



Changes in polysaccharides structure and bioactivity during *Mesona chinensis* Benth storage

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ABSTRACT

Mesona chinensis Benth has been consumed as a functional food for many years. It is widely believed that storage times affect its health benefits. In this study, *Mesona chinensis* Benth polysaccharides with two different storage times (fresh and storage for 1 year) were prepared, namely, FMP and AMP. The physicochemical properties and bioactivity were comparatively assessed. Results indicated that FMP was mainly composed of galacturonic acid, galactose, and glucose with a molecular weight of 44.39 kDa. AMP was composed of galacturonic acid, galactose, and fructose with a molecular weight of 64.34 kDa. However, the principal structural characteristics of polysaccharides remained stable. Furthermore, assays of antioxidant activity showed that *Mesona chinensis* Benth polysaccharide had an antioxidant effect against DPPH radical, ABTS radical cation, among which FMP was stronger. Additionally, flow cytometry indicated that the apoptosis rate of FMP and AMP on HepG2 tumor cells was $22.50 \pm 1.25\%$ and $15.49 \pm 1.30\%$, respectively. In general, antioxidant and antitumor activities of *Mesona chinensis* Benth polysaccharides were decreased as the storage for 1 year. The change of physicochemical properties was responsible for the enhanced bioactivities. These results explained how polysaccharides contributed to the decreased health benefits of *Mesona chinensis* Benth during storage.

1. Introduction

Mesona chinensis Benth is a well-known Chinese supplement and medicinal plant also known as the *Hsian-tsao*. It is a popular supplement with the function of detoxifying and heat-clearing use in Asia. It is used to be processed into the popular tea, soup, black jelly (Tang et al., 2017). There are several active components in the *Mesona chinensis* Benth including polysaccharide, campesterol, apigenin, saponins and flavonoid (Lin et al., 2018). *Mesona chinensis* Benth polysaccharide (MP) as the main bioactive substance of water-soluble *Mesona chinensis* Benth extracts, has become a hot topic of research owing to its various bioactivities. It is generally recognized that the bioactivity of *Mesona chinensis* Benth is closely related to the storage time. The health benefits and application of *Mesona chinensis* Benth are different depending on the storage time. In the market, generally only sell *Mesona chinensis* Benth within one year, and the price varies depending on the storage time. However, why storage time can afford *Mesona chinensis* Benth bioactivity remains unclear. As polysaccharides are important bioactive components in *Mesona chinensis* Benth, they may contribute to the

changed bioactivity of *Mesona chinensis* Benth due to storage time.

There are some reports have shown that long-term storage will lead to changes in the structure of the active ingredients in the raw material (Nagarajan et al., 2021). For example, The longer Chachi is stored, the more obvious the changes in the structure of its cell wall polysaccharides, and the better bioactivity it brings (Zhou et al., 2021). In addition, the change of polysaccharide structure affects its application in food processing. In Chinese folks, people utilize the gel-forming properties of the *Mesona chinensis* Benth by boiling its stems in water and then combining with starch to make jelly (often called "herbal jelly"), which is a popular food to quench heat and thirst (Ren et al., 2020a,b; Tang et al., 2017; Wang et al., 2020a, 2020b). It is unknown whether the storage time also affects the properties of the jelly produced by the blending of jelly with polysaccharides.

Therefore, in this work, fresh *Mesona chinensis* Benth by hot air-dried and *Mesona chinensis* Benth sample with storage times of 1 year were selected. Their polysaccharides were obtained, and the physicochemical characteristics and properties of these polysaccharides were comparatively discussed in this study. Various chemical methods such as high-

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performance anion exchange chromatography (HPAEC), fourier-transform infrared spectroscopy (FT-IR), high-performance gel permeation chromatography (HPGPC) were applied to analyze the physicochemical properties. The rheological and texture of polysaccharides mixing with wheat starch (WS) were used to evaluate the gel feature of the polysaccharides. The free radical scavenging ability and antitumor activities of polysaccharides were also evaluated.

2. Materials and methods

2.1. Materials and reagents

Mesona chinensis Benth was purchased from Yichun, Jiangxi, China. Coomassie brilliant blue G250, bovine serum albumin, ascorbic acid and the standard monosaccharides including mannose, fructose, rhamnose, xylose, glucose, galactose, arabinose, ribose, and fucose were purchased from Sigma-Aldrich (Sigma, USA). Wheat starch was purchased from Enmiao Food Co., Ltd. (Henan, China). Human hepatocellular carcinoma cell lines (HepG2) were purchased from the Chinese Academy of Sciences (Shanghai, China). CCK8 was purchased from Meilunbio (Dalian, China). The ultrapure water was utilized from a Milli-Q water purification system (Millipore, USA). DMEM medium was purchased from Solarbio (Solarbio, China). All other chemicals and reagents were of analytical grade.

2.2. Preparation of polysaccharides

Fresh *Mesona chinensis* Benth by hot air-dried and *Mesona chinensis* Benth sample with storage times of 1 year were soaked with 95% ethanol overnight at room temperature, and extracted with distilled water for 2 times at 100 °C. Then the supernatant was precipitated by the 80% ethanol overnight. polysaccharides were obtained after dialysis and lyophilization, namely FMP and AMP.

2.3. Physicochemical properties of polysaccharides

2.3.1. Chemical analysis

The sulfuric acid-carbazole method (Blumenkrantz and Asboe-Hansen, 1973), phenol-sulfuric acid method (Dubois et al., 1956), and Coomassie brilliant blue method (Bradford, 1976) were used to measure the contents of uronic acid, total sugar and protein of FMP and AMP, respectively.

2.3.2. Molecular weight (Mw) determination

The Mw was analyzed using high-performance gel permeation chromatography (HPGPC) method (Tang et al., 2017) on a Waters e2695 system coupled with a Waters Ultrahydrogel™ Linear column (7.8 mm × 300 mm). The Dextrans were applied as standard to establish a standard curve. Dextran standards (Mw: 10000 Da, 40000 Da, 70000 Da, 500000 Da, 2000000 Da) were used for calibration.

2.3.3. Monosaccharide composition

Monosaccharide composition of FMP and AMP was analyzed using high-performance anion exchange chromatography equipped with pulsed amperometric detection (HPAEC-PAD) (Xie et al., 2013). Briefly, under ice bath conditions, 5 mg sample was hydrolyzed with 0.5 mL of sulfuric acid (12 M). Then hydrolyzed for 4 h at 105 °C and then made up to 50 mL with deionized water. The FMP and AMP were determined by the Dionex ICS-2500 ion chromatography system (Dionex, USA) coupled with a CarboPAC™ PA10 column (2.0 × 250 mm) analytical column.

2.3.4. FT-IR spectra determination

FT-IR spectra of the FMP and AMP were obtained using fourier transform infrared spectroscopy as a film between two potassium bromide (KBr) plates on a Nicolet 5700 FT-IR spectrometer (Sheikh et al., 2021) (Thermo Nicolet Nexus-870, USA).

2.3.5. UV absorption peak determination

The UV-Vis spectrum of AMP and FMP was obtained using a spectrophotometer (TU-1900, Pgenenal, Beijing, China) in the wavelength range of 200–500 nm.

2.3.6. Microstructure

FMP and AMP were scanned using a scanning electron microscopy SEM (Jeol, Japan) at 5.0 kV and atomic force microscope (AFM), then the microstructure and surface morphology of polysaccharides were observed.

2.4. Biological activities of polysaccharides

2.4.1. Antioxidant assays

2.4.1.1. DPPH assay. The DPPH radical-scavenging capabilities of FMP and AMP were determined in the 96-well plates according to the previous method with some modifications (Shao et al., 2014). 100 μL of 0.1 M DPPH dissolved in 95% ethanol was mixed with 100 μL of polysaccharide samples. Then the mixture reacted for 30 min in the dark at room temperature. The absorbance was recorded at 517 nm. The result was expressed by the following equation (Xie et al., 2010):

$$\text{DPPH radical scavenging rate (\%)} = [1 - (A_1 - A_2)/A_0] \times 100\%$$

Where A_0 is the absorbance of the blank (ethanol instead of the sample), A_1 is the absorbance of samples, A_2 is the absorbance of background (ethanol instead of DPPH).

2.4.1.2. ABTS assay. ABTS radical-scavenging capabilities of polysaccharides were determined according to previous method with some modifications (Shi et al., 2016). Various concentrations of polysaccharides (0.05, 0.1, 0.25, 0.5, 1 mg/mL) reacted with ABTS working liquid at room temperature for 10 min in the dark followed by absorbance determination at 734 nm. The ABTS radical scavenging rate was expressed by the following equation (Gu et al., 2020):

$$\text{ABTS radical scavenging rate (\%)} = [1 - (A_1 - A_2)/A_0] \times 100\%$$

Where A_0 : the absorbance without sample, A_1 : the absorbance of sample, A_2 : the absorbance without ABTS+ solution.

2.4.2. Antitumor assay

2.4.2.1. Cell and cell culture. HepG2 cells were adherently grown in media containing DMEM with 10% (v/v) FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin. The cells were cultured in 5% (v/v) CO₂ humidified incubator at 37 °C.

2.4.2.2. Assessment of cell viability. The cell viability of FMP and AMP on HepG2 cells was measured. Cells (2×10^5 cells/mL) were cultured in 96-well plates for 12 h and next treated by two samples with different concentrations (50, 100, 200, 400 μg/mL). After 24 h, these cells were pulsed with 10 μL of CCK8 solution, and the absorbance at 450 nm was assessed by a microplate reader.

2.4.2.3. Assessment of cell apoptosis by flow cytometry. To measure the antitumor activity of FMP and AMP, HepG2 cells (2×10^5 cells/mL) were grown in 6-well plates for 12 h. Then cells were exposed to FMP and AMP (400 μg/mL), respectively. After 24 h, the cells were resuspended in binding buffer (500 μL), FITC Annexin-V (5 μL) and propidium iodide (PI) (5 μL) in dark at room temperature for 10 min. The percentages of cells apoptosis were analyzed on the flow cytometer.

2.5. Gel properties of polysaccharides with wheat starch (WS)

Mesona chinensis Benth is a significant edible and medicinal plant

resource, which has the functions of clearing heat, relieving heat, cooling blood, and detoxifying. In Chinese folks, people utilize the gel-forming properties of the *Mesona chinensis* Benth by boiling its stems in water and then combining with starch to make jelly (often called "herbal jelly"), which is a popular food to quench heat and thirst (Ren et al., 2020a,b; Tang et al., 2017; Wang et al., 2020a, 2020b).

FMP (0.05, 0.1 and 0.2, w/v)-WS (6%, w/v) and AMP (0.05, 0.1 and 0.2, w/v)-WS (6%, w/v) mixtures (FMP-WS and AMP-WS) were prepared as follows. The polysaccharides and WS were added in distilled water while constantly stirring at 95 °C for 30 min to get FMP-WS and AMP-WS suspension.

2.5.1. Gel strength

The gel strength of FMP-WS and AMP-WS was analyzed via applying the texture analyzer (TA-XTplus, Stable Co., England) coupled with a P/0.5R probe (Ren et al., 2020a,b). FMP-WS and AMP-WS gel were obtained as the method described in Section 2.5. The FMP-WS and AMP-WS were placed for 1 h at room temperature, then remove to 4 °C to stabilize gel (12 h). The parameters were set as followed: the test distance was 10.0 mm, the pre-test, the test, and the latter test speed were all set as 2.0 mm/s, the trigger type was automatic, and the trigger force was 5 g.

2.5.2. Gel texture analysis

The samples were placed for 12 h at room temperature to stabilize gel and the rheological properties were measured using an ARES rheometer (TA Instruments, New Castle, DE, USA) fitted with a parallel plate (500 μm gap, 40 mm diameter). The MP-WS were removed to the parallel plate. The steady shear was determined with a shear rate range from 0.01 to 10 s⁻¹ at 25 °C. The relationships of shear rate and apparent viscosity were tested via a continuous shear test. The dynamic oscillatory rheological properties of MP-WS were determined via the frequency sweep test at the 1% strain within the frequency range from 0.1 to 25 Hz. The values of complex viscosity storage modulus (G'), loss modulus (G''), and loss tangent ($\tan\delta = G''/G'$) were obtained.

2.6. Statistical analysis

All experiments were performed at least in triplicate and data were expressed as mean ± standard deviation (SD). Differences among data mean values were tested for statistical significance at the $p < 0.05$ level using analysis of variance and Duncan's multiple range test followed by SPSS 20.0 (IBM, USA).

3. Results and discussion

3.1. Chemical characterization

The extraction yields and the chemical composition of the FMP and AMP are listed in Table 1. The resulting yields of the two samples were 2.58% for AMP and 0.84% for FMP. Apparently, the yield of AMP was higher than FMP, which suggested the AMP has high production. However, FMP had the higher uronic acid and total sugar, which were higher than AMP. On the contrary, the content of protein of AMP is higher than FMP. Polysaccharides from diverse origins have been found to possess few protein substances that may be present as a pollutant during the co-precipitated and extraction in the polysaccharides (Olawuyi et al., 2020). However, the protein content of the separated components was applied to assess the purity of obtained polysaccharide. The discrepancies in polysaccharide content reflect structural changes caused by the raw materials and purity of the polysaccharides. Therefore, it can be observed from the results of low protein contents and significantly higher polysaccharide content that *Mesona chinensis* Benth polysaccharides of higher purity could be extracted from fresh *Mesona chinensis* Benth.

Table 1

Chemical composition, molecular weight and monosaccharide composition of two polysaccharides.

Samples	AMP	FMP
Yield (%)	2.59	0.84
Chemical composition (Weight percentage)		
Total sugar (%)	17.08 ± 6.17	30.69 ± 6.98
Uronic acid (%)	11.85 ± 0.28	20.86 ± 1.31
Protein (%)	36.22 ± 1.92	25.30 ± 5.77
Molecular weight (Weight-average, Da)		
	64.34 kDa	44.39 kDa
Monosaccharide composition (Amount ratios)		
Ara	0.24	0.30
Fru	1.98	0.66
Fuc	N.D.	N.D.
Gal	1.42	1.97
GalA	1.50	1.69
GlcA	N.D.	N.D.
Glu	0.64	1.12
Man	N.D.	0.50
Rha	0.30	0.42
Rib	N.D.	N.D.
Xyl	N.D.	N.D.

N.D.: Not detectable or lower than the limit of quantification.

Values are mean ± SD, n = 3.

3.2. Mw and monosaccharide composition analysis

According to Table 1, The average Mw of the main fraction of AMP and FMP was 64.34 kDa and 44.39 kDa respectively. Obviously, the molecular weight of *Mesona chinensis* Benth increased after storage.

HPLC-PAD is a common method used to analyze the monosaccharide composition via comparisons of retention times with standards (Xie et al., 2016). Based on the retention times with the standards, the results are summarized in Table 1. AMP has six types of monosaccharides, whereas FMP contained seven monosaccharides. These results showed that two samples had different monosaccharide types, which reconfirmed that FMP and AMP possessed different structural characteristics. The monosaccharide compositions results indicated that the monosaccharides of FMP consisted of arabinose, fructose, galactose, glucose, mannose, rhamnose and galacturonic acid, while AMP is an acidic heteropolysaccharide containing arabinose, fructose, glucose, galactose, galacturonic acid and rhamnose, which is consistent with chemical characterization. Furthermore, galactose, galacturonic acid and glucose were the main monosaccharides of FMP with the ratios of 1.97:1.69:1.12. And galactose, galacturonic acid and fructose were the main monosaccharides of AMP ratios was 1.42:1.50:1.98 (Table 1). Apparently, the monosaccharide compositions and content differed between the two samples. Compared with AMP, the contents of galacturonic acid and galactose of FMP were higher (Tang et al., 2017). reported that MP extraction thought different methods were mainly composed of glucose, galactose and galacturonic acid with ratios of 1.00:2.49:0.19, 1.00:2.95:0.84 and 1.00:1.34:0.25, which was discrepant with our dates possibly for the different extraction methods and raw material origins. The monosaccharide compositions can even be influenced via the growth stage of plants (Yan et al., 2019). Thus, raw materials may also be an important factor in the monosaccharide compositions of MP.

3.3. UV-vis and FT-IR spectrum analysis

FMP and AMP were recorded at 200–500 nm in UV-vis spectrophotometer. In Fig. 1A, compared with the FMP, AMP shared similar maximum absorption peaks, which indicates that the substances were relatively single and confirm that AMP still retained their nature main material component during the storage.

The data of FT-IR spectra (Fig. 1B) indicated two samples have similar structural features suggesting that the functional group conformation of *Mesona chinensis* Benth polysaccharide did not be affected

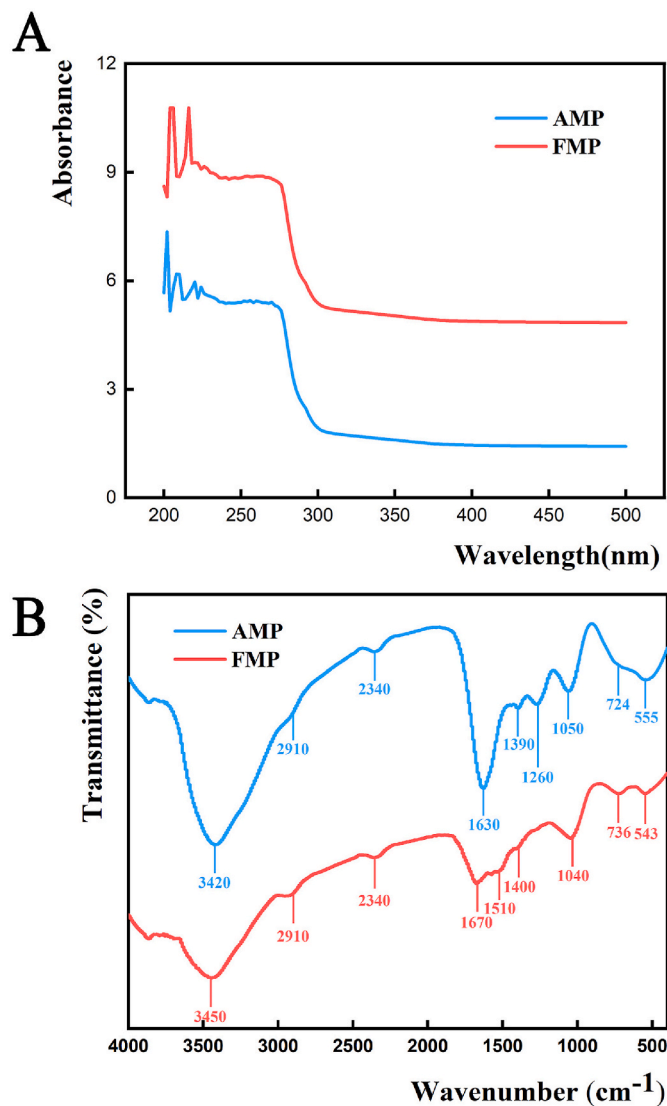


Fig. 1. UV-vis and FT-IR spectra of AMP and FMP. (A) UV-vis spectra were recorded in the range of 200–400 nm. (B) FT-IR spectrum.

after storage for 1 year. The broad band around 3400 cm^{-1} and small band appeared around $2960\text{--}2855\text{ cm}^{-1}$ assigned to the O–H stretching vibration, methylene group and methyl group respectively, which are typical absorption band areas of polysaccharides (Li et al., 2020; G. Liu et al., 2020). The absorptions in the range of 1610 cm^{-1} and 1260 cm^{-1} attributed to the C=O and O–H of COOH, respectively, which suggested the occurrence of the uronic acid (Tang et al., 2017). The band at 730 cm^{-1} shown that the existence of α -linked glycosyl residues. The bands near 1038 cm^{-1} and $500\text{--}800\text{ cm}^{-1}$ showed the existence of pyranose rings (Y. Liu et al., 2020). The results confirmed that FMP and AMP may be polysaccharides with α -type glycosidic linkages and pyranose rings. The results of FT-IR band assignments of FMP and AMP are consistent with previous research of MP (Huang et al., 2019; Tang et al., 2017).

3.4. Microstructure analysis

Microstructure images can be applied to observation of the surface morphology of polysaccharides to reveal the molecular morphological features of polysaccharides (Qin et al., 2018). The SEM analysis was carried out to check for the differences, if any, from the microstructures of AMP and FMP. The micrographs of AMP and FMP at various magnifications such as $500\times$ and $2000\times$ are shown in Fig. 2. FMP was diverse

appearances, such as rod-like, sheet-like and thin slice shapes with a smooth surface. Compare with FMP, AMP exhibited a different appearance consisting of lump-like particles with a rough surface in different sizes and the surfaces of these structures were even with bead-like or blistered structures thereon. This distinction can be ascribed to the different monosaccharide composition and the content of uronic acid (Romdhane et al., 2017), which well confirmed the above results and is consistent with other studies (Ma et al., 2019). The AFM analysis revealed the aggregation extent of the two samples. In general, sugar chains of different compositions always tend to form the conformation with the lowest free energy. The bulk structure of AMP is more irregular compared to that of FMP. This suggests that the inter- and intramolecular aggregation caused by hydroxyl group interactions on the polysaccharide chains may have occurred as a disordered spontaneous self-assembly process during the storage of the raw material (Lin et al., 2020). These results are consistent with the results of Section 3.2 (Molecular weight increase after raw material storage). The difference in the microstructure of the two samples indicates that the storage of *Mesona chinensis* Benth for one year has an effect on the surface morphology of the macromolecules, which may have changed the polysaccharides with physical properties that could determine their applications in cosmetics and medicine (Lin et al., 2018, 2020; Olawuyi et al., 2020; Zhu et al., 2014).

3.5. Antioxidant activity

3.5.1. DPPH assay

DPPH free radical scavenging model is a common antioxidant assay to assess the scavenging ability of free radical scavengers (Yuan et al., 2020). Polysaccharides that could deliver hydrogen have been shown to decrease the stabilized DPPH radical to yellow diphenylpicrylhydrazine (Qin et al., 2018). The effects of AMP and FMP on the DPPH scavenging capability are shown in Fig. 3A, the DPPH scavenging assay of two samples exhibits a concentration-dependent scavenging activity against DPPH. When the concentration of polysaccharides up to $1000\text{ }\mu\text{g/mL}$, the scavenging effects of AMP and FMP were $75.59 \pm 0.13\%$ and $86.78 \pm 0.15\%$ respectively, which exhibited an excellent capability against DPPH free radicals. Previous studies have exhibited MP had excellent antioxidant activity (Tang et al., 2017). Interestingly, among the two samples, FMP showed higher scavenging activity than AMP in all concentrations. Therefore, this result implies that the polysaccharide extracted from fresh *Mesona chinensis* Benth might have better antioxidant activity.

3.5.2. ABTS assay

ABTS free radical scavenging assay is commonly used to assess antioxidant activity owing to its convenience, rapidity and sensitivity (Yuan et al., 2020). In the ABTS free radical test, antioxidants can supply electrons or hydrogen atoms to transform the color of the ABTS and the antioxidant capacity of the sample is calculated by determining the absorbance at 734 nm (Ma et al., 2020). The results of the ABTS assay showed AMP and FMP to exhibit ABTS radical scavenging activities in an obvious dose-dependent manner (Fig. 3B). Previous studies have shown that polysaccharides exhibit changes in structure owing to changes in storage time (Ren et al., 2020) and MP to exhibit remarkable antioxidant activity as well as important differences in structure, have been observed due to their extraction methods (Tang et al., 2017). The IC_{50} value of FMP ($280.54\text{ }\mu\text{g/mL}$) was lower than that of AMP ($332.34\text{ }\mu\text{g/mL}$), which exhibited an excellent capability against ABTS free radicals. The AMP shows the lower antioxidant activity may be a result of longer storage time. Therefore, combined with the result of DPPH, the FMP may have indeed better antioxidant activity. Furthermore, the mechanism of the polysaccharide's antioxidant activity may be owing to the released hydrogen ions (H^+) binding to ABTS radicals, it could form more stabilized radicals, thus ceasing the free radical chain reaction. Besides, the antioxidant activity of polysaccharides has also depended

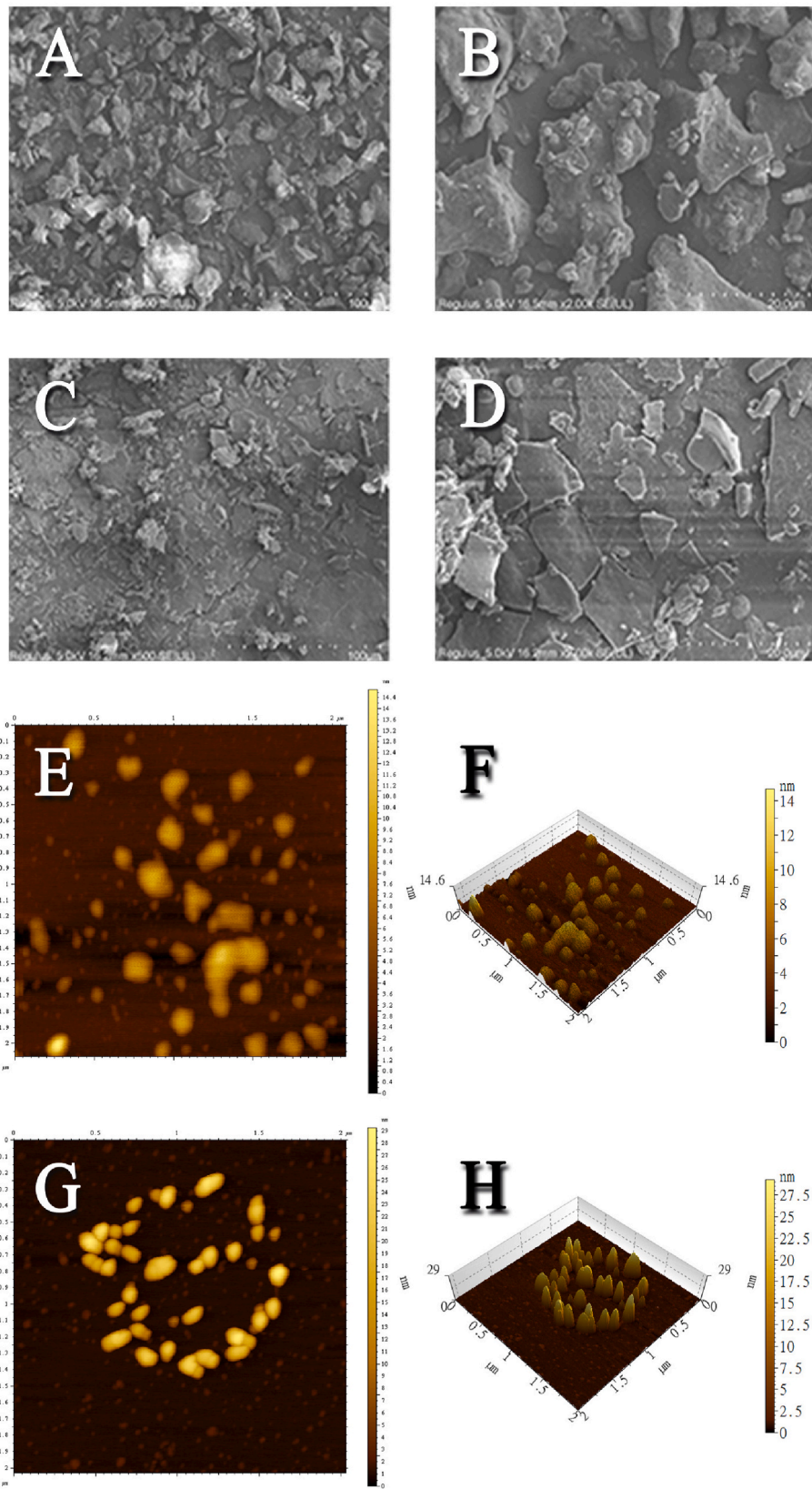


Fig. 2. Microstructure images of AMP and FMP. SEM images of AMP (A: $\times 500$, B: $\times 2000$) and FMP (C: $\times 500$, D $\times 2000$); AFM images of AMP and FMP.

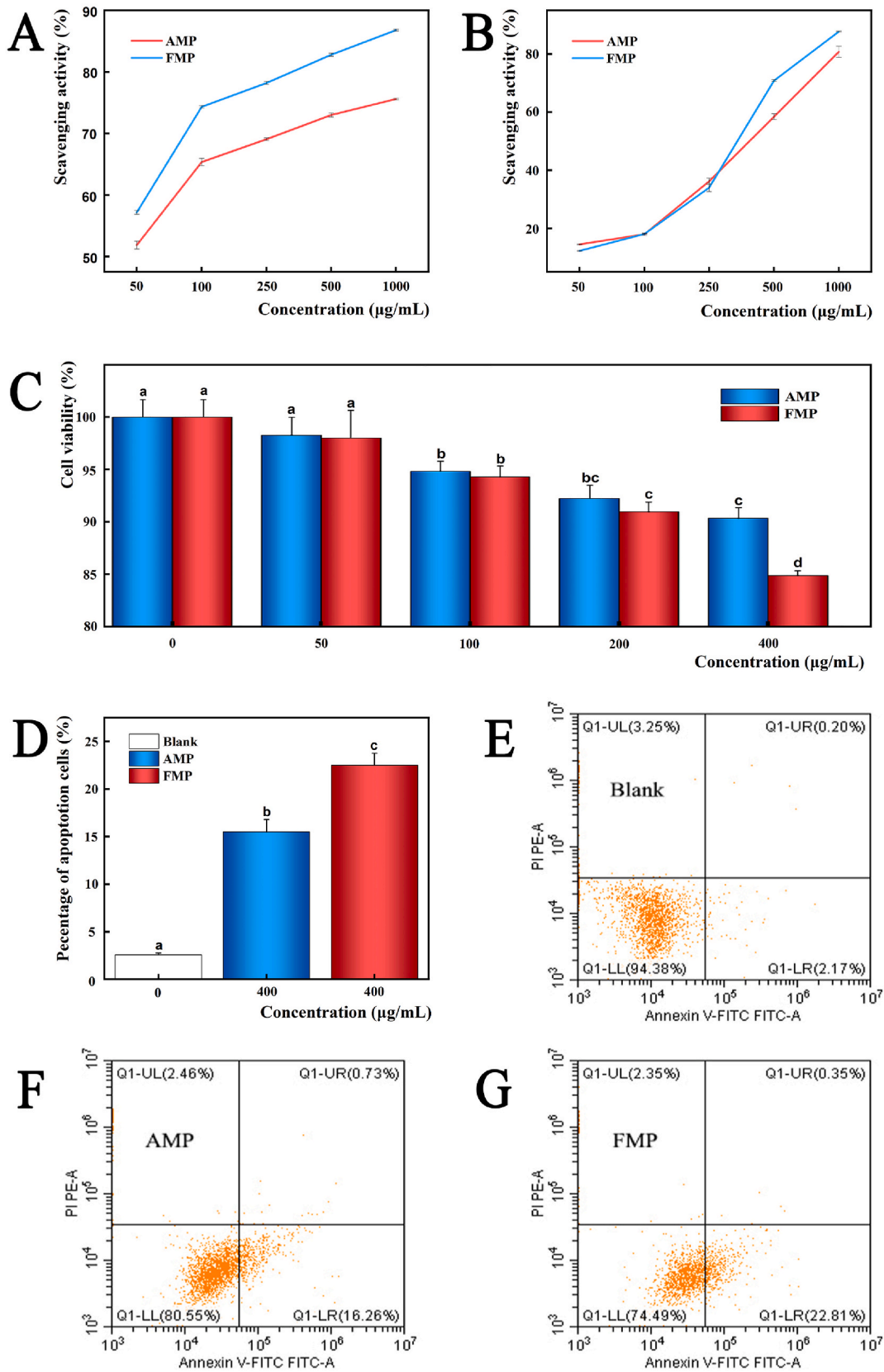


Fig. 3. Biological activities of AMP and FMP. (A) DPPH assay; (B) ABTS assay; (C) Cell viability assay; Effects of polysaccharide treatment on the apoptosis in HepG2 cells ((D) Quantitative presentation of apoptosis; (E) Blank (untreated) cells; (F) cells treated with 400 µg/mL AMP; (G) cells treated with 400 µg/mL FMP). Values are expressed as means ± SD (n = 3), different letters indicated a different significance ($p < 0.05$) among all the groups.

on their structure and purity (Olawuyi et al., 2020; Tang et al., 2017).

This could be attributed to higher molecular weight tending to exhibit greater steric hindrance and poorer water solubility. In addition, High-molecular-weight polysaccharides have a dense structure and stronger intramolecular hydrogen bonds, which limits the activity of hydroxyl and amino groups (Chen et al., 2021). Therefore, the increase in molecular weight of *Mesona chinensis* Benth after storage may be the main reason for the decrease in its antioxidant activity. These results are similar to previous researches (Yuan et al., 2020).

3.6. Antitumor activity

Liver cancer was the most commonly seen of all cancer cases and is a major cause of cancer deaths around the world. The finding of effective drugs has been a crucial challenge for decades (Zhang et al., 2019). Polysaccharide is a natural product that is currently drawing much attention owing to its antitumor activity without obvious side effects (Yuan et al., 2019).

3.6.1. Cell viability

To test the potential cytotoxicity of AMP and FMP, CCK8 assay was used to detect the inhibitory effect of AMP and FMP at different concentrations on the proliferation of human hepatoblastoma HepG2 (Liu et al., 2018). As shown in Fig. 3C, after HepG2 cells were incubated with AMP or FMP from 0 $\mu\text{g}/\text{mL}$ to 400 $\mu\text{g}/\text{mL}$, the cell viability decreased from 100% to 89.86% and from 100% to 81.46%, respectively, demonstrating that AMP and FMP had a prominent inhibiting effect on HepG2 cells in dose-dependent manners. Interestingly, among the two samples, FMP showed higher growth inhibition of HepG2 cells than AMP. In addition, the maximum inhibitory ability of AMP and FMP both were reached at a concentration of 400 $\mu\text{g}/\text{mL}$. Moreover, when the addition of polysaccharide is 400 $\mu\text{g}/\text{mL}$, FMP showed significant ($p < 0.05$) stronger cells cytotoxicity than AMP.

3.6.2. Cell apoptosis

To better quantify the apoptosis caused by FMP and AMP on HepG2 cells, the apoptosis levels were measured using the concentration that works best in section 3.6.1 (400 $\mu\text{g}/\text{mL}$) by flow cytometry. As shown in Fig. 3D–G, after cells were treated with FMP and AMP, the numbers of apoptotic cells were significantly increased ($p < 0.05$) as compared to the cells without treatment of polysaccharides. Interestingly, among the two samples, FMP showed a higher apoptosis rate of HepG2 cells than AMP. These results indicated that FMP showed better antitumor activity than AMP.

3.7. Gel strength

Gel strength is an important index of food texture and is chiefly useful for predicting the physical properties of foods (Ren et al., 2020a, b). As shown in Fig. 4A, the Gel strength of the WS gel was very low (12.14 g), the addition of FMP was significant for improving the gel strength of FMP-WS gel (20.95–34.47 g) ($p < 0.05$), and the improvement was a concentration dependent. This result indicated that FMP could promote the formation of FMP-WS gel networks, which may be owing to *Mesona chinensis* Benth polysaccharides is an anionic polysaccharide that has a negative charge (Wang et al., 2020a, 2020b). After FMP was added, hydrophobic, hydrogen bonding, and electrostatic interactions were enhanced in the FMP-WS system (Wang et al., 2020a, 2020b). As a result, WS and FMP form a dense network structure via these interactions, leading to an enhance in the gel strength of the FMP-WS gel, which is consistent with other studies (Ren et al., 2020a,b; Wang et al., 2020a, 2020b). On the contrary, AMP not only didn't increase the gel strength of WS, but also resulted in the weakening of WS gel-forming ability. This may be caused by changes in the internal structure of the raw material and its surface potential during storage for one year, which reduces the interaction strength between

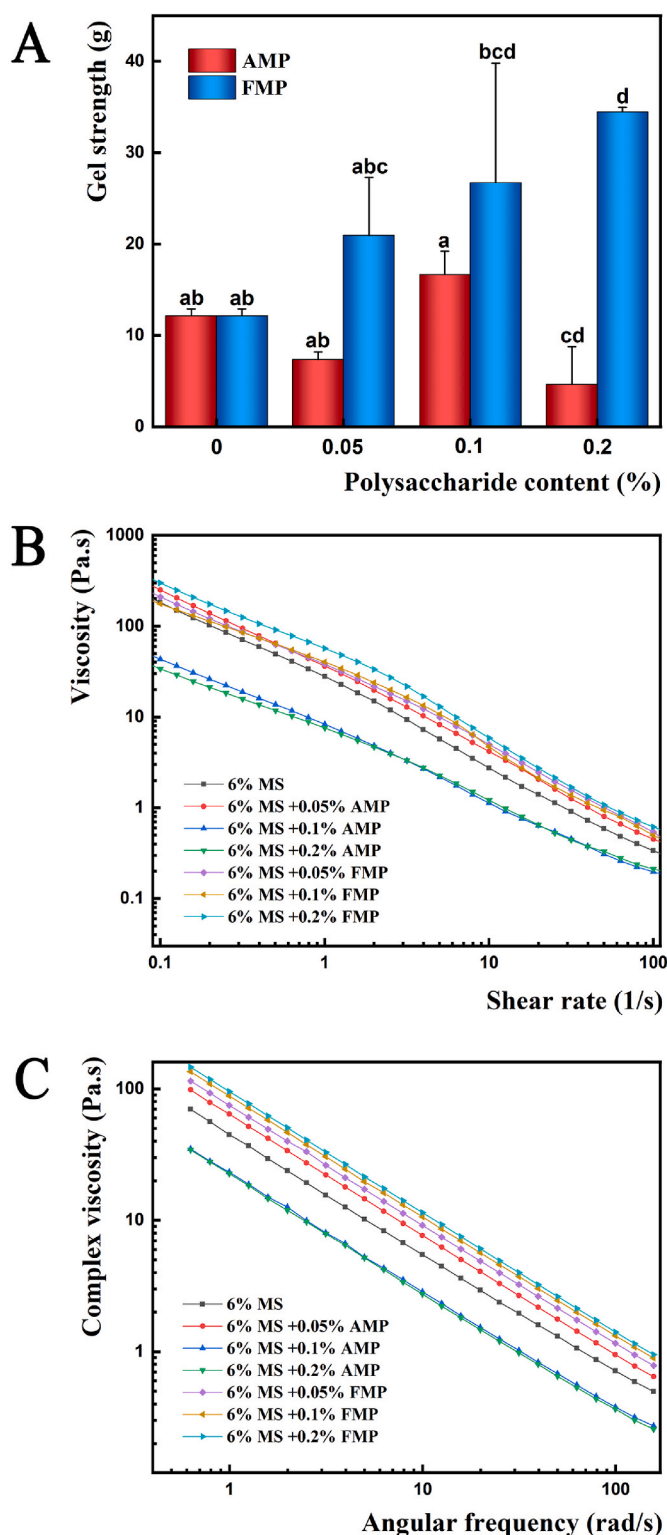


Fig. 4. Functional properties of AMP-WS and FMP-WS. (A) Gel strength; (B) steady shear viscosity; (C) complex viscosity. Values are expressed as means \pm SD ($n = 3$), different letters indicated a different significance ($p < 0.05$) among all the groups.

polysaccharides and WS (Famá et al., 2007; Wang et al., 2020a, 2020b).

3.8. Rheological measurements

The results of rheological tests can provide the information of

solution state, solution phase and fluid type transition (Wang et al., 2020a, 2020b). For better application and development of polysaccharides in food industries, it is of great importance to study the rheological properties of polysaccharides affecting other substances (Bai et al., 2020). In this work, the rheological properties of the MP-WS gel were measured. The relationship between the WS mixing with MP (different concentrations) and apparent viscosity was systematically researched. The apparent viscosity of the MP-WS gel reduced as the shear rate increased, demonstrating that these gels showed shear-thinning behavior (Fig. 4). It can be noticed that the apparent viscosity of FMP-WS gel was increased in dose-dependent manners with FMP was added compared with WS gel. At low levels of shear rate, polysaccharide molecules usually exist as aggregates. When the shear rate increased, the aggregates progressively dissociate as a result of shear forces, and the individual molecules are rearranged in the form of disordered coils (Ghebremedhin et al., 2021). High concentrations of FMP could enhance the entanglement and aggregation of the macromolecule chains. Thus, the apparent viscosity enhances as the entanglement increases. However, when AMP was added at a concentration greater than 0.1%, the apparent viscosity decreased in contrast. Previous studies have shown that apparent viscosity is closely related to the side chains of polysaccharides (Xu et al., 2019). Thus, this result means that the raw materials after a certain storage time may affect the structure of the polysaccharide and the MP-WS interaction.

Cox-Merz rule is an empirical relationship between steady shear viscosity and dynamic complex viscosity (Bai et al., 2020; Wang et al., 2019). It was observed that steady shear viscosity was always lower than that of the complex viscosity at the equivalent shear rate and angular frequency (Fig. 4). The steady shear viscosity and complex viscosity curves could not superimpose with each other, indicating the failure of the Cox-Merz rule. This result indicated that AMP-WS and FMP-WS might have a rigid and ordered chain conformation in water (Wang et al., 2019).

The dynamic rheological properties of MP-WS gel were researched in the frequency around 0.1–25 Hz. The G' , G'' and $\tan \delta$ of MP-WS gel were shown in Fig. 5. The values of G' were higher than G'' within the oscillation frequency range, it was suggested that MP-WS gel exhibited a solid-like behavior (Ren et al., 2020a,b). For FMP-WS, the values of G' and G'' enhanced with increase of frequency, indicating that strong interactions between WS and FMP and significant frequency dependence. $\tan \delta$ value was less than 1 and enhanced with the enhance of the angular frequency for all MP-WS. With addition of FMP, the value of $\tan \delta$ of FMP-WS gel was reduced, suggesting that FMP could increase the interaction in FMP-WS system and solid-like behaviors. However, AMP-WS didn't exhibit this property. Our previously study found that *Mesona chinensis* Benth polysaccharides has excellent gelling performance and can be coated on the surface of starch granules to establish a stable gelling network and improvement of gel system (Lin et al., 2018). The results showed that FMP can dramatically improve the viscoelasticity of the hybrid gel system of FMP-WS. Compared with FMP, AMP has a weaker gelling performance capability and prevents the interaction between WS molecules to forming gelation, which well confirmed the results of gel strength.

4. Conclusion

In this study, two kinds of crude polysaccharides (FMP and AMP) were prepared from *Mesona chinensis* Benth, and their physicochemical properties, antioxidant and antitumor were systematically investigated and compared. The physicochemical analyses indicated that FMP had functional groups to AMP, while difference in monosaccharide composition, M_w and surface morphology. In addition, FMP was characterized by the lower yield and protein content, as well as higher total sugar and uronic acid content, compared to AMP. Furthermore, FMP exhibited better antioxidant and antitumor bioactivity, as well as gel-forming properties than AMP, which attributed to their distinctive structure

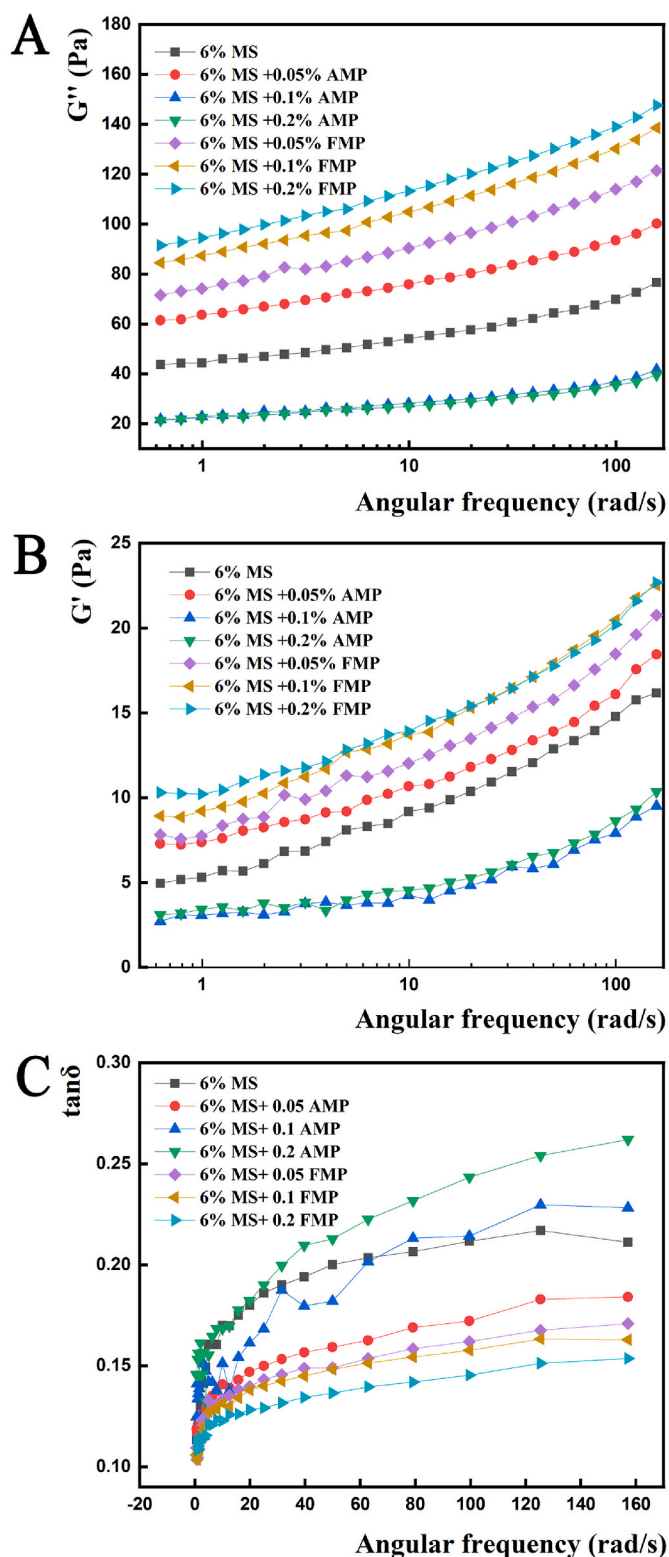


Fig. 5. Rheological properties of AMP-WS and FMP-WS. (A) Storage modulus G' ; (B) Loss modulus G'' ; (C) The phase angle ($\tan \delta$).

characteristics. In summary, the results indicated that the storage time affect the structure and function of the polysaccharide. Thus, this study provided a basis for the utilization of polysaccharide in *Mesona chinensis* Benth. Further studies will focus on investigating the underlying mechanism of action and explore the feasible methods of *Mesona chinensis* Benth preservation.

CRedit authorship contribution statement

Xianxiang Chen: Writing – original draft, Methodology, Conceptualization, Visualization, Data curation. **Wenhao Xiao:** Writing – review & editing, Methodology. **Mingyue Shen:** Writing – review & editing. **Qiang Yu:** Writing – review & editing. **Yi Chen:** Writing – review & editing. **Jun Yang:** Writing – review & editing. **Jianhua Xie:** Writing – original draft, Resources, Conceptualization, Validation, Supervision.

Declaration of competing interest

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.

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References

- Bai, L., Zhu, P., Wang, W., Wang, M., 2020. The influence of extraction pH on the chemical compositions, macromolecular characteristics, and rheological properties of polysaccharide: the case of okra polysaccharide. *Food Hydrocolloids* 102, 105586.
- Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uronic acids. *Anal. Biochem.* 54 (2), 484–489.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72 (1–2), 248–254.
- Chen, X., Wang, Y., Shen, M., Yu, Q., Chen, Y., Huang, L., Xie, J., 2021. The water-soluble non-starch polysaccharides from natural resources against excessive oxidative stress: a potential health-promoting effect and its mechanisms. *Int. J. Biol. Macromol.* 171, 320–330.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28 (3), 350–356.
- Famá, L., Goyanes, S., Gerschenson, L., 2007. Influence of storage time at room temperature on the physicochemical properties of cassava starch films. *Carbohydr. Polym.* 70 (3), 265–273.
- Ghebremedhin, M., Seiffert, S., Vilgis, T.A., 2021. Physics of agarose fluid gels: rheological properties and microstructure. *Curr. Res. Food Sci.* 4, 436–448.
- Gu, J., Zhang, H., Wen, C., Zhang, J., He, Y., Ma, H., Duan, Y., 2020. Purification, Characterization, Antioxidant and Immunological Activity of Polysaccharide from *Sagittaria Sagittifolia* L. *Food Research International*, p. 109345.
- Huang, L., Huang, M., Shen, M., Wen, P., Wu, T., Hong, Y., Yu, Q., Chen, Y., Xie, J., 2019. Sulfated modification enhanced the antioxidant activity of Mesona chinensis Benth polysaccharide and its protective effect on cellular oxidative stress. *Int. J. Biol. Macromol.* 136, 1000–1006.
- Li, C., Dong, Z., Zhang, B., Huang, Q., Liu, G., Fu, X., 2020. Structural characterization and immune enhancement activity of a novel polysaccharide from *Moringa oleifera* leaves. *Carbohydr. Polym.* 234, 115897.
- Lin, L., Shen, M., Liu, S., Tang, W., Wang, Z., Xie, M., Xie, J., 2018. An acidic heteropolysaccharide from *Mesona chinensis*: rheological properties, gelling behavior and texture characteristics. *Int. J. Biol. Macromol.* 107, 1591–1598.
- Lin, X., Liu, K., Yin, S., Qin, Y., Shen, P., Peng, Q., 2020. A novel pectic polysaccharide of Jujube Pomace: structural analysis and intracellular antioxidant activities. *Antioxidants* 9 (2), 127.
- Liu, G., Ye, J., Li, W., Zhang, J., Wang, Q., Zhu, X., Miao, J., Huang, Y., Chen, Y., Cao, Y., 2020. Extraction, structural characterization, and immunobiological activity of ABP Ia polysaccharide from *Agaricus bisporus*. *Int. J. Biol. Macromol.* 162, 975–984.
- Liu, Y., Tang, T., Duan, S., Li, C., Lin, Q., Wu, H., Liu, A., Hu, B., Wu, D., Li, S., 2020. The purification, structural characterization and antidiabetic activity of a polysaccharide from *Anoectochilus roxburghii*. *Food Funct.* 11 (4), 3730–3740.
- Liu, Y., Zhang, J., Meng, Z., 2018. Purification, characterization and anti-tumor activities of polysaccharides extracted from wild *Russula griseocarnosa*. *Int. J. Biol. Macromol.* 109, 1054–1060.
- Ma, J., Liu, H., Han, C., Zeng, S., Xu, X., Lu, D., He, H., 2020. Extraction, characterization and antioxidant activity of polysaccharide from *Pouteria campechiana* seed. *Carbohydr. Polym.* 229, 115409.
- Ma, Y., Zhao, Y., Chi, Y., 2019. Changes in the gel characteristics of two hen egg white powders modified by dry heating and the Maillard reaction during long-term storage. *Lebensm. Wiss. Technol.* 109, 123–129.
- Nagarajan, M., Rajasekaran, B., Benjakul, S., Venkatchalam, K.J., 2021. Influence of chitosan-gelatin edible coating incorporated with longkong pericarp extract on refrigerated black tiger Shrimp (*Penaeus monodon*). *Curr. Res. Food Sci.* 4, 345–353.
- Olawuyi, I.F., Kim, S.R., Hahn, D., Lee, W.Y., 2020. Influences of combined enzyme-ultrasonic extraction on the physicochemical characteristics and properties of okra polysaccharides. *Food Hydrocolloids* 100, 105396.
- Qin, Y., Xiong, L., Li, M., Liu, J., Wu, H., Qiu, H., Mu, H., Xu, X., Sun, Q., 2018. Preparation of bioactive polysaccharide nanoparticles with enhanced radical scavenging activity and antimicrobial activity. *J. Agric. Food Chem.* 66 (17), 4373–4383.
- Ren, Y., Sun, P., Wang, X., Zhu, Z., 2020a. Degradation of cell wall polysaccharides and change of related enzyme activities with fruit softening in *Annona squamosa* during storage. *Postharvest Biol. Technol.* 166, 111203.
- Ren, Y., Jiang, L., Wang, W., Xiao, Y., Liu, S., Luo, Y., Shen, M., Xie, J., 2020b. Effects of *Mesona chinensis* Benth polysaccharide on physicochemical and rheological properties of sweet potato starch and its interactions. *Food Hydrocolloids* 99, 105371.
- Romdhane, M.B., Haddar, A., Ghazala, I., Jeddou, K.B., Helbert, C.B., Ellouz-Chaabouni, S., 2017. Optimization of polysaccharides extraction from watermelon rinds: structure, functional and biological activities. *Food Chem.* 216, 355–364.
- Shao, P., Chen, X., Sun, P., 2014. Chemical characterization, antioxidant and antitumor activity of sulfated polysaccharide from *Sargassum horneri*. *Carbohydr. Polym.* 105, 260–269.
- Sheikh, M.A., Saini, C.S., Sharma, H.K., 2021. Synergistic effect of microwave heating and hydrothermal treatment on cyanogenic glycosides and bioactive compounds of plum (*Prunus domestica* L.) kernels: an analytical approach. *Curr. Res. Food Sci.* 5, 65–72.
- Shi, J., Zhang, J., Sun, Y., Qu, J., Li, L., Prasad, C., Wei, Z., 2016. Physicochemical properties and antioxidant activities of polysaccharides sequentially extracted from peony seed dreg. *Int. J. Biol. Macromol.* 91, 23–30.
- Tang, W., Shen, M., Xie, J., Liu, D., Du, M., Lin, L., Gao, H., Hamaker, B.R., Xie, M., 2017. Physicochemical characterization, antioxidant activity of polysaccharides from *Mesona chinensis* Benth and their protective effect on injured NCTC-1469 cells induced by H₂O₂. *Carbohydr. Polym.* 175, 538–546.
- Wang, S., Yang, J., Shao, G., Qu, D., Zhao, H., Yang, L., Zhu, L., He, Y., Liu, H., Zhu, D., 2020a. Soy protein isolated-soy hull polysaccharides stabilized O/W emulsion: effect of polysaccharides concentration on the storage stability and interfacial rheological properties. *Food Hydrocolloids* 101, 105490.
- Wang, W., Shen, M., Jiang, L., Song, Q., Liu, S., Xie, J., 2020b. Influence of *Mesona chinensis* polysaccharide on the gel properties and microstructure of acid-induced soy protein isolate gels. *Food Chem.* 313, 126125.
- Wang, X., Xu, R., Yin, J., Wang, Y., Ma, L., Nie, S., Xiong, T., Xie, M., 2019. Physicochemical, structural and rheological properties of alkali-extracted polysaccharide from fruiting body of *Hericium erinaceus*. *Lebensm. Wiss. Technol.* 115, 108330.
- Xie, J., Shen, M., Nie, S., Liu, X., Zhang, H., Xie, M., 2013. Analysis of monosaccharide composition of *Cyclocarya paliurus* polysaccharide with anion exchange chromatography. *Carbohydr. Polym.* 98 (1), 976–981.
- Xie, J., Wang, Z., Shen, M., Nie, S., Gong, B., Li, H., Zhao, Q., Li, W., Xie, M., 2016. Sulfated modification, characterization and antioxidant activities of polysaccharide from *Cyclocarya paliurus*. *Food Hydrocolloids* 53, 7–15.
- Xie, J., Xie, M., Nie, S., Shen, M., Wang, Y., Li, C., 2010. Isolation, chemical composition and antioxidant activities of a water-soluble polysaccharide from *Cyclocarya paliurus* (Batal.) Iljinskaja. *Food Chem.* 119 (4), 1626–1632.
- Xu, L., Qiu, Z., Gong, H., Zhu, C., Li, Z., Li, Y., Dong, M., 2019. Rheological behaviors of microbial polysaccharides with different substituents in aqueous solutions: effects of concentration, temperature, inorganic salt and surfactant. *Carbohydr. Polym.* 219, 162–171.
- Yan, L., Xiong, C., Xu, P., Zhu, J., Yang, Z., Ren, H., Luo, Q., 2019. Structural characterization and in vitro antitumor activity of A polysaccharide from *Artemisia annua* L. (Huang Huahao). *Carbohydr. Polym.* 213, 361–369.
- Yuan, D., Li, C., Huang, Q., Fu, X., 2020. Ultrasonic degradation effects on the physicochemical, rheological and antioxidant properties of polysaccharide from *Sargassum pallidum*. *Carbohydr. Polym.* 239, 116230.
- Yuan, Y., Che, L., Qi, C., Meng, Z., 2019. Protective effects of polysaccharides on hepatic injury: a review. *Int. J. Biol. Macromol.* 141, 822–830.
- Zhang, Y., Cui, Z., Mei, H., Xu, J., Zhou, T., Cheng, F., Wang, K., 2019. Angelica sinensis polysaccharide nanoparticles as a targeted drug delivery system for enhanced therapy of liver cancer. *Carbohydr. Polym.* 219, 143–154.
- Zhou, T., Jiang, Y., Wen, L., Yang, B., 2021. Characterization of polysaccharide structure in Citrus reticulata ‘Chachi’ peel during storage and their bioactivity. *Carbohydr. Res.* 508, 108398.
- Zhu, Z., Pang, W., Li, Y., Ge, X., Chen, L., Liu, X., Lv, Q., Dong, G., Liu, A., Zhang, Y., 2014. Effect of ultrasonic treatment on structure and antitumor activity of mycelial polysaccharides from *Cordyceps gunnii*. *Carbohydr. Polym.* 114, 12–20.