Alcohol precipitation ratio. As shown in Fig. 8, when the alcohol precipitation ratio was in the range of 1 ∶ 3 to 1 ∶ 4, the polysaccharide extraction yield increased with the rise in the alcohol precipitation ratio, reaching a maximum value of 43.7% at a ratio of 1 ∶ 4. However, when the alcohol precipitation ratio was further increased, the polysaccharide extraction yield showed a downward trend. This phenomenon may be attributed to the following reasons: the increase in ethanol content in the alcohol precipitation system reduces the dielectric constant of the medium, which weakens the stability of the binding between water molecules and the hydroxyl groups of carbohydrates. As a result, the hydroxyl groups on polysaccharide molecular chains become more prone to intertwining and forming stable hydrogen bonds, promoting tighter entanglement and aggregation among polysaccharide molecules, thereby facilitating precipitation. Alternatively, increasing the amount of ethanol within a certain range may reduce the solubility of polysaccharides, which also favors their precipitation[16-17]. However, when the amount of ethanol is further increased, some impurities may co-precipitate, which adversely affects polysaccharide precipitation and consequently leads to a decrease in the polysaccharide extraction yield.

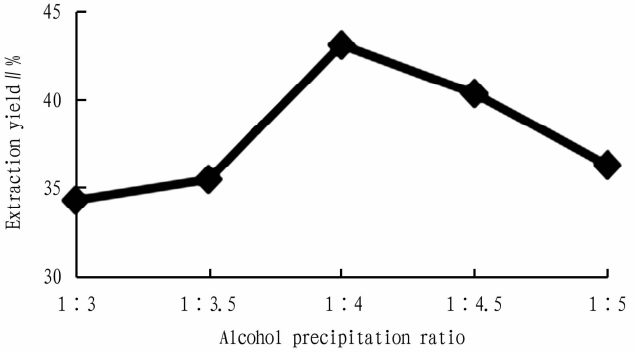

Fig. 8 Effects of different alcohol precipitation ratios on OJPS extraction yield

3.3 Results of orthogonal experiment

3.3.1 Analysis of orthogonal results. Based on the single-factor investigations, solid-to-liquid ratio, extraction time, and alcohol

precipitation ratio were selected to design an $L_9(3^4)$ orthogonal experiment to optimize the IH extraction process of polysaccharides. The levels for each factor are shown in Table 1. Considering the comprehensive effects of the extraction solvent volume and the extraction ratio on the extraction yield, and to ensure the highest polysaccharide extraction yield while using a low volume of petroleum ether, an extraction ratio of 2∶1 was adopted. The orthogonal experimental data (Table 2) were processed and analyzed using SPSS 24.0, resulting in the range analysis table (Table 3), the mean value plot of factor levels (Fig. 9), and the analysis of variance (ANOVA) table (Table 4).

Table 1 Orthogonal factor level

Level	Factor		
	A (solid-to-liquid ratio)	B (extraction time)//min	C (alcohol precipitation ratio)
1	1∶55	65	1∶3.75
2	1∶60	70	1∶4.00
3	1∶65	75	1∶4.25

Table 2 Orthogonal experimental data

No.	A	B	C	D (blank)	OPJS extraction yield//%
1	1	1	1	1	45.18
2	1	2	2	2	49.64
3	1	3	3	3	48.20
4	2	1	2	3	45.34
5	2	2	3	1	44.56
6	2	3	1	2	45.14
7	3	1	3	2	44.59
8	3	2	1	3	46.14
9	3	3	2	1	48.50

As shown in Table 3, the solid-to-liquid ratio, extraction temperature, and alcohol precipitation ratio all had significant effects on the OJPS extraction yield ($P<0.05$). According to Table 4, the range values R followed the order: RA > RC > RB, indicating that the order of factors affecting the polysaccharide extraction yield was: solid-to-liquid ratio > alcohol precipitation ratio > extraction time. Furthermore, based on Table 3 and the mean value plot of factor levels (Fig. 9), the optimal conditions for the novel IH extraction process were determined as $A_1B_3C_2$, specifically corresponding to a solid-to-liquid ratio of 1∶55, an extraction time of 75 min, and an alcohol precipitation ratio of 1∶4.

Table 3 Range analysis results

Item	Level	A (solid-to-liquid ratio)	B (extraction time)	C (alcohol precipitation ratio)
K	1	143.02	135.11	136.46
	2	135.04	140.34	143.48
	3	139.23	141.84	137.35
K avg	1	47.67	45.04	45.49
	2	45.01	46.78	47.83
	3	46.41	47.28	45.78
Optimal level	1	3	2	
R	2.66	2.24	2.34	
Level quantity	3	3	3	
Number of repetitions per level (*r*)	3	3	3	

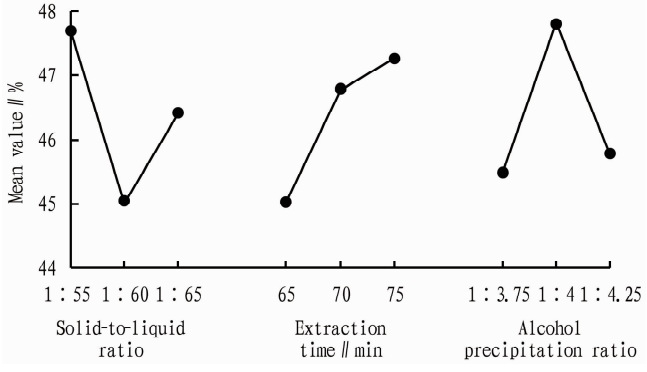

Fig. 9 Mean value of each factor level

Table 4 Variance analysis results

Source of variation	Class III sum of squares	Degree of freedom	Mean square	*F*	Significance
Modified model	28.683a	6	4.780	24.966	0.039
Intercept	19 347.883	1	19 347.883	101 045.055	0.000
Solid-to-liquid ratio	10.622	2	5.311	27.738	0.035
Extraction time	8.322	2	4.161	21.730	0.044
Alcohol precipitation ratio	9.739	2	4.869	25.431	0.038
Error	0.383	2	0.191		
Total	19 376.949	9			
Total after modification	29.066	8			

3.3.2 Validation of the optimal extraction process. The test results showed that the average absorbance from three extraction trials was 0.631. Under the optimal OJPS extraction conditions, the extraction yield reached 51.74%, with an *RSD* of 1.11% across the three trials, indicating that the process is stable and feasible.

3.4 Results of methodological validation The methodological validation results confirmed the reliability of the analysis: the precision experiment showed an *RSD* of 1.23% (*RSD* < 2%), demonstrating excellent instrument reproducibility; the stability test yielded an *RSD* of 1.69% (*RSD* < 2%), indicating the polysaccharide solution remained stable within 24 h; in addition, the repeatability test obtained an *RSD* of 1.13% (*RSD* < 2%), verifying the high consistency of the method for polysaccharide content determination.

From Table 5, it can be known that the recovery rates of each group were 99.05%, 101.46% and 99.31%, the average recovery rate was 99.94% (97% ≤*P*≤103%), and the *RSD* was less than 2%, indicating that the method had a good recovery rate.

Table 5 Results of the spike recovery test

Group	No.	Absorbance	Mean	Recovery rate//%	*RSD*//%
Control	①	0.312	0.316		1.11
	②	0.319			
	③	0.316			
80%	①	0.502	0.510	99.05	1.26
	②	0.512			
	③	0.514			
100%	①	0.551	0.564	101.46	2.22
	②	0.564			
	③	0.576			
120%	①	0.616	0.607	99.31	1.48
	②	0.607			
	③	0.598			

As shown in Table 6, the average *RSD* of the three extraction methods was less than 2%, indicating that all methods were stable and feasible. The OJPS extraction yields for reflux extraction, ultrasonic extraction, and the novel IH extraction were 26.67%, 40.70%, and 51.74%, respectively. The extraction yield of the novel IH method was 1.94 times that of reflux extraction and 11.04% higher than that of ultrasonic extraction. These results demonstrate that the novel IH extraction technology significantly improves the OJPS extraction yield and offers distinct advantages over both reflux and ultrasonic extraction methods.

Table 6 Effects of different extraction methods on OJPS extraction yield

Group	No.	Absorbance	Mean	Recovery rate//%	*RSD*//%
IH extraction	①	0.624	0.631	51.74	1.11
	②	0.638			
	③	0.632			
Ultrasonic extraction	①	0.491	0.498	40.70	1.41
	②	0.497			
	③	0.505			
Reflux exraction	①	0.326	0.327	26.67	1.24
	②	0.331			
	③	0.323			

4 Discussion

By comparing the experimental results of IH extraction, reflux extraction, and ultrasonic extraction in parallel, it was found that the novel IH extraction exhibited significant advantages in improving polysaccharide extraction efficiency, with a much higher yield than reflux extraction. Ultrasonic extraction also showed certain benefits, yielding more than reflux extraction but less than IH extraction. The superior performance of ultrasonic extraction over reflux extraction may be attributed to the cavitation, mechanical, and thermal effects generated by ultrasound at specific frequencies. The cavitation effect instantly produces numerous microscopic bubbles in the liquid, and the high temperature and pressure released upon bubble collapse help loosen tightly bound plant tissues and even disrupt plant cell walls. The mechanical effect causes solvent water molecules to vibrate, enhancing the propagation and diffusion of the solvent and facilitating the dissolution and diffusion of polysaccharides. The thermal effect enables rapid energy transfer between the solvent and polysaccharides, increasing the dissolution rate. Under the combined influence of these three effects, the release, dissolution, and diffusion of polysaccharides are promoted, allowing more polysaccharides to transfer more quickly into the distilled water, thereby improving the polysaccharide extraction yield[18-20].

The excellent performance of the novel IH extraction compared to ultrasonic extraction may be attributed to the following mechanism: IH technology utilizes an electric current passing through a coil to generate an alternating magnetic field. Under this field, a reaction kettle with magnetic permeability and electrical conductivity develops eddy currents. These currents cause the randomly oriented polar molecules in the material to be influenced by electromagnetic waves from the high-frequency alternating magnetic field, leading them to align in specific orientations that continuously change. During this realignment, the molecules move and collide incessantly, converting electromagnetic field energy into thermal energy in the water. This rapidly raises the intracellular temperature, vaporizing the liquid water inside the cells and continuously increasing the internal pressure until it exceeds the cell wall's maximum bearing capacity, resulting in cell rupture and pore formation. In addition, the heating process further reduces water vaporization within the cells and cell walls, causing cell contraction and crack formation. These micropores and cracks enhance substance exchange inside and outside the cells, accelerating solvent penetration into the cells and promoting the dissolution and release of polysaccharides into the external medium, thereby improving polysaccharide extraction efficiency[21-23]. In practice, due to the hard texture or thick cell walls of many medicinal materials, traditional extraction methods often fail to effectively release polysaccharides. In contrast, the cell-disrupting effect of IH extraction technology offers a promising alternative and opens up new possibilities for extracting polysaccharides from such challenging sources.

Furthermore, compared with traditional polysaccharide extraction methods, IH technology achieves a thermal efficiency of over 90%, which increases the heating rate by 60% and significantly shortens equipment preheating time. As a result, it offers remarkable energy-saving benefits, with energy consumption reduced by more than 30%[24-25]. By further integrating IH technology into deeper stages of polysaccharide extraction, the extraction efficiency of polysaccharides from TCM can be substantially improved. This approach not only provides valuable insights for the

development of TCM polysaccharide-based formulations but also fully leverages the advantages of IH technology in terms of energy savings, high efficiency, and environmental friendliness. Ultimately, it will help promote the innovative development, energy conservation, emission reduction, and sustainable industrial production of TCM preparations.

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