

Original Research Paper

# Optimization of Ultrasonic/Microwave Assisted Extraction (UMAE) and Rheological Properties of Polysaccharides from *Auricularia polytricha*

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**Abstract:** Polysaccharides from *Auricularia polytricha* (APPS) were extracted using Ultrasonic/Microwave Assisted Extraction (UMAE) technology. UMAE conditions of APPS were optimized with Response Surface Methodology (RSM). Rheological properties of APPS were investigated. The results suggested that the optimal UMAE conditions for APPS were ratio of water to raw material 22 (mL/g), extraction time 15.3 min and microwave power 59W. APPS solution exhibited Newtonian flow behavior at low concentration, formed entanglement network at lower concentration and established weak gel structures with solid-like properties at higher concentration. The viscosity of APPS solutions decreased when temperature increased. The viscosity, storage ( $G'$ ) and loss ( $G''$ ) modulus of APPS solution increased when sucrose concentration increased. Heating time and freeze-thaw changes had no significant effect on rheological properties of APPS. The results suggested that UMAE was a suitable and efficient method for APPS extraction and APPS had potential as a novel food additive hydrocolloid in food industry.

**Keywords:** *Auricularia polytricha*, Polysaccharide, Ultrasonic/Microwave Assisted Extraction, Rheological Properties

## Introduction

Mushrooms have been considered as edible and medicinal resources for thousands of years and many biologically active molecules have been isolated and identified from mushrooms (Wang *et al.*, 2018). In addition, many mushrooms have been shown to contain bioactive polysaccharides in fruiting bodies (Chen *et al.*, 2008; Zhao *et al.*, 2010).

*Auricularia polytricha* belonging to the Auriculariaceae family is widely distributed in many places in China, such as Hebei, Heilongjiang, Anhui, Jiangxi and Guangdong. It has high contents of carbohydrates in the fruiting bodies traditionally used as food and medicine in China (Fan *et al.*, 2006) and has lowering blood-fat, antioxidant, antitumor, antinociceptive and immunomodulatory activities (Mau *et al.*, 2001).

In recent years, various new extraction techniques for extracting polysaccharides including Hot Water Extraction (HWE), Ultrasonic Assisted Extraction (UAE) and Microwave Assisted Extraction (MAE) have been developed (You *et al.*, 2014). HWE requires high temperature and long extraction time (Li *et al.*, 2007).

UAE has the benefits of mass transfer enhancement, cell destruction, increased permeability and capillary effects (Hmelkov *et al.*, 2018). The main advantage of MAE is saving time (Zhang and Liu, 2008). Therefore, to take full advantage of the cavitation of ultrasonic vibration and the high-energy effect of microwave, combining ultrasonic and microwave extraction technology to extract polysaccharides is the future development trend. However, to date, there have been no reports of Ultrasound/Microwave Assisted Extraction (UMAE) of polysaccharides from *A. polytricha* (APPS).

Polysaccharides can be used as emulsifiers and thickeners in the food industry to improve the appearance characteristics of food. As an important feature of polysaccharides, rheological behavior not only affects the manufacture, storage and texture characteristics of food greatly, but also has an intrinsic relationship with molecular structure and chain conformation (Huang *et al.*, 2009; Xu *et al.*, 2008).

Polysaccharides as a colloidal substance are involved in the rheological properties in many practical applications. Therefore, an important prerequisite for

expanding the application range of polysaccharides as a food additive hydrocolloid is to reveal the rheological properties of polysaccharides. APPS had anticancer activities (Song and Du, 2010), antitumor effects (Song and Du, 2012), antioxidant (Chen and Xue, 2018) and anti-hypercholesterolemic effect (Zhao *et al.*, 2015). However, no rheological properties of APPS have been reported in the literature.

Based on the above analysis, the present study aimed to optimize the UMAE conditions for APPS using response Surface Analysis Methodology (RSM) for finding a suitable and efficient method for APPS extraction. In addition, rheological properties of APPS were also investigated to explore further the applications and the functional properties as a novel food additive hydrocolloid in food industry.

## Materials and Methods

### Materials

The dried fruiting bodies of *A. polytricha* were purchased from Changshu city, China. The dried materials were crushed into a fine powder in a high disintegrator (Micron Co. LTD, China) for the subsequent studies. Ethanol, phenol, sulphuric acid and glucose were purchased from the Chengdu Kelong Chemical Factory (Chengdu, China). All reagents were of analytical grade.

### Experimental

#### Extraction of APPS with UMAE

Ultrasonic/microwave assisted extraction was performed using an UMAE apparatus (CW-2000, Shanghai Xintuo Microwave Instrument Co. Ltd. China) with maximal microwave power of 800 W at a frequency of 2450 MHz and an ultrasonic transducer with a fixed power of 50 W at a frequency of 40 KHz (fixed parameter of equipment). 2.0 g fine powder of *A. polytricha* and 100 mL deionized water were added into a 250 mL flask and then transferred into the chamber of the apparatus connected with condensing tubes.

Extraction of APPS with UMAE was performed under different extraction time and microwave power conditions. After the extraction was completed, the proteins in polysaccharide extract were separated for 6 times with seväge method. The polysaccharide extract was then centrifuged at 4500 rpm for 10 min. The

supernatant was concentrated in a rotary evaporator at 50°C and then precipitated with four volumes of 95% ethanol for 48 h at 4°C. The precipitate was collected by centrifugation (5000 rpm, 15 min), washed with pure ethanol and acetone and finally lyophilized to obtain APPS. With D-glucose as the standard, the total carbohydrate content of APPS was determined by phenol sulfuric acid colorimetric method (Dubois *et al.*, 1956). Yield and purity of APPS were measured using the following equation:

$$APPS \text{ yield} = \frac{\text{weight of crude polysaccharide extract (g)}}{\text{weight of each powder sample (g)}} \times 100\% \quad (1)$$

$$APPS \text{ purity} = \frac{\text{weight of determined polysaccharide (g)}}{\text{weight of crude polysaccharide extract (g)}} \times 100\% \quad (2)$$

### Experimental Design of RSM and Statistical Analysis

Response Surface Methodology (RSM) is used to explore the effect of independent variables on the response within the investigation scope. Statistical techniques can be collected by RSM to design experiments, build models, evaluate the effects of factors and find the optimal conditions for factors to obtain the desirable responses (Li *et al.*, 2002).

Independent variables and their ranges were selected based on our preliminary single tests. A central composite design with three independent variables ( $X_1$ , ratio of water to raw materials;  $X_2$ , extraction time;  $X_3$ , microwave power) (Table 1) was performed to optimize extraction conditions and investigate the influence of the above independent variables on the dependent variables (yield and purity of polysaccharides).

To predict the optimal parameters of UMAE of APPS, a second-order polynomial model was fitted to establish the relationship between the independent and response variables as shown below:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (3)$$

where,  $y_k$  is the response,  $b_{k0}$ ,  $b_{ki}$ ,  $b_{kii}$  and  $b_{kij}$  are coefficients for intercept, linear, quadratic and interactive terms, respectively;  $x_i$ ,  $x_i^2$  and  $x_i x_j$  represent linear, quadratic and interactive terms of coded independent variables, respectively.

**Table 1:** Independent variables and their levels in the response surface design

Independent variables	Symbol	Coded factor level		
		-1	0	1
Ratio of water to raw material (mL/g)	$X_1$	10	20	30
Extraction time (min)	$X_2$	10	15	20
Microwave power (W)	$X_3$	20	50	80

The experimental data was analyzed using the Design Expert program (version 8.0.6). The statistical significance of the coefficients in the regression equation was checked by the ANOVA program. When  $p < 0.05$ , the significance of all terms in the polynomial was considered statistically different.

### Chemical Composition Analysis of APPS

The total carbohydrates were determined by the phenol-sulfuric acid colorimetric method with D-glucose as a standard at 490 nm (Dubois *et al.*, 1956).

Determination of uronic acid content was performed by measuring the absorbance at 525 nm using a meta-hydroxybiphenyl colorimetric method with glucuronic acid as a standard (Wang *et al.*, 2017). The protein content was measured based on the reported method (Vakondios *et al.*, 2014).

### FT-IR Spectroscopy

1 mg APPS was ground with dried potassium bromide (KBr) powder and then pressed into pellets for the analysis. The IR spectrum was recorded in the wavenumber range of 400-4000  $\text{cm}^{-1}$  on a Nicolet IS10 FT-IR spectrometer (Thermo Nicolet Co., USA).

### Rheological Properties of APPS

The dried APPS powder was dissolved in distilled water and stirred at 25°C for 2 h. Thus, aqueous APPS solution with various concentrations of 0.1 to 3.0% (W/V) was obtained for the steady shear tests. A rheometer (Physica MCR 301, Anton Paar, Austria) was used to perform stable shear and oscillation tests using a parallel plate (40 mm diameter, 1.0 mm gap). The stable shear viscosity of various APPS concentrations was measured at 25°C. Viscoelasticity, storage modulus ( $G'$ ) and loss modulus ( $G''$ ) were measured using a small amplitude oscillation test at a frequency of 0.1 to 10 Hz. A temperature ramp from 20 to 80°C was applied using a parallel plate at a rate of 10°C/min. All rheological measurements were performed at least three replicates and the obtained data was the average values.

The APPS solution (0.5%, W/V) (10 mL) was heated at 50°C for 20, 40, 60 and 80 min in a water bath, respectively. In addition, the APPS solution (0.5%, W/V) (10 mL) was refrigerated at 4°C, frozen at eighteen degrees below zero (-18°C) and left at room temperature for 40 min, respectively. After the above two treatment, the shear rate dependency of APPS solution viscosity was measured at 25°C to investigate the effect of heating time and temperature on the rheological properties of APPS. After stirring at room temperature for 40 min, 5 mL sucrose solution with a concentration of 20% and 50% (W/V) was added into the APPS solution (0.5%, W/V) (10 mL), respectively. Then, the shear rate dependency of APPS solution viscosity

was measured at 25°C to investigate effect of sucrose on rheological properties.

## Results and Discussion

### Statistical Analysis and the Model Building

Results of response surface analysis of the variation of the yield and the purity of APPS with ratio of water to raw material ( $X_1$ ), time ( $X_2$ ) and microwave power ( $X_3$ ) were presented in Table 2. The regression coefficients and model analysis of the two response variables were summarized in Table 3. A second-order polynomial model that establishes the relationship between independence and yield ( $Y_1$ ) and purity ( $Y_2$ ) was obtained as follows:

$$Y_1 = 4.2 + 0.14X_1 + 0.025X_2 - 0.15X_3 - 0.36X_1^2 + 0.073X_1X_2 - 0.027X_1X_3 - 0.39X_2^2 - 0.089X_2X_3 - 0.61X_3^2 \quad (4)$$

$$Y_2 = 49.01 + 1.06X_1 + 0.33X_2 - 0.16X_3 - 1.49X_1^2 + 0.37X_1X_2 - 0.49X_1X_3 - 1.81X_2^2 + 0.020X_2X_3 - 1.94X_3^2 \quad (5)$$

As shown in Table 3, the proposed regression model for yield and purity was sufficient to have satisfactory  $R^2$  values (0.9692 and 0.9411, respectively), which indicated that the experimental results were very consistent with the theoretical values predicted by the polynomial models. Linear ( $X_1$  and  $X_3$ ) and quadratic coefficients ( $X_1^2$ ,  $X_2^2$  and  $X_3^2$ ) were significant for yield ( $P < 0.05$  or  $P < 0.01$ ). From the model of purity, Linear ( $X_1$ ) and quadratic coefficients ( $X_1^2$ ,  $X_2^2$  and  $X_3^2$ ) were significant ( $p < 0.01$ ). The independent variables including ratio of water to material and microwave power were the most important factors with a greater effect on the yield of APPS. At the same time, ratio of water to material affected purity of APPS most obviously.

According to Equation (4) and (5), 3D response surface plots and 2D contour plots (Fig. 1) were obtained to evaluate effect of independent variables and their interaction on the yield and purity of APPS. It could be seen from Fig. 1A and 1B that when extraction time was constant (15.3 min), the yield of APPS gradually increased with the increase of ratio of water to raw material ( $X_1$ ) and microwave power ( $X_3$ ) in the range of 10-22 mL/g and 20-59.4 W, respectively. However, when ratio of water to raw material and microwave power exceeded 22 mL/g and 59.4 W, respectively, the yield of APPS decreased slightly, which indicated that effect of ratio of water to raw material ( $X_1$ ) and microwave power ( $X_3$ ) on yield was significant. Similarly, effect of ratio of water to raw material ( $X_1$ ) and microwave power ( $X_3$ ) on purity was also significant (Fig. 1C and 1D).

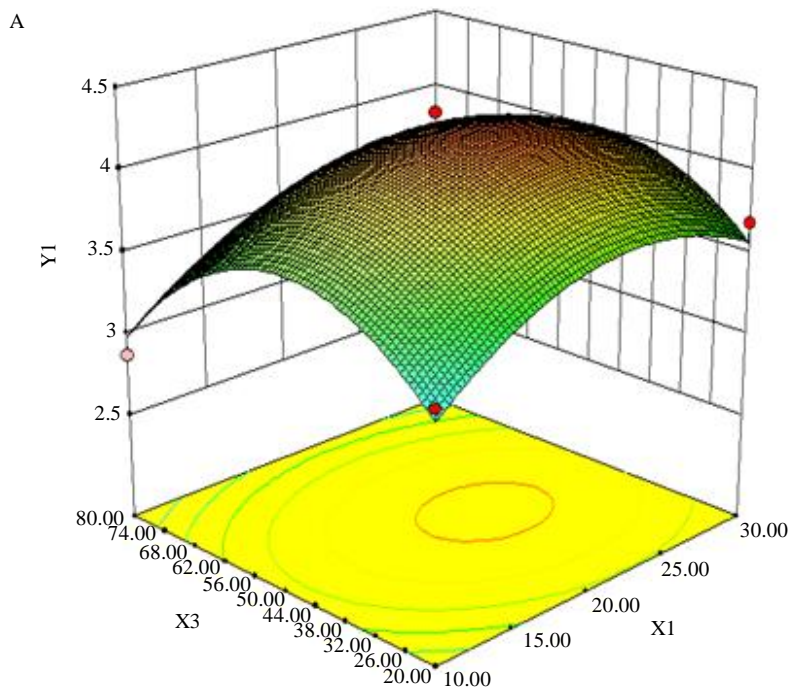
**Table 2:** Results of response surface analysis of the variation of the yield and the purity of polysaccharides from *Auricularia polytricha* with ratio of water to raw material ( $X_1$ ), time( $X_2$ ) and microwave power ( $X_3$ )

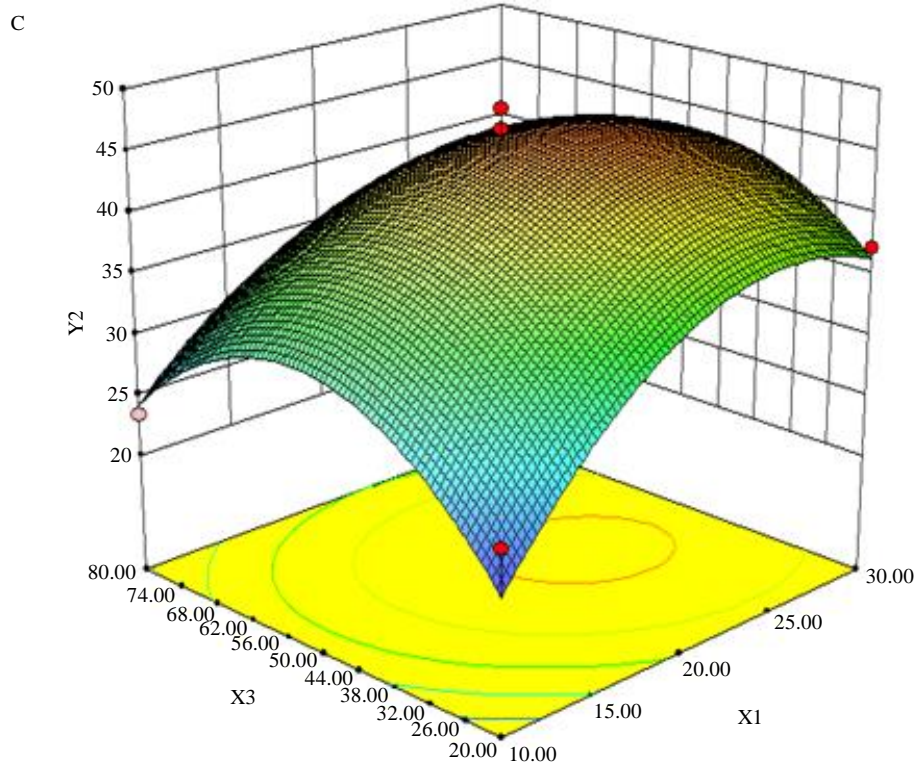
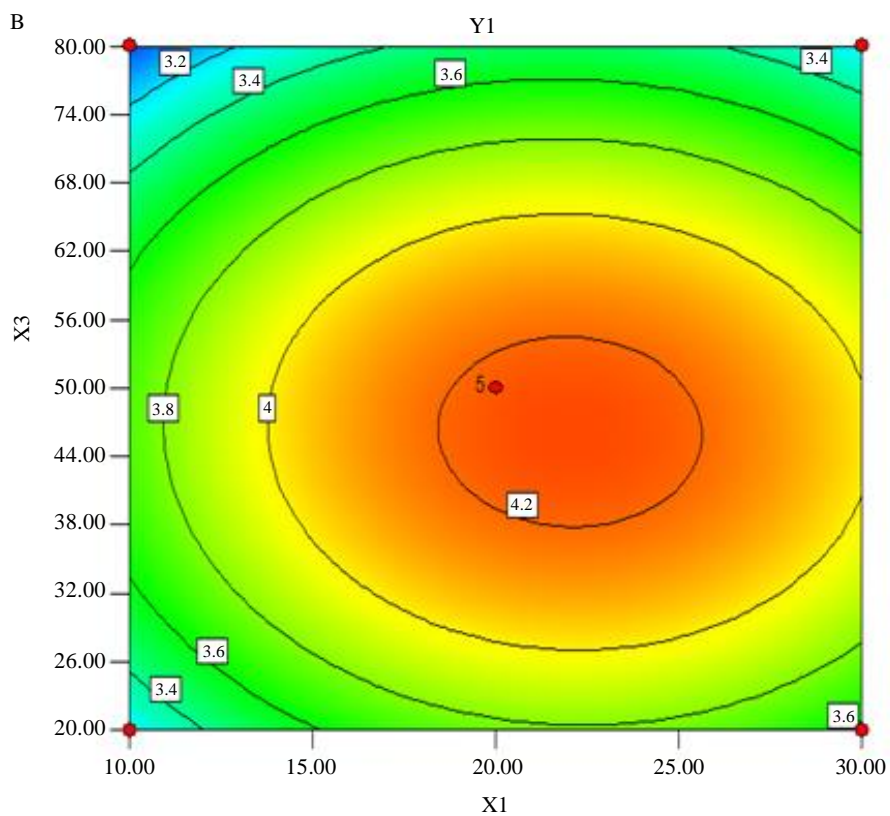
Number	$X_1$	$X_2$	$X_3$	Yield (%)	Purity (%)
1	-1	-1	0	3.21	44.37
2	-1	1	0	3.49	44.34
3	1	1	0	3.60	46.34
4	1	1	0	3.59	47.78
5	0	-1	-1	3.22	44.67
6	0	-1	1	3.28	45.22
7	0	1	-1	3.36	45.26
8	0	1	1	3.06	45.89
9	-1	0	-1	3.30	44.94
10	1	0	-1	3.69	47.45
11	-1	0	1	2.87	44.69
12	1	0	1	3.14	45.23
13	0	0	0	4.16	48.98
14	0	0	0	4.19	49.68
15	0	0	0	4.20	48.48

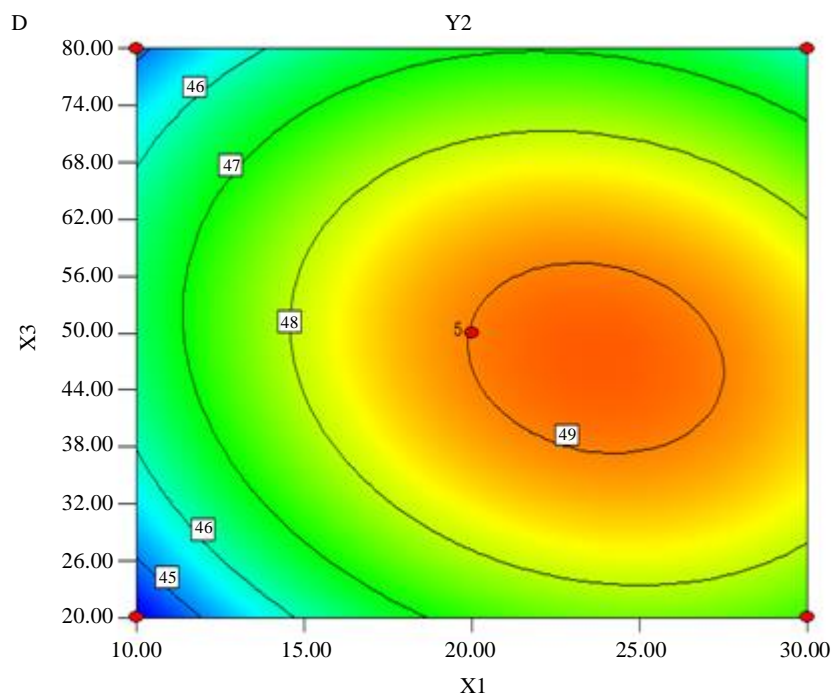
**Table 3:** Regression coefficient and analysis of the model for two response variables

coefficient	Yield (%)	Purity (%)
$b_0$	4.22	49.01
$b_1$	0.14 <sup>a</sup>	1.06 <sup>b</sup>
$b_2$	0.025	0.33
$b_3$	-0.15 <sup>a</sup>	-0.16
$b_{11}$	-0.36 <sup>a</sup>	-1.49 <sup>b</sup>
$b_{12}$	0.073	0.37
$b_{13}$	-0.027	-0.49
$b_{22}$	-0.39 <sup>a</sup>	-1.81 <sup>b</sup>
$b_{23}$	-0.089	0.020
$b_{33}$	-0.61 <sup>b</sup>	-1.94 <sup>b</sup>
$R^2$	0.9692	0.9411

Note: 'a' means  $P < 0.05$ ; 'b' means  $P < 0.01$ .







**Fig. 1:** Response surface (A and C) and contour (B and D) plots showing the effect of solid/water ratio and microwave power (extraction time 15.3min) on yield ( $Y_1$ ) and purity ( $Y_2$ ) of APPS

### Optimization of UMAE Process of APPS and Validation of the Models

Based on the response surface plots, contour plots and analysis of variance, effect of extraction conditions on yield and purity of APPS was optimized and analyzed. The optimal UMAE process of APPS was obtained as follows: Ratio of water to raw material was 22 mL/g, extraction time was 15.3 min and microwave power was 59W.

To verify the adequacy of the model equations, three verification experiments were performed under optimal conditions as described above. The average yield and purity of APPS were 4.10% and 47.98%, which were in good agreement with the predicted values (4.12% and 48.92%). The results showed that the regression models were accurate and sufficient for UMAE of APPS.

### Chemical Analysis of APPS

In the present study, APPS were extracted by UMAE under the optimal extraction conditions. Chemical analysis of APPS was performed. The result showed that content of total carbohydrate and uronic acid in APPS was  $78.3 \pm 1.57\%$  (W/W) and  $19.5 \pm 1.21\%$  (W/W), respectively. However, no proteins were found.

### FT-IR Spectroscopy

FT-IR analysis of APPS was carried out and the result was shown in Fig. 2. A broad and strong absorption peak

around  $3434 \text{ cm}^{-1}$  was due to the presence of the hydroxyl groups stretching vibration. A weak-CH stretching peak near  $2949 \text{ cm}^{-1}$  existed. A peak at  $1740 \text{ cm}^{-1}$  suggested carboxyl group existed. A absorption peak at  $1633 \text{ cm}^{-1}$  attributed to carboxyl group in uronic acid. In addition, the absorptions at  $1026$  and  $1106 \text{ cm}^{-1}$  indicated the presence of  $\alpha$ -pyranose ring (Wang *et al.*, 2014).

### Rheological Properties of Apps

#### Flow Behavior of APPS

Steady shear flow curve of APPS solution with different concentration (0.1, 0.2, 0.5, 1.0, 2.0 and 3.0%, W/V) at  $25^\circ\text{C}$  was illustrated in Fig. 3. When the shear rate increased, the apparent viscosity of APPS solution with high concentration (1.0, 2.0 and 3.0%, W/V, respectively) decreased significantly, which suggested that APPS solution exhibited pseudoplastic flow behavior at high shear rate region (shear rate  $> 25 \text{ s}^{-1}$ ). However, at low concentration (0.1, 0.2 and 0.5%, respectively), APPS solution with relatively low apparent viscosity had almost Newtonian flow behavior.

At a specified shear rate, apparent viscosity increased when APPS concentration increased. Interpenetration of polymer chains can form a dynamic 'entangled' network structure in concentrated solutions. New interactions between different chains replaced those entanglements destroyed by the imposed deformation at low rates of shear and there is no net change in the degree of

entanglement, so the viscosity is not lowered (Wu *et al.*, 2012). Shear thinning occurs when the externally applied damage rate becomes larger than the formation rate of new entanglements. In this case, the density of the temporarily formed "crosslinking" of the network is depleted and thus the viscosity is lowered (Morris *et al.*, 1981). Therefore, the crosslink density decreased further with the increase of shear rate, which could be proved by a decrease in apparent viscosity.

### Viscoelastic Behavior of APPS

Because polysaccharides are viscoelastic materials with solid and liquid properties, the moduli  $G'$  and  $G''$  refer to the elastic and viscous properties of a given material, respectively. The advantages of solid or liquid

properties of a sample can be quantified by dynamic measurements. The frequency dependence of  $G'$  and  $G''$  can be characterized by four most common classifications of dispersions, such as a dilute solution, an entangled network, a weak gel and a strong gel (Sun *et al.*, 2014). A dilute solution shows  $G''$  higher than  $G'$  over the entire frequency range (Nishinari, 1997). An entangled network shows that the  $G'$  and  $G''$  curves intersect in the test frequency range, indicating that there is a clear trend toward more solid-like behavior at higher frequencies (Xu *et al.*, 2008). The weak gel indicates that  $G'$  is larger than  $G''$  and that they are almost parallel to each other. Strong gel also shows that  $G'$  is greater than  $G''$ , but the slope of the  $G'$  curve is close to 0 and  $G''$  has a minimum at the intermediate frequencies (Moreira *et al.*, 2014).

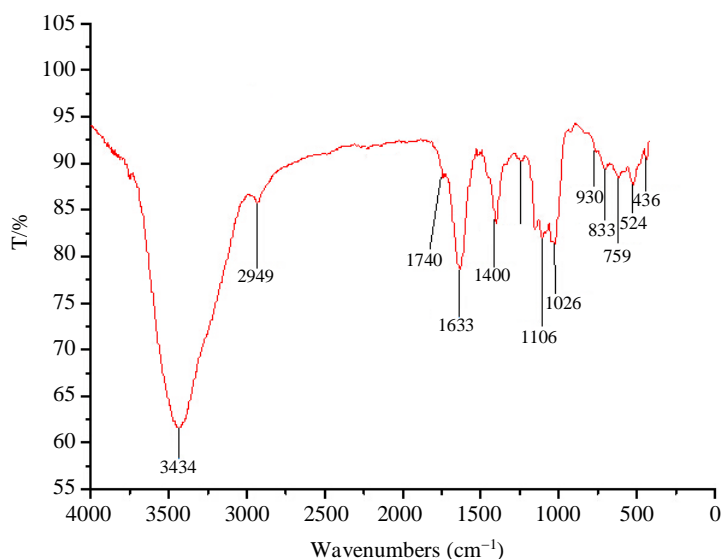


Fig. 2: FT-IR spectrum of APPS

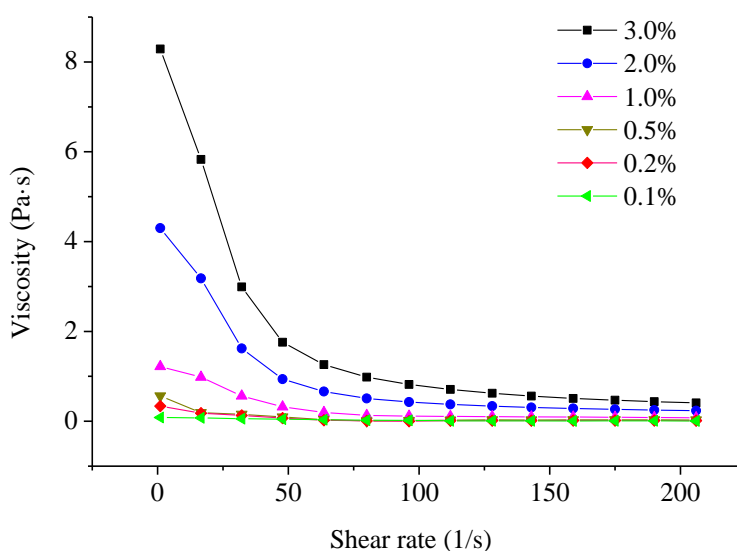


Fig. 3: Steady shear flow curves of different concentration of APPS at 25°C

Based on analysis of frequency dependence of storage ( $G'$ ) and loss ( $G''$ ) modulus of APPS solutions at 25°C, the viscoelastic behavior of APPS solutions (0.5-3.0%, W/V) was shown in Fig. 4. It was observed that in the low frequency range ( $f < 3$  Hz), the  $G'$  and  $G''$  of all APPS solutions increased with the increase of frequency. The concentration of APPS ( $c$ ) had a significant effect on  $G'$  and  $G''$ . When  $c$  was 0.5% or 1.0% and the frequency was lower than 6 Hz, a crossover between  $G'$  and  $G''$  was observed. When  $c$  increased, the cross point moved toward low frequency.  $G'$  was always larger than  $G''$  over the entire frequency range and when  $c$  exceeded 2.0%, the two moduli were almost parallel to each other. Based on the classifications mentioned above, 0.5% and 1.0% APPS solution formed an entangled network, APPS solution with higher  $c$  ( $\geq 2.0\%$ ) established weak gel structures with solid-like properties.

More interestingly,  $G'$  dropped while  $G''$  increased at  $c$  (0.5% and 1.0%) when frequency exceeded 3 Hz. The two moduli crossed at a certain frequency and the crossover of  $G'$  and  $G''$  exhibited a shift to high frequency with the increase of  $c$ . The reason may be that the junctions formed by weak interactions between molecules were destroyed under high frequency oscillatory stress, resulting in decrease in the elastic response  $G'$  and increase in the viscous response  $G''$ . The number and strength of intermolecular junctions increases with the increase of  $c$ . Therefore, the frequency oscillation stress is high enough to destroy these junctions, which leads to an increase in the frequency of intersection of  $G'$  and  $G''$  (Moreira *et al.*, 2014). It had been reported that  $G'$  and  $G''$  of the Ice Cream Mix (ICM) initially increased as the oscillation frequency increased and then decreased (Dogan *et al.*,

2013; Toker *et al.*, 2013). Similar phenomenon was also found in polysaccharides from *Ulva fasciata* (Shao *et al.*, 2015).

#### Effect of Temperature on Rheological Property of APPS Solution

Effect of temperature on rheological property of APPS solution (0.5%, w/v) was shown in Fig. 5A. The results suggested that the apparent viscosity of APPS decreased nonlinearly when solution temperature increased. An increase of solution temperature usually results in a rapid decrease in the ratio of the radius of gyration to the average molecular weight, which increases the flexibility and tightness of the molecular chain and reduces the apparent viscosity (Vardhanabhuti and Ikeda, 2006). The heating curve of 0.5% APPS solution was obtained at a heating rate of 10°C/min at constant frequency (1.59 Hz) shown in Fig. 5B. Both the storage modulus ( $G'$ ) and the loss modulus ( $G''$ ) decreased slightly when temperature increased, but the amplitude of the change was small.

#### Effect of Heating Time on APPS Viscosity

Effect of heating time on APPS viscosity was shown in Fig. 6A. Under different heating time conditions, the viscosity of APPS decreased gradually with the increase of shear rate. The lowest and the highest viscosity was obtained when heating time was 40 min and 60 min, respectively. However, the heating time had little effect on the viscosity of APPS, which was beneficial to the heat treatment for APPS as a novel food additive in food processing.

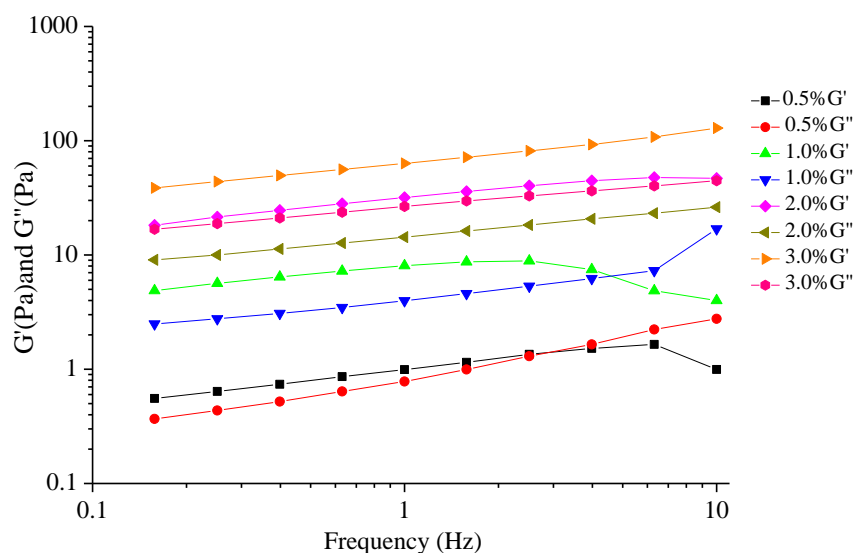
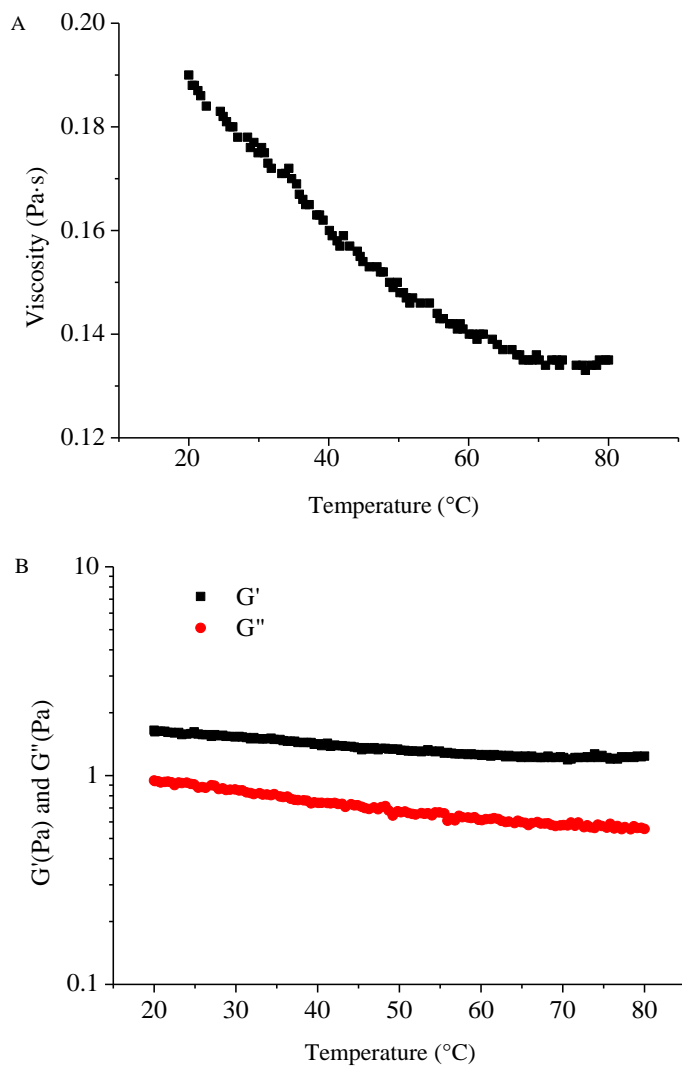
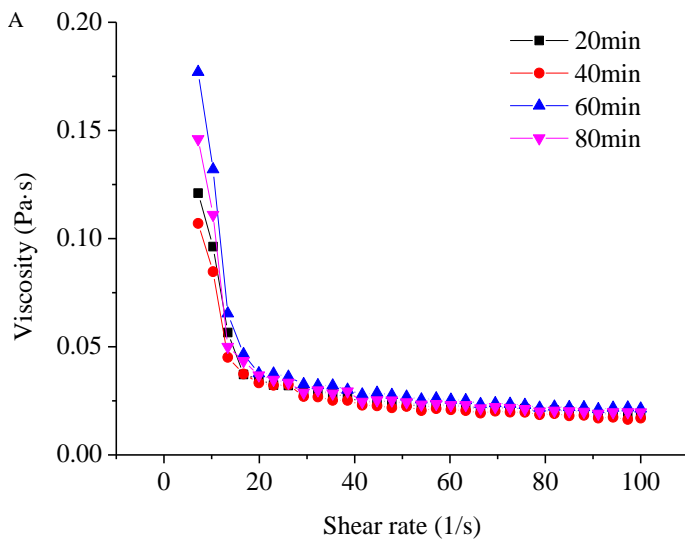


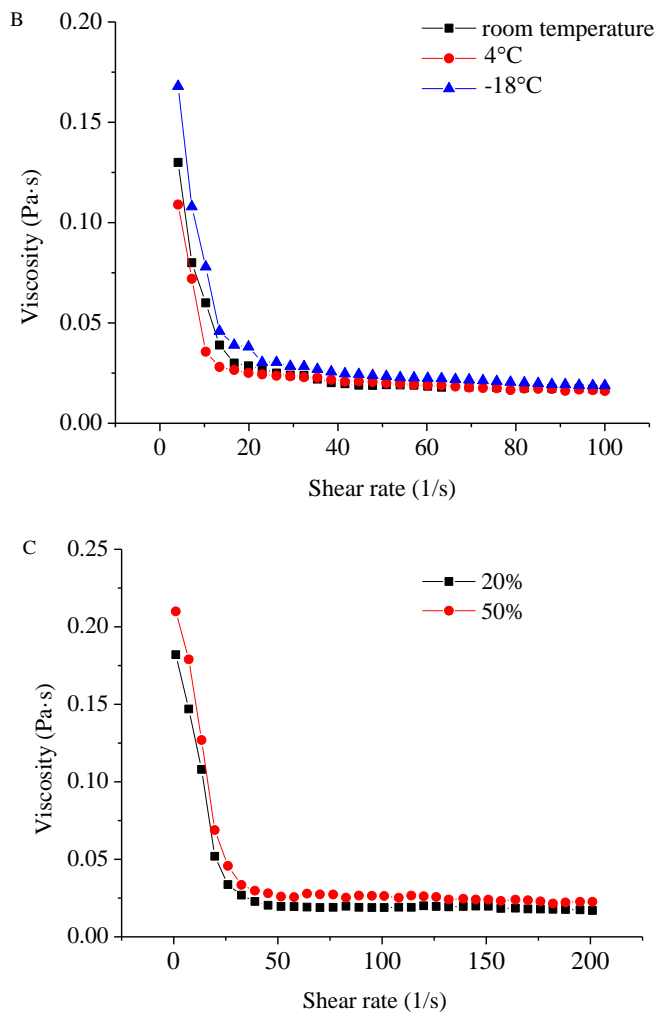
Fig. 4: Frequency dependence of storage ( $G'$ ) and loss ( $G''$ ) modulus of APPS solutions at different concentrations at 25°C



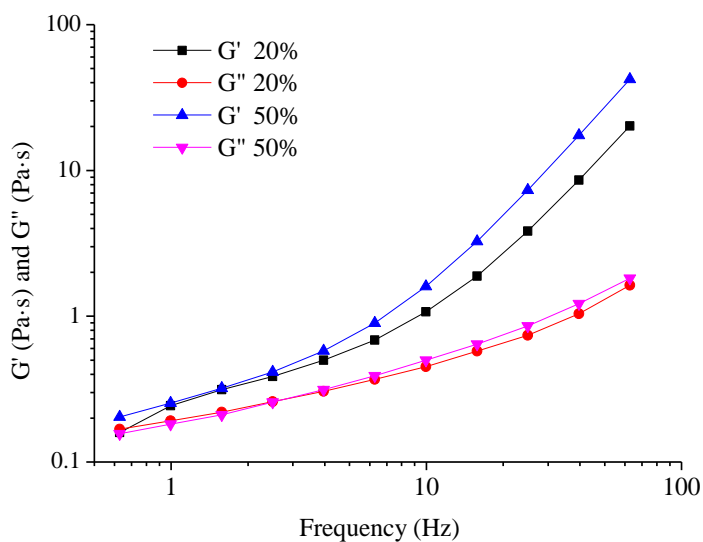


**Fig. 5:** Effect of temperature on viscosity of APPS solution (0.5%) (A) and changes of storage (G') and loss (G'') modulus of APPS solution (0.5%) at a heating rate 10°C/min from 20 to 80°C (B)





**Fig. 6:** Shear rate dependency of viscosity of APPS with different factors. (A) heating time; (B) freeze-thawing and (C) sucrose



**Fig. 7:** Frequency dependence of storage ( $G'$ ) and loss ( $G''$ ) modulus of APPS with 20% and 50% sucrose at 25°C

### Effect of Freeze-Thawing on APPS Viscosity

Effect of freeze-thawing on APPS viscosity was shown in Fig. 6B. Under different temperature conditions, the viscosity of APPS decreased gradually with the increase of shear rate. The viscosity of APPS decreased at 4°C refrigeration, while the viscosity increased under the frozen (-18°C) compared with that at room temperature. However, the change of viscosity of APPS under different temperature was small, which indicated that the freeze-thawing change has little effect on the viscosity of APPS. Some hydrocolloids such as xanthan gum, guar gum exhibited excellent stability during freeze-thaw cycling known as freeze-thaw stability (Vardhanabhuti and Ikeda, 2006). Our results indicated that APPS could be used as a novel food additive in food products that required stability after freezing.

### Effect of the Sucrose on APPS Viscosity

Effect of the sucrose on APPS viscosity was shown in Fig. 6C. Under different sucrose concentration (20% and 50%) conditions, the viscosity of APPS decreased gradually with the increase of shear rate. The viscosity of APPS increased with the increase of sucrose concentration. However, the change of viscosity of APPS under different sucrose concentration was small. The frequency dependence of storage ( $G'$ ) and loss ( $G''$ ) modulus of APPS with 20% and 50% sucrose at 25°C was shown in Fig. 7. The storage ( $G'$ ) and loss ( $G''$ ) modulus increased with the increase of sucrose concentration, which indicated that the elastic and gel structure of APPS were enhanced.

## Conclusion

Ultrasonic/Microwave Assisted Extraction (UMAE) technology was applied to extract APPS. UMAE conditions of APPS were optimized with RSM on basis of single factor experiment as follows: Ratio of water to raw material 22 mL/g, extraction time 15.3 min and microwave power 59W. Chemical characterization analysis showed that APPS contained 78.3±1.57 of total carbohydrate, 19.5±1.21%(w/w) of uronic acid and no proteins. APPS had the general characteristic absorption peaks of polysaccharides by FT-IR analysis. APPS solution exhibited Newtonian flow behavior at low concentration (0.1, 0.2 and 0.5%, respectively), formed entanglement network at lower concentration (<2.0%) and established weak gel structures with solid-like properties at higher concentration (≥2.0%). The viscosity of APPS solutions decreased with the increase of temperature. However, the viscosity, storage ( $G'$ ) and loss ( $G''$ ) modulus of APPS solution increased with the increase of sucrose concentration, which indicated that the elastic and gel structure of APPS were enhanced. Heating time and freeze-thaw changes did not show a significant effect on

rheological properties of APPS. The lowest and the highest viscosity was obtained when heating time was 40 min and 60 min, respectively. Our results suggested that UMAE was a suitable and efficient method for APPS extraction and APPS had potential as a novel food additive hydrocolloid for application in food industry.

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## Author's Contributions

**Ze Zhang and Xiao Ding:** Participated in all experiments and article writing; also responsible for data analysis.

**Ziyi Xu and Xinyi Wu:** Participated in optimization of UMAE conditions of APPS on basis of single factor experiment and rheological properties of APPS and data analysis.

**Chenzhi Yang and Jing Hao:** Participated in chemical characterization analysis and rheological properties of APPS and data processing.

**Yiyong Chen:** Participated in project design and experimental guidance of this manuscript.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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