BIBLIOGRAPHIC INFORMATION SYSTEM

JOURNAL FULL TITLE: Journal of Biomedical Research & Environmental Sciences
ABBREVIATION (NLM): J Biomed Res Environ Sci **ISSN:** 2766-2276 **WEBSITE:** https://www.jelsciences.com

SCOPE & COVERAGE

- **Sections Covered:** 34 specialized sections spanning 143 topics across Medicine, Biology, Environmental Sciences, and General Science
- Ensures broad interdisciplinary visibility for high-impact research.

PUBLICATION FEATURES

- **Review Process:** Double-blind peer review ensuring transparency and quality
- **Time to Publication:** Rapid 21-day review-to-publication cycle
- **Frequency:** Published monthly
- **Plagiarism Screening:** All submissions checked with iThenticate

INDEXING & RECOGNITION

- **Indexed in:** Google Scholar, IndexCopernicus (**ICV 2022: 88.03**)
- **DOI:** Registered with CrossRef (**10.37871**) for long-term discoverability
- **Visibility:** Articles accessible worldwide across universities, research institutions, and libraries

OPEN ACCESS POLICY

- Fully Open Access journal under Creative Commons Attribution 4.0 License (CC BY 4.0)
- Free, unrestricted access to all articles globally

GLOBAL ENGAGEMENT

- **Research Reach:** Welcomes contributions worldwide
- **Managing Entity:** SciRes Literature LLC, USA
- **Language of Publication:** English

SUBMISSION DETAILS

- Manuscripts in Word (.doc/.docx) format accepted

SUBMISSION OPTIONS

- **Online:** https://www.jelsciences.com/submit-your-paper.php
- **Email:** support@jelsciences.com, support@jbresonline.com

HOME ABOUT ARCHIVE SUBMIT MANUSCRIPT APC

Vision: The Journal of Biomedical Research & Environmental Sciences (JBRES) is dedicated to advancing science and technology by providing a global platform for innovation, knowledge exchange, and collaboration. Our vision is to empower researchers and scientists worldwide, offering equal opportunities to share ideas, expand careers, and contribute to discoveries that shape a healthier, sustainable future for humanity.

JOURNAL OF BIOMEDICAL RESEARCH & ENVIRONMENTAL SCIENCES
ISSN: 2766-2276
SUBJECT AREA(S): BIOTECHNOLOGY | BIOCHEMISTRY
DOI: 10.37871

RESEARCH ARTICLE

Extraction and Antioxidant Activity of Polysaccharides from *Tremella fuciformis* by Deep Eutectic Solvents

Hua Zhang[1†], Yashu Wei[1†], Xingjian Ma[2], Yan He[1] and Xia Ma[1*]

[1]School of Perfume and Aroma Technology, Shanghai Institute of Technology, No. 100 Haiquan Road, Shanghai 201418, PR China

[2]Software Engineering, Harbin University of Science and Technology, Wei Hai. No.2006 Xueyuan Road, Rongcheng City, Shandong 264300, PR China

[†]Hua Zhang and Yashu Wei have contributed equally to this work.

Abstract

Tremella fuciformis polysaccharidess possess broad application potential in diverse industrial fields. Submerged fermentation is an efficient technology for producing fermented Tremella fuciformis polysaccharides FTP. In this study, Deep Eutectic Solvents (DES) were adopted to extract FTP from fermentation broths after comparing multiple extraction strategies. The structural characteristics, antioxidant activity, and viscosity stability of the purified components were systematically characterized. Extraction parameters were optimized *via* Box–Behnken design of response surface methodology. Under optimal conditions, the FTP extraction yield reached 75.35 mg/g, representing an 88.06% improvement relative to the traditional hot water extraction and ethanol precipitation. The characterization results of purified components (FTP1 and FTP2) revealed the presence of α-pyranoside bonds and a triple-helix conformations in both compounds. Assays for antioxidant activities demonstrated that FTP1 exhibited significantly greater DPPH and hydroxyl radical scavenging activities compared to FTP2. The results of the viscosity stability test indicate that both FTP1 and FTP2 are suitable for various applications in the food industry. Notably, this is the first report employing deep eutectic solvents (DES) to extract Tremella fuciformis polysaccharidess (FTP) from *Tremella fuciformis* fermentation broth. This study systematically validates the comprehensive advantages of DES against conventional extraction techniques and offers a sustainable green protocol for the preparation of polysaccharidess derived from fungal submerged fermentation. Collectively, FTP1 and FTP2 hold great industrial development value as natural functional biomaterials.

***Corresponding author(s)**

Xia Ma, School of Perfume and Aroma Technology, Shanghai Institute of Technology, No. 100 Haiquan Road, Shanghai 201418, PR China
Email: maxia@sit.edu.cn

DOI: 10.37871/jbres2309

Submitted: 16 June 2026

Accepted: 26 June 2026

Published: 29 June 2026

Keywords

- Deep eutectic solvent
- *Fermented Tremella* fuciformis polysaccharidess
- Antioxidant activity
- Viscosity

VOLUME: 7 ISSUE: 6 - JUNE, 2026

How to cite this article: Zhang H, Wei Y, Ma X, He Y, Ma X. Extraction and Antioxidant Activity of Polysaccharides from *Tremella fuciformis* by Deep Eutectic Solvents. J Biomed Res Environ Sci. 2026 June 29; 7(6): 18. Doi: 10.37872/jbres2309

Introduction

Tremella fuciformis, a member of the *Tremellales* order and *Tremellaceae* family, is a widely distributed fungus commonly found on decaying branches of broadleaf trees in tropical regions [1]. The polysaccharidess derived from *Tremella fuciformis*, known as *Tremella fuciformis* polysaccharidess (FTP), have been scientifically proven to possess various physiological activities, including hypoglycemic lipid, anti-aging, antitumor, wound healing, immunomodulation, and neuroprotective effects [2]. Yang, et al. [3] in our research team confirmed that *Tremella* polysaccharidess has good moisturizing properties and has potential application in the field of beauty. With modern submerged fermentation technology, high-purity FTP can be continuously produced without relying on rare wild fruiting bodies, laying a foundation for large-scale industrial manufacture of functional food additives.

Tremellam is predominantly found within the solid cells of *Tremella* fruiting bodies and developing mycelium, or it is secreted into the fermentation broth during liquid cultivation. The utilization of submerged culture represents an innovative approach for the production of edible fungal polysaccharidess. In comparison to the extraction of polysaccharidess from fruiting bodies, the extraction from fermentation broth offers several advantages, including controllable processing, shorter duration, reduced costs, and enhanced stability of the resulting polysaccharidess. Meanwhile, submerged fermentation serves as an efficient technique for the production of fermented *Tremella fuciformis* polysaccharidess (FTP) [4,5].

At present, the extraction of FTP primarily relies on hot water extraction, enzyme-assisted extraction, and ultrasound-assisted extraction methods [1,6,7]. Although the hot water extraction method is relatively straightforward, it necessitates a lengthy extraction period and Requires prolonged high-temperature heating (180-240 min), consumes massive volumes of water and ethanol, and weakens polysaccharidess triple-helical structures via thermal hydrolysis, leading to low extraction yield (~40 mg/g) and deteriorated antioxidant activity [8,30]. In addition, enzyme preparations tend to be more costly, and the extraction of enzyme-assisted methods are narrow optimal pH-temperature windows; residual protein impurities complicate downstream purification and reduce food safety grade [9]. Moreover, ultrasound-assisted extraction requires more sophisticated equipment [10]. Additionally, traditional separation workflows frequently adopt toxic volatile organic solvents for impurity removal, generating hazardous wastewater and solvent residue risks for food-grade products. Therefore, the development of novel environmentally friendly solvents is a crucial area of focus for enhancing the efficiency and sustainability of chemical processes. The global demand for solvent substitution is increasing due to the extensive use of organic reagents in the extraction of fermented polysaccharidess and the associated environmental, health, and safety concerns related to organic solvents [11]. In 2001, deep eutectic solvent (DES) were first introduced as a group of emerging solvents with the potential to serve as alternatives to traditional solvents and conventional ionic liquids [12]. DES have demonstrated their efficacy as excellent solvents for extracting numerous phytochemicals from biomass and agricultural by-products, surpassing the selectivity and extractability of conventional volatile and toxic organic solvents [13]. DES offers several advantages, including its environmentally benign nature, low cost, biodegradability, and ease of preparation. In addition, due to its unique properties, DES has the potential to significantly enhance extraction efficiency [14]. As a result, DES has been predominantly employed as a green solvent in separation and extraction processes. Multiple studies validated DES's remarkable

extraction efficiency for plant, mushroom polysaccharidess and phenolic compounds [15-17]. However, to the best of our knowledge, there have been no reports on the extraction of polysaccharidess from fermentation broth using DES, which constitutes the core novelty of this study.

Tremella fuciformis polysaccharidess, as a heteropolysaccharides, has different biological activities with different components. The bioactivity of this heterosaccharide has been extensively investigated. However, the relationship between FTP isolation, the structure of purified components, and their bioactivity remains unclear. In this study, The FTP1 and FTP2 polysaccharidess were extracted and purified from crude FTP, and their characteristics were analyzed using Fourier Transform Infrared (FTIR) spectroscopy, Congo red test, and Scanning Electron Microscopy (SEM). In addition, the antioxidant activity and viscosity stability of the purified components were tested *In vitro*, further validating the superiority of the DES extraction strategy proposed herein [18,19]. The findings of this study offer a theoretical foundation advancement and utilization of FTP.

Materials and Methods

Materials

Tremella fuciformis spores (strain *Tremella fuciformis* Beck) were purchased from the Guangdong Province Microbial Culture Collection Center (GDMCC, deposit number GDMCC 5.39). The following analytical-grade chemicals were procured from Shanghai Titan Scientific Co. Ltd.: choline chloride (99%), ethylene glycol (AR, ≥ 99.0%), glycerol (AR), lactic acid (AR, ≥ 80.0%), urea (AR, ≥ 99.0%), oxalic acid (AR, ≥ 99.0%), glucose (AR), and sulfuric acid (AR, 95-98%). Neutral protease was acquired from OXOID, while phenol was purchased from Sinopharm Chemical Reagent Co. Ltd. All reagents and chemicals utilized were of analytical grade in this work. Dialysis bags with a molecular weight cut-off range of 8000-14000 Da were obtained from Beijing Solarbio Science & Technology Co., Ltd., China

Preparation and properties determination of DES

The preparation of various types of DES in this experiment was carried out using a heating and stirring method, as described by Kandanelli, et al. [20]. For the purpose of extracting FTP from freeze-dried *Tremella fuciformis* spore fermentation broth, six different DES systems were prepared (DES-1: Choline chloride-urea; DES-2: Choline chloride-glycerol; DES-3: Choline chloride-glucose; DES-4: Choline chloride-lactic acid; DES-5: Choline chloride-oxalic acid; DES-6: Choline chloride-ethylene glycol. Mole ratio: 1:2), to establish an efficient solvent system.

Single factor test and response surface method (RSM)

For the initial screening of the extraction solvent and method, a precise weight of 1.0000 g of freeze-dried *Tremella fuciformis* fermentation broth powder was combined with 20 mL of DES in a molar ratio of 1:2, with a water content of 60% (every 100g of extraction solvent contains 60g of distilled water and 40 g of DES). The mixture was then subjected to heating in a water bath at 60°C for 60 min within a flask. Subsequently, the mixture underwent centrifugation at a speed of 8000 r/min for 15 min. The resulting supernatant was identified as the *Tremella fuciformis* polysaccharidess extract. To determine the content of total polysaccharidess in the DES extract, the phenol-sulfuric acid method was employed, utilizing glucose as the standard. The standard curve was represented by the equation $Y = 1.4343x - 0.0528$, $R^2 = 0.9958$. It is important to note that the polysaccharidess content mentioned in the text refers to the total sugar content. The extraction

yield of total polysaccharidess was calculated using the following formula:

Extraction yield (mg/g) = weight of polysaccharidess (mg)/weight of sample (g) (1)

This study firstly investigated the effects of varying water content (40, 50, 60, 70, 80%), extraction temperature (30, 40, 50, 60, 70˚C), extraction time (30, 60, 90, 120, 150 min), and solid-liquid ratio (1:10, 1:20, 1:30, 1:40, 1:50 g/mL) on the extraction yield of FTP through a single-factor experimental design. Subsequently, it was observed that three parameters (water content, extraction temperature and extraction time) exerted a more significant influence on the extraction yield of FTP. Therefore, these three parameters were selected for further experimentation.

According to the single-factor experimental results, a Box-Behnken design (BBD) with a 3-factor-3-level configuration was employed to investigate the influence of three variables and their interactions. The selection of factors and levels for the RSM was based on the preliminary single-factor experiments. The factors considered in the RSM were DES water content (50, 60, 70%), extraction time (90, 120, 150 min), and extraction temperature (30, 40, 50˚C). In this study, RSM was utilized to optimize the process conditions for DES extraction of FTP. The data obtained were subjected to multiple regression analysis, and a quadratic polynomial model was fitted as follows:

$$Y = A_0 + \sum_{i=1}^{3} A_i + \sum_{i=1}^{3} A_i X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} A_j X_i X_j \quad (2)$$

Where Y represent the response variable; A_0, A_i, A_{ii}, and A_{ij} denote the regression coefficients for intercept, linearity, square, and interaction, respectively; X_i and X_j refer to the different independent vari

Characterization of purified component

Extraction and purification of FTP: Freeze-dried powder of *Tremella fuciformis* fermentation broth was mixed with DES, followed by water-bath heating. The mixture was then centrifuged at 8000 r/min for 15 min to collect the supernatant. Four volumes of 95% ethanol were added to the supernatant, and the mixture was incubated at 4˚C for 28 h. After centrifugation (8000 r/min, 15 min), crude polysaccharides precipitate was harvested.

Crude FTP solution (20 mg/mL) was loaded onto a DEAE Sepharose Fast Flow anion-exchange column (2.6 cm×30 cm) for purification. Stepwise gradient elution was performed with aqueous NaCl solutions at concentrations of 0, 0.2, 0.4, 0.6 and 0.8 mol/L. Eluent fractions (7 mL per tube) were collected at a constant flow rate of 1 mL/min via an automatic fraction collector (BSZ-100, Shanghai Huxi Analytical Instrument Factory Co., Ltd., China). The total carbohydrate content of each fraction was quantified by the phenol-sulfuric acid colorimetric method. Homogeneous polysaccharides fractions were combined, dialyzed against distilled water for 3 days, and finally lyophilized to obtain purified FTP components.

Fourier transform infrared (FTIR) spectroscopy analysis: The samples were analyzed using a FTIR spectroscopy spectrophotometer (Nicolet iN10 model from Thermo Fisher Scientific). The FTIR spectra were recorded in the wavenumber range of 400-4000 cm^{-1} with a resolution of 2cm^{-1}.

Congo red assay: The two purified components, FTP1 and FTP2 were prepared into 12 mL 1 mg/mL solution, respectively, and 6 mL (80μmol/L) Congo red solution was added. Put 1 mol/L of 0, 0.1, 0.2, 0.3, 0.4, 0.5 mL of NaOH solution into a test tube and fill with liquid A to 1 mL. After mixing, the maximum absorption wavelength (λ_{max}) in the range of 400 - 600 nm is scanned and recorded with an ultraviolet spectrophotometer. The curve is drawn with the

NaOH concentration as the abscissa and λ_{max} as the ordinate.

Scanning electron microscopy (SEM) analysis: The powdered FTP1 and FTP2 samples were observed using a scanning electron microscopy (S-3400N, Hitachi Gaoxin, Japan) at an accelerating voltage of 5 kV.

***In vitro* Antioxidant activity of purified FTPs:** The DPPH, ABTS, hydroxyl radical (·OH) scavenging activities and reducing power of FTP1 and FTP2 were determined. The detailed experimental steps are as follow:

DPPH radical scavenging activity: 3 mL of DPPH (0.2 mmol/L) in methanol were added to 1 mL of sample solutions with varying concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL). The resulting mixture was thoroughly mixed and placed in a dark environment at room temperature for a duration of 30 min. Subsequently, the absorbance values of DPPH were measured at a wavelength of 517 nm. As a positive control, the sample was replaced by Vc. The formula used for the calculation is as follows:

$$\text{DPPH radical scavenging activity (\%)} = 1-(A_i - A_j)/A_c \times 100 \quad (3)$$

Where A_i represents the absorbance of the free radical solution with sample solution; A_j denotes the absorbance of the sample solution; and A_c refers to the free radical solution.

ABTS radical scavenging activity: 2 mL of ABTS (10 mmol/L) in phosphate buffer was introduced to 2 mL of sample solutions possessing varying concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL). Subsequently, the resulting mixture was thoroughly agitated. Following a 30 min incubation period at ambient temperature, the absorbance values of the mixture were measured at 734 nm. It is worth noting that Vc was employed as the positive control. The calculation formula employed in this study remained consistent with formula 3.

Hydroxyl radical scavenging activity: 1 mL of H_2O_2 (8.8 mmol/L), 1 mL of salicylic acid-ethanol (9 mmol/L), and 1 mL of $FeSO_4$ (9 mmol/L) were combined with 1 mL of sample solutions possessing varying concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL). Subsequently, the resulting mixture was subjected to a 30 min incubation period at 37°C. Following this incubation, the absorbance of the mixture was measured at 510 nm. It is important to note that Vc was utilized as the positive control. The calculation formula employed in this study remained consistent with formula 3.

Determination of reducing power: 1 mL of sample solutions with varying concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) were combined with 1 mL of 1% (w/v) $K_3[Fe(CN)_6]$ and 1 mL of Phosphate buffer (pH = 6.6). The resulting mixture was allowed to react at a temperature of 50°C for a duration of 20 min. Subsequently, 2 mL of 0.3% trichloroacetic acid was added to the reaction solution. Following this, 2 mL of distilled water and 0.4 mL of 0.3% (w/v) $FeCl_3$ were added to a 2 mL mixed solution, and the reaction was carried out at 50°C for 10 min. The absorbance of the solution was then measured at 700 nm. The formula employed is as follows:

$$X = A_1 - A_2 \quad (4)$$

Where, X represents reducing capacity; A_1 denotes the absorbance value of the sample group; A_2 refers to the absorbance value of the blank group.

Viscosity stability of purified FTP: The viscosity curve of the sample solution over time was determined using a DV3T viscometer (Brookfield, Middleboro, MA, USA). The purified component solutions (50 mL) was added to the sample pool. To ensure accurate measurements, the appropriate rotor was selected to maintain the torque between 10% and 100%. The rotation speed was set at 30 r/min, the temperature was maintained at 25°C, and the measurement

duration was 1 min.Effect of temperature on viscosity: Purified component solutions (4 mg/mL) were kept at 30, 40, 50, 60, 70, 80 and 90°C for 10 min. The viscosity was determined at 30 r/min, respectivelyEffect of concentration on viscosity: The purified components were accurately weighed and prepared by dissolving them in distilled water to obtain concentrations of 1, 2, 3, 4, 5, 6, and 7 mg/mL. The viscosity of each solution was then determined at a constant rotation speed of 30 r/min.

Effect of pH on viscosity: The viscosity of the purified component solutions (4 mg/mL) was measured at 30 r/min. The pH of each solution was adjusted using 1 mol/L HCl solution and 1 mol/L NaOH solution to achieve pH values of 2, 4, 6, 8, 10, and 12.

Effect of salt ions on viscosity: 0.5% and 1.0% NaCl and $CaCl_2$ were added to the purified component solutions (4 mg/mL). This resulted in final concentrations of NaCl and $CaCl_2$ of 5% in the solutions. However, it was observed that the addition of these inorganic salts caused the gel solution system to become unstable. After allowing the solution to stand, the viscosity was measured at 30 r/min.

Statistical Analysis

All experimental determinations were performed in three independent biological replicates (n = 3). All quantitative data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed byLSDandS–N–Kpost-hocmultiplecomparison tests were conducted via SPSS Statistics 27 software to evaluate inter-group significant differences. Statistical significance thresholds: $p < 0.05$ (marked with different lowercase letters in all figures), $p < 0.01$ (extremely significant). Origin 2022 software was adopted for all graph plotting; every figure explicitly labels sample number (n = 3), significance letter notation and unit specifications in captions and axis labels to enhance readability.

Results and Discussion

Screening of DES system for extraction of FTP

This study investigated the efficacy of six distinct choline chloride-based DES in extracting freeze-dried *Tremella fuciformis* fermentation broth to obtain FTP. The extraction yields of FTP using different solvents were depicted in figure 1. The results displayed significant variations in the ability and efficiency of DES for FTP extraction. The total sugar extraction yields of the six DES preparations from *Tremella fuciformis* were as follows: 15.75, 57.95, 25.27, 16.30, 16.29, and 36.22 mg/g, respectively. These findings indicated that all DES were capable of extracting FTP; however, DES-2 exhibited the highest yield of 57.95 ± 5.17 mg/g in terms of polysaccharides content, surpassing the other DES. It is worth noting that DES derived from different combinations of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) possess distinct physicochemical properties, including surface tension, polarity, and solubility [21]. The varying extraction rates of FTP among different DES systems have been established. Furthermore, Radošević, et al.

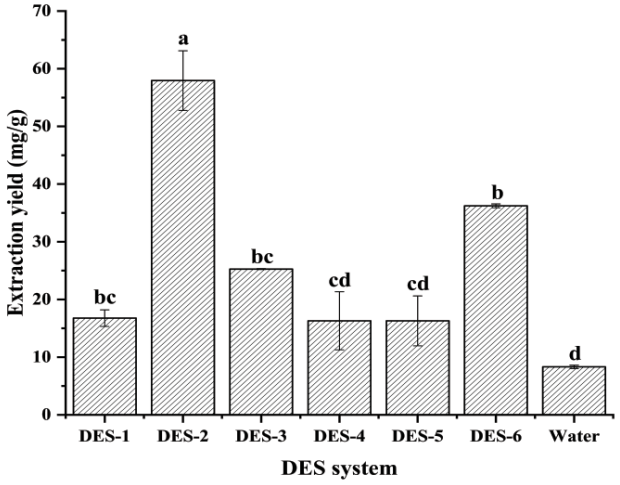

Figure 1 Extraction yields of FTP obtained from deep eutectic solvent (DES). Mean ± standard deviation (n = 3). Different lowercase letters on the columns represent significant differences ($p < 0.05$).

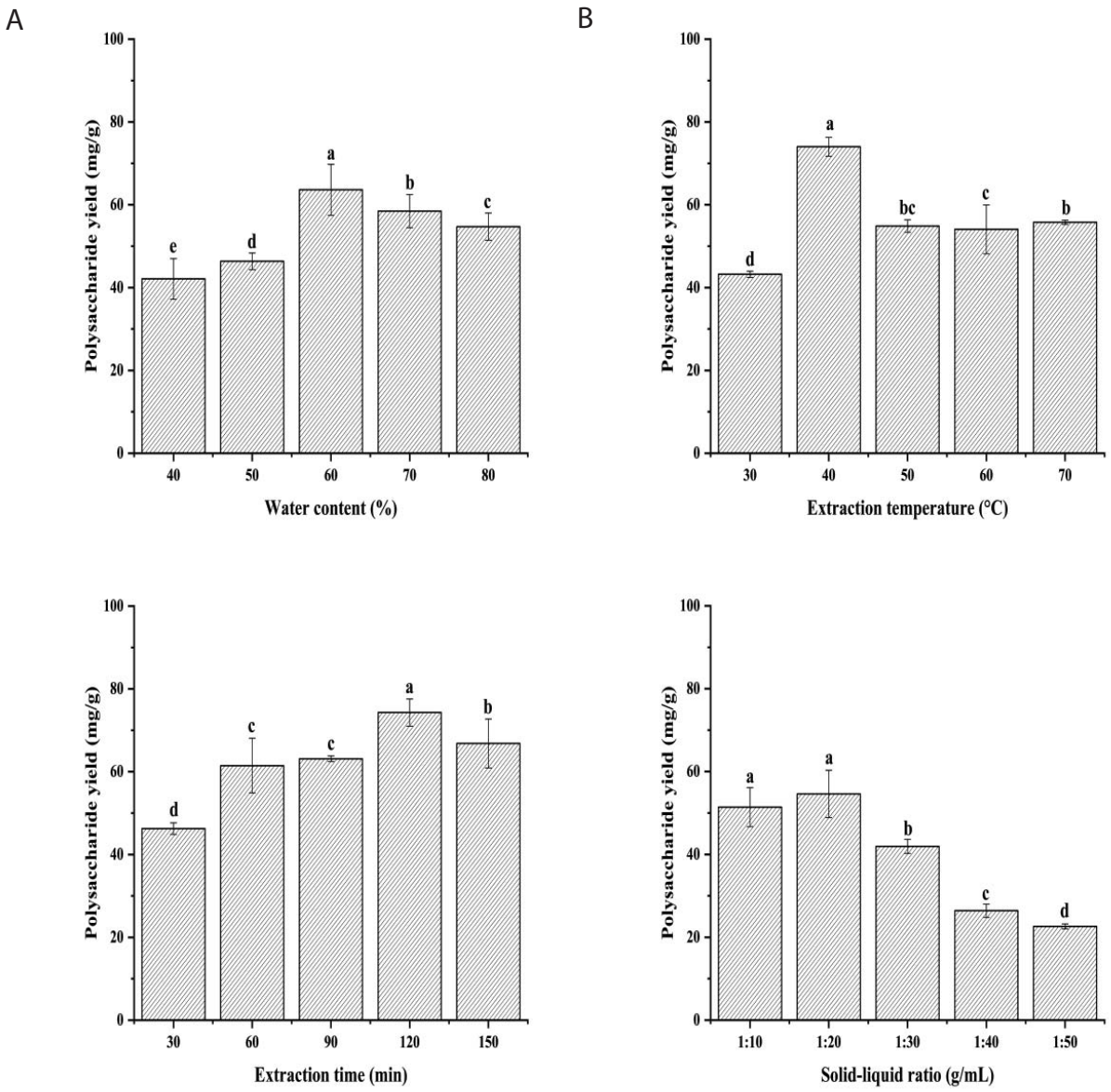

Figure 2 Single factor test results of FTP extraction by deep eutectic solvent (DES).
(A): Water content (B): Temperatures (C): Extraction time (D): Solid-liquid ratio on extraction yield of FTP. Mean ± standard deviation (n = 3). Different lowercase letters on the columns represent significant differences (p < 0.05).

[22] confirmed the low cytotoxicity of choline chloride-glycerol and its non-inhibitory effect on wheat seed germination through cytotoxicity and phytotoxicity data. Jamshaid and Ahmed [23] employed choline chloride-glycerol as an extraction solvent for flavonoids from Koenigia weyrichii, and valuable compounds from the fruit of Melia azedarach, respectively. Therefore, choline chloride-glycerol appears to be the most suitable choice for FTP extraction

Analysis of single factor experimental results

According to figure 2A in this study, it is evident that the polysaccharides extraction yield exhibited an upward trend, reaching its peak at 63.63 mg/g when the water content ranged from 50% to 60%. Conversely, a decrease in extraction yield was observed when the water content ranged from 60% to 80%. This decline

can be attributed to the excessive viscosity of the low water content DES, which hampers the fluidity of its constituents and impedes molecular diffusion, thereby compromising the extraction efficiency. However, as the water content increased, the viscosity gradually diminished, leading to an increment in the extraction volume.

The effect of extraction temperature on the polysaccharides yield was illustrated in figure 2B. It was observed that the maximum yield of 74.01 mg/g was achieved at a temperature of 40°C. Subsequently, as the temperature increased, the polysaccharides yield exhibited a declining trend. This decrease can be attributed to the effects of excessive heating on the density and viscosity of DES, as well as the conductivity. The vibrational motion of anions and cations within the DES can induce molecular rearrangement, leading to changes in the distribution of vacancies or holes and weakening the interactions between ions [24]. Therefore, precise temperature control during the extraction process is vital.

Figure 2C reveals a notable trend in the relationship between extraction time and the extraction rate of polysaccharidess. As the extraction time extended from 30 min to 120 min, the extraction rate exhibited a gradual increase. The peak extraction rate of DES was observed at 74.28 mg/g after 120 min of extraction. However, when the extraction time was further extended from 120 min to 150 min, the yield of polysaccharidess displayed a gradual decline. This decrease could potentially be attributed to the hydrolysis of certain components, such as FTP, during prolonged exposure to high temperatures. Therefore, based on these findings, an optimal extraction time of 120 min was determined.

The findings depicted in figure 2D demonstrate the relationship between the volume of DES and the extraction rate of polysaccharidess. As the volume of DES increased, the extraction rate of polysaccharidess exhibited an upward trend, reaching its peak of 54.6 ± 5.7 mg/g at 1:20 (g/mL). However, as the volume of DES continued to increase, the extraction rate of polysaccharidess gradually decreased. This

Table 1: Box-Behnken design (BBD) matrix and response values for the extraction yield of fermented *Tremella* polysaccharidess (FTP).

Group	Water content (%)	Extraction temperature (°C)	Extraction time (min)	Extraction yield (mg/g)	Predicted yield (mg/g)
1	70	40	90	70.5	66.27
2	70	40	150	51.91	57.27
3	70	50	120	68.97	67.58
4	60	40	120	71.49	73.91
5	60	40	120	69.42	73.91
6	60	40	120	72.11	73.91
7	50	30	120	32.53	33.92
8	60	30	150	52.72	47.09
9	50	40	90	36.91	36.91
10	60	40	120	83.25	83.25
11	70	30	120	49.48	49.48
12	60	50	90	49.38	49.39
13	60	40	120	73.28	73.28
14	50	50	120	42.55	42.55
15	60	50	150	60.44	60.44
16	60	30	90	34.22	34.22
17	50	40	150	46.66	46.66

Table 2: Analysis of the variance (ANOVA) for the second-order polynomial model.

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value	
Model	3569.97	9	396.66	8.98	0.0043	significant
A	844.81	1	844.81	19.13	0.0033	
B	53.61	1	53.61	1.21	0.3070	
C	343.22	1	343.22	7.77	0.0270	
AB	200.79	1	200.79	4.55	0.0704	
AC	22.42	1	22.42	0.5076	0.4992	
BC	13.88	1	13.88	0.3142	0.5926	
A^2	567.79	1	567.79	12.86	0.0089	
B^2	491.34	1	491.34	11.13	0.0125	
C^2	815.27	1	815.27	18.46	0.0036	
Residual	309.16	7	44.17			
Lack of Fit	192.27	3	64.09	2.19	0.2314	Not significant
Pure Error	116.89	4	29.22			
Cor Total	3879.13	16				
		$R^2 = 0.9203$		$R^2_{Adj} = 0.8178$		

decline can be attributed to the achievement of an extraction equilibrium in smaller volumes of DES. While a larger quantity of DES may enhance the leaching rate of FTP, it also results in the wastage of DES and complicates the extraction process. Therefore, based on these observations, a material-to-liquid ratio of 1:20 (g/mL) was determined to be the optimal choice.

In summary, the preliminary optimized experimental conditions for the extraction of polysaccharidess are as follows: a water content of 60%, an extraction temperature of 40˚C, an extraction time of 120 min, and a solid-liquid ratio of 1:20 (g/mL).

Modeling of the extraction process

Based on the experimental design of RSM combined with BBD, the data of 17 runs are presented in table 1. Design-Expert (version 8.06) software was used to perform second-order polynomial regression analysis and statistical analysis of variance (ANOVA) on the data in table 2. The quadratic multinomial regression model, expressing the relationship between the response variable and the three independent variables, is represented by formula (5).

$$\text{Extraction yield} = 6.2 + 0.3175A + 0.3988B + 0.0688C + 0.2125AB - 0.1075AC - 0.5250BC - 1.11A^2 - 0.5290B^2 - 1.24C^2 \quad (5)$$

The significance of each parameter was evaluated using the *p*-value and the *F*-value. The variance analysis of the regression model reveals the model's regression equation to be statistically significant ($p < 0.05$), indicating the reliability of the test method. The lack of fit term was found to be non-significant ($p = 0.2314 > 0.05$), suggesting that the regression equation effectively explains the results and predicts optimal conditions. The model's correlation coefficient $R^2 = 0.9203$ indicates a moderate deviation between the experimental and predicted values. Analysis from table 2 demonstrates that the impact of X1 on the model was highly significant ($p < 0.01$), while the effect of X3 was significant ($p < 0.05$). Based on the *F*-value of each factor, the order of significance for the extraction of tea polysaccharides was as follows: A (water content) > C (extraction temperature) > B (extraction time).

Verification of predictive model

The optimal values of independent variables and response variable for the proposed extraction by using the software Design-Expert

were determined as follows: The water content (A) was found to be 64.81% (w/w), the extraction time (B) was 127.71 min, and the extraction temperature (C) was 44.34˚C. The maximum

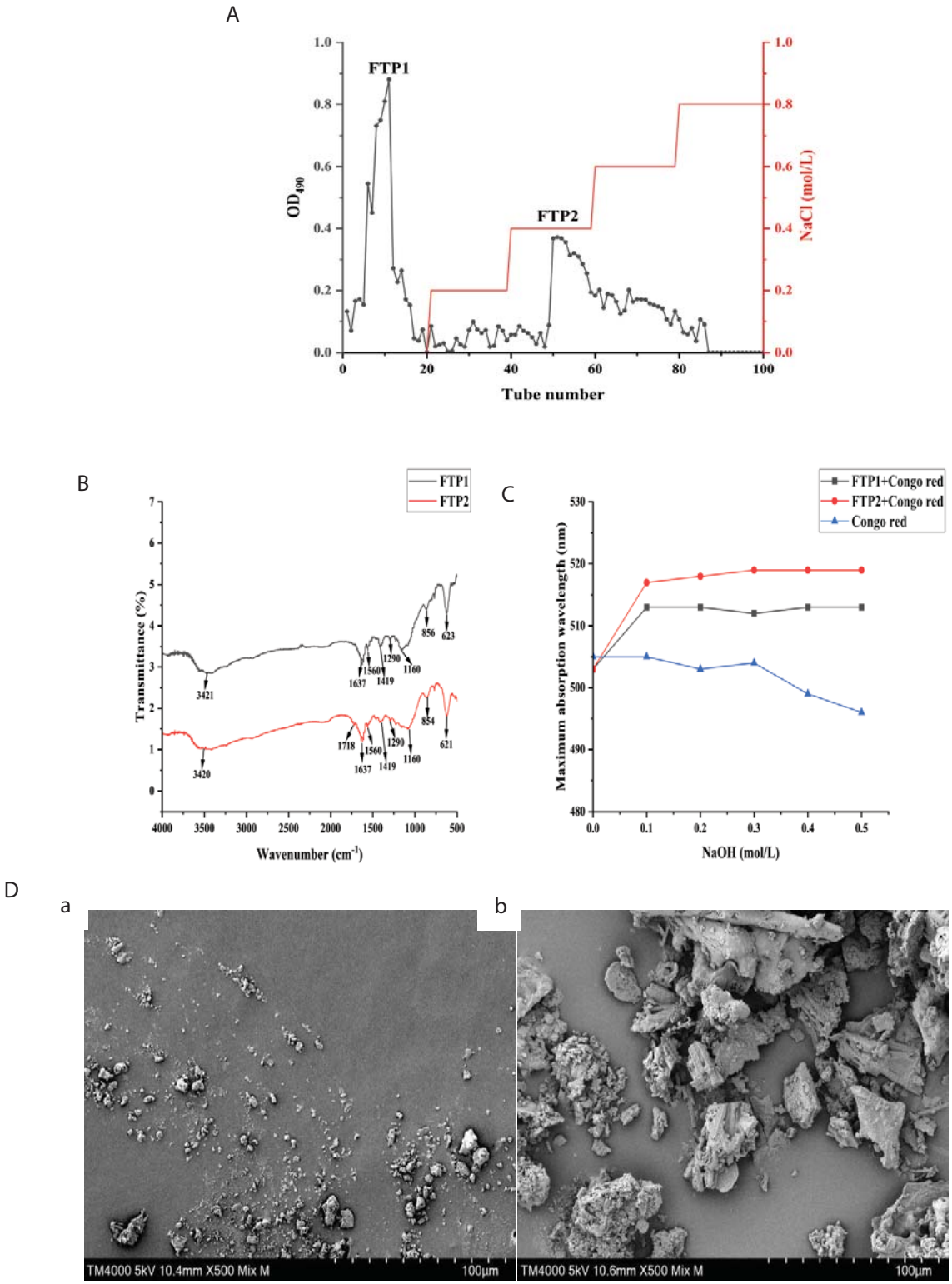

Figure 3 Characterization analysis of purified FTP1 and FTP2. (A): DEAE-Sepharose Fast Flow chromatography of FTP (B): FTIR spectra (C): Congo red λ_{max} shift curve for triple-helix identification (D): SEM micrographs (500×, a: FTP1; b: FTP2).

predicted extraction yield was calculated to be 75.75 mg/g. The actual experimental conditions employed were a water content of 64.8% (w/w), an extraction time of 127.7 min, and an extraction temperature of 44°C. Through three validation experiments conducted under these actual conditions, the average extraction yield of FTP was determined to be 75.35 mg/g.

The observed FTP extraction rate, which was in good agreement with the predicted value, demonstrates the accuracy of the regression model and its potential for predicting FTP extraction rates. Notably, the extraction rate achieved using this method was 88.06% higher than that of the traditional water extraction and alcohol precipitation method. Hence, this model proves to be well-suited for optimizing FTP extraction in DES. Therefore, the findings presented here highlight the utility of DES extraction in isolating specific compounds from microbial fermentation products. Moreover, the environmentally friendly, rapid, efficient, and cost-effective nature of DES solvents should be duly considered for various biological applications.

Cross-Comparison with Previously Reported FTP Extraction Yields published. Traditional hot water extraction yields range from 32-45 mg/g; ultrasonic-assisted extraction ranges from 43-50 mg/g; enzyme composite extraction reaches 41-48 mg/g; prior DES extraction for solid Tremella fruiting bodies achieves 52-61 mg/g [18]. Our optimized DES method for fermentation broth FTP reaches 75.35 mg/g, 24-88% higher than all reported conventional and solid-matrix DES extraction yields, fully demonstrating the unique advantage of DES for liquid fermentation raw materials.Characterization analysis of FTP1 and FTP2

Upon purification, two components of FTP, namely FTP1 and FTP2, were obtained, as depicted in figure 3A.

The infrared spectra of polysaccharidess FTP1 and FTP2 are presented in figure 3B. Through spectrogram analysis, it is evident that both FTP1 and FTP2 exhibit nearly identical infrared spectral characteristic peaks, which are indicative of polysaccharides absorption peaks. Notably, as depicted in figure 3B, both polysaccharidess display pronounced absorption peaks at approximately 3420 cm^{-1}, primarily attributed to the O-H stretching vibration. FTP2 exhibits a relatively feeble absorption peak at 1718 cm^{-1}, which corresponds to the characteristic absorption peak of uronic acid, thereby signifying the presence of uronic acid in FTP2. Conversely, FTP1 lacks a distinctive peak at this wavenumber, indicating its neutral polysaccharides nature. The absorption peak observed around 1640 cm^{-1} arises from the stretching vibration of the C = O bond of the aldehyde. The absorption peak observed at a wavelength of 1560 cm^{-1} corresponds to the deformation vibration of N-H. Similarly, the absorption peak at 1419 cm^{-1} signifies the C-H variable angular vibration. In addition, the absorption peak at 1290 cm^{-1} is attributed to the elastic vibration of O-H in COOH. Notably, the presence of pyranoside in the polysaccharides is indicated by the three absorption peaks between 1000 cm^{-1} and 1200 cm^{-1}, which arise from the stretching vibrations of C-O-C and C-O-H in the pyranoid ring. Moreover, the absorption peak at approximately 850 cm^{-1} corresponds to the scissor-like vibration of the C-H bond of alpha-pyranose. Lastly, the CO-NH bond is detected at a wavelength of 620 cm^{-1}.

Conducting Congo red experiments becomes a valuable endeavor to ascertain the presence of a triple-helix structure in polysaccharidess. In these experiments, the maximum absorption wavelength (λ_{max}) of the polysaccharides's Congo red complex undergoes a redshift when exposed to solutions with varying concentrations of sodium hydroxide. As depicted in figure 3C, when the NaOH concentration was 0.1 mol/L, both FTP1 and FTP2 exhibited a pronounced and

significant redshift in the maximum absorption wavelength of the Congo red complex. This observation strongly suggests the presence of a triple helix structure in the eluent fractions, aligning with the findings reported by Li, et al. [25], the biological activities of polysaccharidess are closely linked to their triple-helical conformation. Research has demonstrated that polysaccharidess exhibiting a triple helix structure generally possess stronger biological activity compared to those with single helix or random helix structures [26].

Figure 3D present the SEM images of FTP1 and FTP2, respectively, at 500× magnification. At low magnification, FTP1 exhibits dispersed small particles. The microscopic morphology of FTP1, as depicted in the figure 3D a, reveals a compact, block-like structure with quasi-circular and quasi-spherical shapes. The surface appears smooth without any visible pores. In contrast, FTP2 displays a flake-like morphology with larger clumps, indicating strong molecular interactions and tight binding. The size of FTP2 particles is considerably larger than that of FTP1. These findings align with the results reported by Zhang, et al. [27], who observed a significant difference in the size of purified components through 100-fold scanning electron microscopy.

Antioxidant activity of FTP1 and FTP2 in vitro

In this study, a comparative analysis of the two components is conducted based on their *in vitro* antioxidant activity, with Vc as positive control.

Figure 4A illustrates the scavenging efficacy of FTP1 and FTP2 on DPPH free radicals. At a concentration of 2 mg/mL, FTP1 exhibited a DPPH scavenging activity of 27.58%, while FTP2 demonstrated a slightly higher activity of 32.01% compared to FTP1. However, as the concentration increased, the scavenging abilities of the samples on DPPH free radicals followed the order: FTP1 > FTP2. The scavenging activities of FTP1 and FTP2 against ABTS free radicals are presented in figure 4B. Notably, Vc displayed the highest activity in scavenging ABTS free radicals, while FTP1 exhibited a significantly greater scavenging ability against ABTS free radicals compared to FTP2.Figure 4C presents the scavenging capacity of FTP1 and FTP2 on·OH radicals. The order of scavenging ability on·OH radicals was as follows: FTP1 > FTP2. In figure 4D, the reducing power of FTP1 was higher than that of FTP2 at low concentrations and slightly lower than that of FTP2 at high concentrations. At a concentration of 1.0 mg/mL, the maximum reducing capacity of FTP1 and FTP2 was 0.191 and 0.204, respectively.

The analysis of the *In vitro* antioxidant experiments revealed that FTP1 exhibited robust antioxidant activity. This observation led to the speculation that the smaller molecular size of FTP1 compared to FTP2 may contribute to this phenomenon. This inference was supported by the results of SEM. FTP1 has smaller dispersed particles (SEM), providing larger specific surface area for full contact with free radicals to capture DPPH/·OH radicals efficiently Both FTP1 and FTP2 demonstrated favorable antioxidant activity, aligning with the comprehensive assessment of *Tremella fuciformis* polysaccharides's antioxidant properties by Wu, et al. [1]. Hence, this study confirms that FTP1 and FTP2 hold promise as potential sources of functional food.

Viscosity stability of FTP1 and FTP2

According to figure 5A, the viscosity of the solutions of FTP1 and FTP2 gradually decreased from 0.76 and 0.87 Pa·s to 0.49 and 0.53 Pa·s, respectively, as the temperature increased from 30°C to 90°C. The viscosity reduction rate of FTP1 was 35.53%, while that of FTP2 was 39.08%. The viscosity change rates of FTP1 at each temperature interval were as follows: 9.09%, 7.14%, 3.08%, 7.94%, 8.62%, and 11.32%. Similarly, the viscosity change rates of FTP2 at each temperature interval were 2.27%,

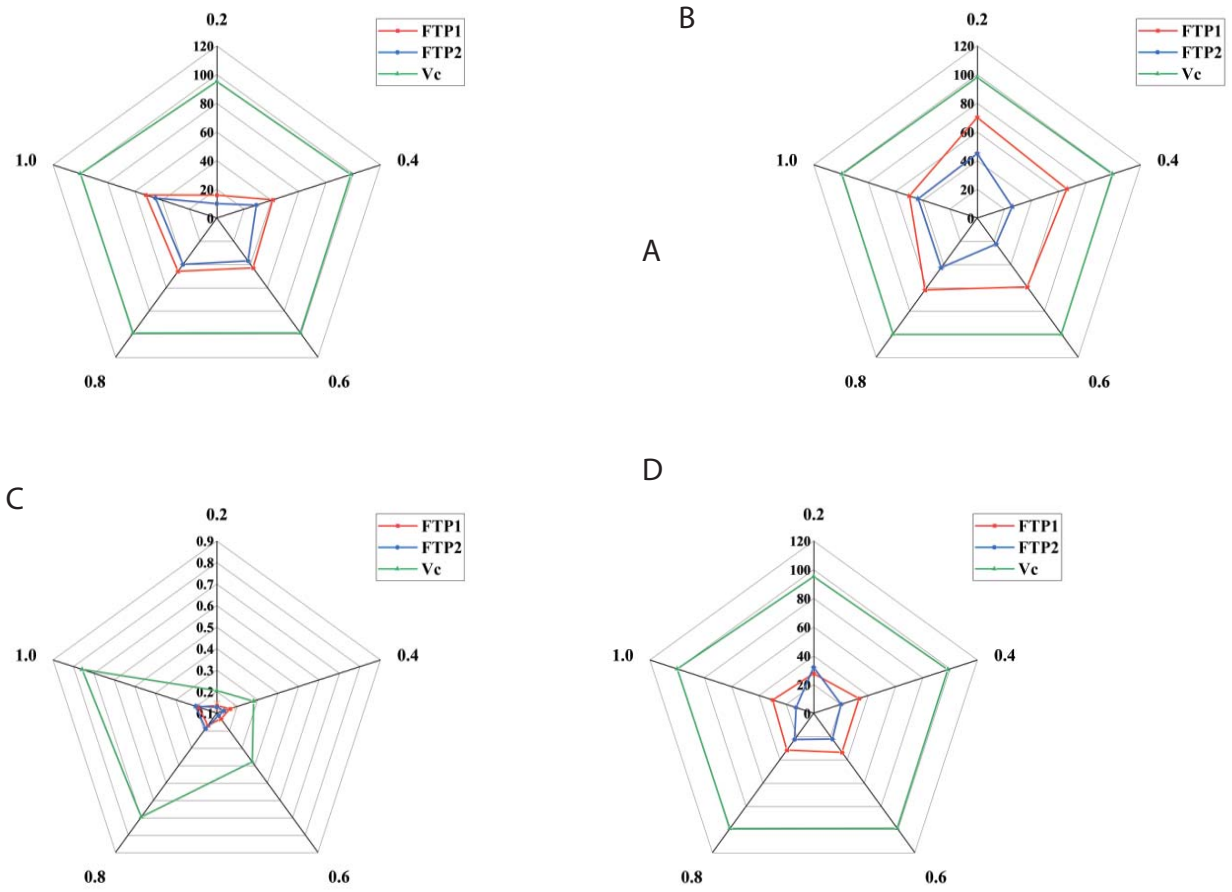

Figure 4 Radar charts of four in vitro antioxidant indicators for FTP1, FTP2 and positive control Vc (A): DPPH (B): ABTS (C): ·OH (D): Determination of reducing power. Mean ± standard deviation (n = 3). Different lowercase letters on the columns represent significant differences ($p < 0.05$).

9.30%, 16.67%, 3.08%, 17.46%, and 3.85%. Xu, et al. [28] suggested that the thermal stability of a polysaccharides improves as the viscosity change rate decreases. Therefore, FTP1 exhibited stronger overall thermal stability compared to FTP2. FTP1 demonstrated the highest thermal stability between 50°C and 60°C, whereas FTP2 exhibited the best thermal stability between 30°C and 40°C. Notably, FTP2 displayed enhanced stability and resistance to decomposition at higher temperatures ranging from 80°C to 90°C. In addition, as the temperature increases, the viscosity of both components gradually decreases. Previous research has indicated that the rise in system temperature leads to increased energy dissipation motion of molecules, resulting in the disruption of hydrogen bonds within and between molecular chains, thereby reducing the overall molecular interaction [29]. As a result, the entanglement between polysaccharides macromolecules weakens, leading to a thermal expansion phenomenon within the solution. This phenomenon, known as the "dilution effect," contributes to the viscosity reduction observed in the FTP1 and FTP2 solutions. These experimental findings align with the studies conducted by Nwokocha and Williams [26] on *Adansonia digitata* leaf polysaccharides and Exopolysaccharides from *Leuconostoc citreum* 1.2461 Fermented in Soybean Whey, as well as Li, et al. [30].

Figure 5B illustrates the influence of concentration on the viscosity of FTP1 and FTP2 solutions. The viscosity of both solutions

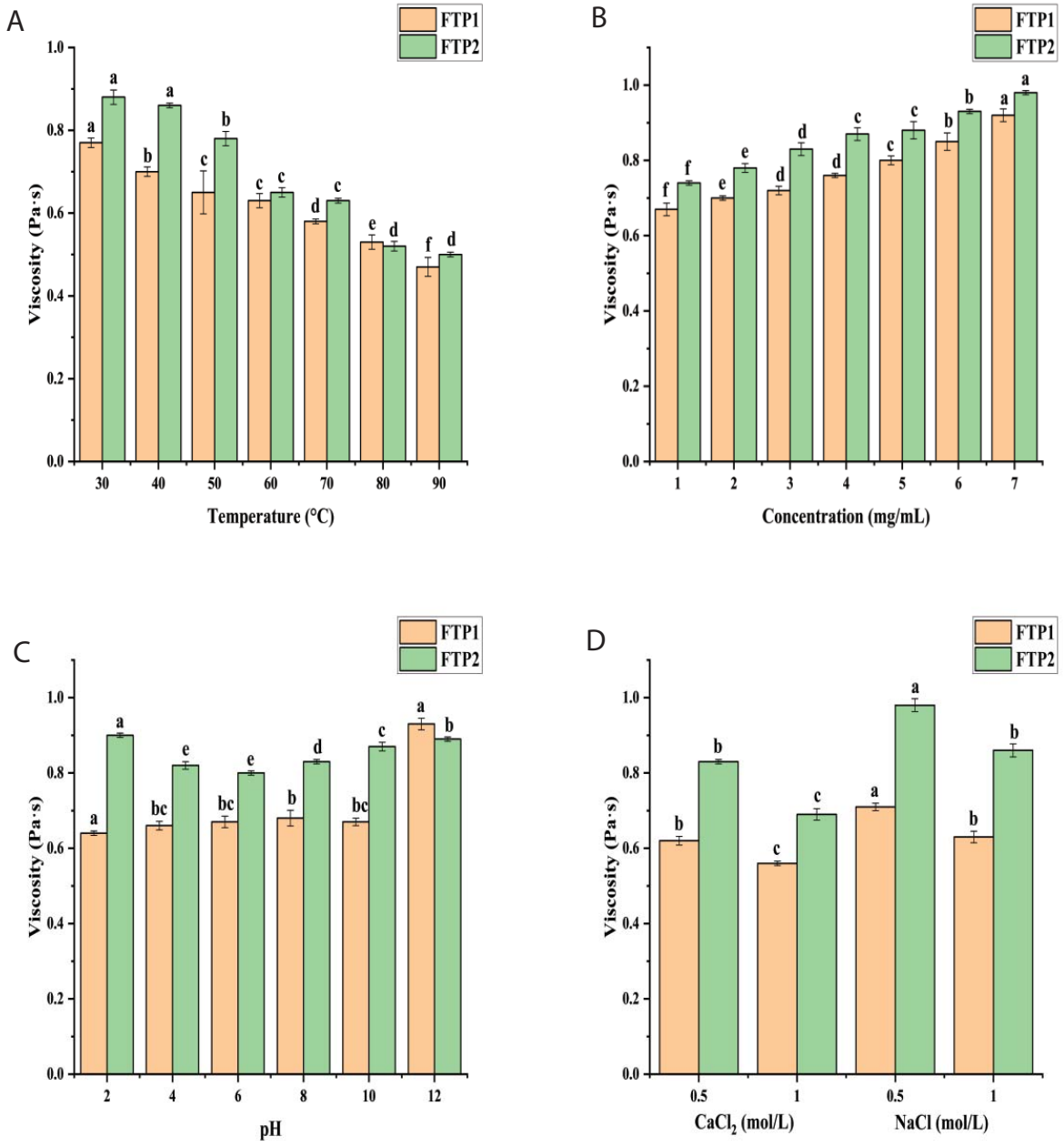

Figure 5 Viscosity stability of purified FTP1 and FTP2. (A): Temperature (B): ConcentrationC): pH (D): Inorganic salt concentration. Mean ± standard deviation (n = 3). Different lowercase letters on the columns represent significant differences (p < 0.05).

shows a positive correlation with concentration. This can be attributed to the increased number of molecules per unit volume at lower concentrations, as well as the presence of branching structures that affect intra- and inter-chain entanglement and interaction, leading to a more compact state and reduced intermolecular forces and viscosity [29,31]. At a polysaccharides concentration of 7 mg/mL, the viscosity of the solutions reached a maximum of 3.79 mPa·s. This finding aligns with the results reported by Xu, et al. [32] on the viscosity increase with the concentration of *Vaccinium bracteatum* Thunb leaves polysaccharidess. In addition, FTP2 exhibited higher viscosity than FTP1 at the same concentration, which can be attributed to the

larger molecular size of FTP2. These results are consistent with the observations produced with SEM.

The influence of pH on the viscosity of FTP1 and FTP2 solutions was depicted in figure 5C. The viscosity of the FTP1 solution exhibited a range from 0.65 to 0.67 Pa·s at pH 2 to 10. Similarly, the FTP2 solution displayed a viscosity range of 0.88-0.9 Pa·s at pH 2 to 12, with no significant changes observed. Notably, when the pH exceeded 10, the viscosity of the FTP1 solution increased from 0.67 Pa·s to 0.93 Pa·s due to alkaline enhancement. Conversely, the viscosity of FTP2 solution exhibits an increase under conditions of both strong acid and strong base. These findings suggest that FTP1 exhibits stability under strong acidic conditions, while FTP2 demonstrates stability under both strong acidic and basic conditions. Previous studies have reported that acidic or alkaline conditions can disrupt the hydrogen bonds of polysaccharidess, leading to conformational changes and subsequent alterations in viscosity [28]. The apparent viscosity of the *Ribes stenocarpum* Maxim polysaccharides prepared by Qiao, et al. [33] was enhanced in an acidic environment, and the increase in FTP2 viscosity under acidic conditions was comparable to that observed in his study. The observed outcome may be attributed to the interaction between H^+ ions and the negatively charged species in the solution, leading to a flocculation reaction that enhances the viscosity of the sample [34].

The addition of salt ions is also a significant factor influencing the viscosity of the solution. Figure 5D illustrates that the viscosity of both FTP1 and FTP2 solutions decreased upon the addition of NaCl and $CaCl_2$ solutions. Specifically, the viscosity of FTP1 decreased by 9.68% following the addition of $CaCl_2$ and 11.27% following the addition of NaCl. Similarly, the viscosity of FTP2 decreased by 16.87% and 12.24% after the addition of $CaCl_2$ and NaCl, respectively. This phenomenon can be attributed to the distinct binding modes between the polysaccharides molecular chains and metal ions, resulting in alterations in the shape and movement of the molecular chains [35]. Moreover, the steady-state and dynamic rheological properties of FTP1 and FTP2 are affected. Experimental findings from the literature have demonstrated varying effects on the viscosity of polysaccharides solutions upon the addition of salt, including increases, decreases, or minimal changes [28]. In summary, the stability of FTP1 exhibited a higher resistance to the addition of salt ions compared to FTP2.

In conclusion, the findings regarding viscosity stability indicate that the viscosity of FTP1 and FTP2 is notably influenced by the concentration, pH, temperature, and salt ions present in the polysaccharides solution. Additionally, it is evident that FTP1 exhibits a higher degree of stability compared to FTP2. The viscosity of FTP2 is higher than that of FTP1 under varying test conditions. FTP2 forms large aggregated flaky clusters (SEM), raising hydrodynamic volume of macromolecules in aqueous solution.FTP1's small isolated particles have weaker interchain entanglement, resulting in lower viscosity under identical concentration, temperature and pH conditions. These characteristics, including viscosity stability, make FTP1 and FTP2 an appealing choice for novel food thickeners, particularly in common food environments where such properties are desirable for processing purposes. **Conclusions**

In this study, the investigation focused on the antioxidant activities and apparent viscosity of the two constituent polysaccharidess derived from DES subsequent to purification. Among the six distinct solvents examined, the selection for the extraction of FTP involved choline chloride-glycerol (with a molar ratio of 1:2) through a process of heating and mixing. The impact of water content, extraction time, and temperature on the concentration of the desired compounds was thoroughly assessed. The response surface

method revealed that the optimal extraction conditions obtained were in good agreement with the experimental values. Under the conditions of water content of 64.8% (w/w), extraction temperature of 44°C and extraction time of 127.7 min, the highest extraction rate of 75.35 mg/g was obtained, and the extraction rate was 88.06% higher than that of traditional water extraction and alcohol precipitation method. The conformation analysis of FTP1 and FTP2, as determined by the congo red test, indicated a trihelical structure. Notably, FTP1 exhibited robust scavenging and reducing capabilities against DPPH, ABTS, and hydroxyl radicals, as demonstrated by the antioxidant activity assays. Moreover, the viscosity stability measurements highlighted the significant influence of polysaccharides solution concentration, pH, temperature, and salt ions (NaCl and $CaCl_2$) on the observed viscosity stability. Therefore, the findings presented herein underscore the potential utility of DES extraction for the isolation of targeted compounds from microbial fermentation products. Moreover, the environmentally friendly, rapid, efficient, and cost-effective nature of DES solvents warrants greater consideration for diverse biological applications. Additionally, this study has successfully purified and characterized FTP, thereby confirming the applicability of FTP1 and FTP2 in various food products.

Overall, this study establishes a low-energy, eco-friendly DES extraction platform for fermented polysaccharidess, fills the research gap of DES application in liquid fermentation separation, and provides comprehensive theoretical data supporting industrial transformation of *Tremella fuciformis* polysaccharides functional biomaterials.

Author Contributions

Hua Zhang: Writing-review and editing, Validation, Formal analysis, Conceptualization, Methodology.

Yashu Wei: Writing-original draft, Data curation, Visualization.

Xingjian Ma: Data curation.

Yan He: Conceptualization, Methodology.

Xia Ma: Conceptualization, Methodology, Writing - review and editing, Supervision, Project administration, funding acquisition.

Funding

This research work was supported by the horizontal project (Controllable preparation, activity evaluation, and application research of water-soluble yeast glucan) (27220H240367-A06-J2024-367).

Availability of data and materials

No datasets were generated or analyzed during the current study.

Acknowledgements

We extend our gratitude to our lab members for their valuable insights and constructive feedback on this review.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare no competing interests.

References

1. Wu YJ, Wei ZX, Zhang FM, Linhardt RJ, Sun PL, Zhang

AQ. Structure, bioactivities and applications of the polysaccharides from *Tremella fuciformis* mushroom: A review. Int J Biol Macromol. 2019 Jan;121:1005-1010. doi: 10.1016/j.ijbiomac.2018.10.117. Epub 2018 Oct 18. PMID: 30342120.

2. Xu JY, Zou Y, Guo LQ, Lin JF, Jiang Z, Zheng QW. Rheological and microstructural properties of polysaccharides obtained from gelatinous *Tremella fuciformis* fungus. Int J Biol Macromol. 2023;228:153-164. doi: 10.1016/j.ijbiomac.2022.12.214.
3. Yang M, Zhang ZL, He Y, Li CL, Wang JM, Ma X. Structural characterization and moisturizing effects of fermented *Tremella fuciformis* polysaccharides from strain GCMCC5.39. Food Sci Hum Wellness. 2021;10:471-479. doi: 10.1016/j.fshw.2021.04.009.
4. Zhou YF, Zhang ZL, He Y, Gao P, Zhang H, Ma X. Multi-sensor fusion combined with chemometrics for monitoring *Tremella fuciformis* submerged fermentation. Talanta. 2024;274:126006. doi: 10.1016/j.talanta.2024.126006.
5. Zhou YF, Zhang H, He Y, Ma X. ATR-MIR spectroscopy coupled with chemometrics to monitor submerged fermentation of *Tremella fuciformis*. Food Chem. 2025;482:144027. doi: 10.1016/j.foodchem.2025.144027.
6. Yuan HJ, Dong L, Zhang ZY, He Y, Ma X. Production, structural features and biological activities of *Tremella fuciformis* polysaccharides. Food Sci Hum Wellness. 2022;11:1010-1017. doi: 10.1016/j.fshw.2022.03.030.
7. Li YX, He Y, Zhang H, Ma X. Physicochemical, hypoglycemic and antioxidant properties of *Tremella fuciformis* polysaccharides prepared by ultrasonic-enzymatic ethanol precipitation. Ultrason Sonochem. 2023;101:106682. doi: 10.1016/j.ultsonch.2023.106682.
8. Zhang YK, Zhang Q, Lu J, Xu JL, Zhang H, Wang JH. Physicochemical characteristics of *Tremella fuciformis* polysaccharides and their interactions with myofibrillar proteins. Bioact Carbohydr Diet Fibre. 2017;11:18-25. doi: 10.1016/j.bcdf.2017.06.002.
9. Sun T, Wang R, Sun DF, Li S, et al. Efficient biosynthesis of *Tremella aurantialba* polysaccharides via basidiospore fermentation. Bioresour Technol. 2020;318:124268. doi: 10.1016/j.biortech.2020.124268.
10. Liu Y, Sun YY, Huang GL. Preparation and in vitro antioxidant properties of typical medicinal plant polysaccharides. Int J Biol Macromol. 2018;111:780-786. doi: 10.1016/j.ijbiomac.2018.01.086.
11. Boateng ID. Recent advances and prospects of hydrophobic deep eutectic solvents in food chemistry research. J Agric Food Chem. 2022;70(38):11860-11879. doi: 10.1021/acs.jafc.2c05079.
12. Abbott AP, Capper G, Davies DL, Munro HL, Rasheed RK, Tambyrajah V. Novel moisture-stable Lewis acidic ionic liquids based on quaternary ammonium salts. Chem Commun (Camb). 2001;(19):2010-2011. doi: 10.1039/B106357J.
13. Jiménez-Ortega LA, Yao S, Mota-Morales JD, Heredia JB. Sustainable application of deep eutectic solvents to mitigate global food insecurity. J Agric Food Chem. 2023;71(13):5027-5029. doi: 10.1021/acs.jafc.3c01460.
14. Redha AA. A review on phenolic compound extraction using green deep eutectic solvents. J Agric Food Chem. 2021;69(3):878-912. doi: 10.1021/acs.jafc.0c06641.
15. Jurić T, Mićić N, Potkonjak A, Milanov D, Dodić J, Trivunović Z, Popović BM. Phenolic, antibacterial and antioxidant profiles of peppermint extracts isolated with natural deep eutectic solvents. Food Chem. 2021;362:130226. doi: 10.1016/j.foodchem.2021.130226.
16. Zhou C, Adeyanju AA. Physical field-assisted deep eutectic solvent processing: A green and water-saving extraction and separation technology. J Food Sci. 2024;89(12):8248-8275. doi: 10.1111/1750-3841.17545.
17. Yadav S, Verma A, Kumar S. Exopolysaccharides from submerged fungal fermentation: Extraction, purification and green separation strategies. Foods. 2024;13(18):2891. doi: 10.3390/foods13182891.
18. Li M, Wang L, Chen T. Deep eutectic solvent ultrasonic-assisted extraction of *Tremella sanguinea* polysaccharides: Structure and gut microbiota regulation. J Food Process Preserv. 2026;50(1):e2616984. doi: 10.1080/19476337.2026.2616984.
19. Fu GQ, Li YX, He Y, Zhang H, Ma X. Extraction, structure and bioactivity of *Tremella fuciformis* polysaccharides: A review. Food Med Homol. 2025;2:9420038. doi: 10.26599/FMH.2025.9420038.
20. Kandanelli R, Thulluri C, Mangala R, Rao PV, Gandham S, Velankar HR. Ternary deep eutectic solvent-alcohol blends for synergistic biomass delignification. Bioresour Technol. 2018;265:573-576. doi: 10.1016/j.biortech.2018.06.002.
21. Wang XS, Wu YF, Li J, Wang AX, Li GY, Ren XL, Yin WP. Ultrasound-assisted deep eutectic solvent extraction of bioactive components from *Syringa pubescens*. Ind Crops Prod. 2020;151:112442. doi: 10.1016/j.indcrop.2020.112442.

22. Radošević K, Bubalo MC, Srček VG, Grgas D, Dragičević TL, Redovniković IR. Toxicity and biodegradability evaluation of choline chloride-based deep eutectic solvents. Ecotoxicol Environ Saf. 2015;112:46-53. doi: 10.1016/j.ecoenv.2014.09.034.

23. Jamshaid S, Ahmed D. Ultrasound-assisted extraction of bioactive compounds from *Melia azedarach* fruit with glycerol-choline chloride deep eutectic solvent. Sustain Chem Pharm. 2022;29:100827. doi: 10.1016/j.scp.2022.100827.

24. Huang J, Guo XY, Xu TY, Fan LY, Zhou XP, Wu SH. Ionic deep eutectic solvents for natural product separation and extraction. J Chromatogr A. 2019;1598:1-19. doi: 10.1016/j.chroma.2019.03.046.

25. Li P, Jiang Z, Sun T, Du B, et al. Structural features and α-glucosidase inhibition of polysaccharide fractions from *Sargassum fusiforme*. Int J Biol Macromol. 2022;194:602-610. doi: 10.1016/j.ijbiomac.2021.11.103.

26. Nwokocha LM, Williams PA. Rheological characteristics of polysaccharides isolated from *Adansonia digitata* leaves. Food Hydrocoll. 2016;58:29-34. doi: 10.1016/j.foodhyd.2016.02.013.

27. Zhang SJ, Hu TT, Chen YY, Wang SY, Kang YF. Fractionated purification and structural analysis of pea polysaccharides. J Food Biochem. 2020;44(8):e13248. doi: 10.1111/jfbc.13248.

28. Xu GY, Liao AM, Huang JH, Zhang JG, Thakur K, Wei ZJ. Rheological behaviors of potato peel polysaccharides extracted via different methods. Int J Biol Macromol. 2019;137:1-7. doi: 10.1016/j.ijbiomac.2019.06.175.

29. Fu GQ, Li YX, He Y, Zhang H, Ma X. A review on extraction, structure and bioactivities of *Tremella fuciformis* polysaccharides. Food Med Homol. 2025;2:9420038. doi: 10.26599/FMH.2025.9420038.

30. Li YY, Xiao LY, Tian JJ, Wang XM, Zhang XL, Fang Y, Li W. Structural, rheological and antioxidant protective properties of exopolysaccharides from fermented *Leuconostoc citreum*. Foods. 2022;11(15):2283. doi: 10.3390/foods11152283.

31. Zhu DY, Ma YL, Thakur K, Wang CH. Effects of extraction methods on rheological properties of onion polysaccharides. Int J Biol Macromol. 2018;112:22-32. doi: 10.1016/j.ijbiomac.2018.01.160.

32. Xu QX, Shi JJ, Zhang JG, Li L, Jiang L, Wei ZJ. Thermal, emulsifying and rheological properties of leaf polysaccharides from *Vaccinium bracteatum*. Int J Biol Macromol. 2016;93:1240-1252. doi: 10.1016/j.ijbiomac.2016.09.098.

33. Qiao YB, Ye Y, Cai TX, Liu YY, Han LJ. Antioxidant and rheological features of polysaccharides from *Ribes stenocarpum*. J Food Process Preserv. 2021;45(12):e16087. doi: 10.1111/jfpp.16087.

34. Ma FY, Wang RJ, Li XJ, Kang WY, Bell AE, Zhao DB, Liu XH, Chen WZ. Physical properties of Chinese yam mucilage polysaccharides. Food Chem. 2020;311:126039. doi: 10.1016/j.foodchem.2019.126039.

35. Geng XQ, Liu CY, Wang LY, Jiang W, Liu XC, Zhu ZY. Concentration-, pH-, temperature- and salt-dependent rheology of *Pholiota nameko* polysaccharides. J Food Sci. 2022;87(8):3632-3643. doi: 10.1111/1750-3841.16223.