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Comparison of structures and properties of gels formed by corn starch with fresh or dried *Mesona chinensis* polysaccharide

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ABSTRACT

Starch is a major dietary carbohydrate, but its digestion properties need to be improved. *Mesona chinensis* polysaccharides (MCPs) had a unique function in improving the flocculation performance of starch. This study investigated the effects of adding *Mesona chinensis polysaccharide* extracted from wet fresh and dry plants with one-year storage, namely WMCP and DMCP, on the physicochemical properties and digestion kinetics of corn starch(CS). The composition analysis showed both WMCP and DMCP were an acidic heteropolysaccharide rich in galacturonic acid and galactose, whereas showed different average main fraction molecular weights (Mw) of 47.36 kDa and 42.98 kDa, respectively. In addition, WMCP showed higher yield, purity and better physicochemical properties to CS than DWCP. Thermal analysis showed WMCP decreased more gelatinization temperatures and enthalpy of CS, and increased more freeze-thaw stability, water holding capacity, and textural parameters of CS gels than DMCP. Structural analysis revealed WMCP induced more changes in crystallinity, short-range order, and microstructure of CS, which inhibited retrogradation than DMCP. In vitro digestion assays demonstrated WMCP addition significantly increased higher resistant starch content by altering starch-starch and starch-MCP interactions than DWCP. Overall, MCPs addition beneficially modulated CS properties and digestion kinetics, providing a novel way to improve starch functionalities. Moreover, WMCP had more advantages to be chosen to form hydrocolloid with CS than DMCP.

1. Introduction

Starch was the leading energy source in animal and human diet and the most abundant and significant polysaccharide in natural plants, either as an important food ingredient (e.g., noodles and bread), or as food thickeners, fat replacers, stabilizers, gell agents, and water holding agents in the food processing industry (Cui, et al., 2018; B. Zhang et al., 2018). Corn starch is also widely applied in the food industry because of its low prices. Whereas, the application of sole starch is restricted in some food industry because of its syneresis, sensitivity to hydrogen ion, retrogradation, and heating instability (Zhou et al., 2017). Thus, CS modification is a way to break through these restrictions (Wang et al., 2018b). Hydrocolloids are becoming more popularly studied and employed in the modification of native starches. Non-starch polysaccharides have attracted intensive attentions due to its richness, non chemical nature and cheapness in decades (Ma et al., 2019; Y. Zhang et al., 2018). Previous studies have elucidated that the thermodynamic characteristics, physicochemical properties and digestibility in vitro of starch foods could be increased by combining with non-starch polysaccharides such as konjac glucomannan(Ma et al., 2019), guar gum (Zheng et al., 2019) and pullulan(Sheng et al., 2018).

Mesona chinensis is widely cultivated in Southeast Asian countries as an herbal tea and medicinal plant. *Mesona chinensis* polysaccharide is an acidic non-starch heteropolysaccharide which shows diverse pharmceutical activities, including antioxidation(Tang et al., 2017), anti-hypertension and anti-inflammation, as well as antidiabetes(Lin et al., 2017). In addition, it is applied in making the yummy dessert Shaoxiancao jelly in folk. *Mesona chinensis* Benth polysaccharides (MCP) are thought to be one of the cost effective and new hydrocolloids for starch modification like other commercial gums. In past ten years some researchers have compared the effects of DMCP from dry plants on the physicochemical properties of various types of starches such as those of rice (Feng et al., 2012), wheat (Liu et al., 2018; Yuris et al., 2017, 2018), maize (Liu et al., 2019a), and tapioca (Xiao et al., 2020).

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So far, no reports focused on the effects of WMCP extracted from fresh plants on CS, and few reports deeply elucidated the mechanisms of polysaccharide and CS interaction. In this study, adding MCPs, especially WMCPs could inhibit CS retrogradation and improve freeze-thaw stability, water holding capacity, textural parameters and digestive properties of CS-MCPs, and the improvement of these properties resulted from the change of colloid structure led by interaction between CS and MCPs. Thus, the aim of our paper was to compare the physicochemical and rheological differences between CS-WMCP and CS-DMCP. Besides, the interactions of CS-MCPs were discussed in detail. The results provided in our study will provided a new way for the utilization of MCP and a new scheme for improving the properties of CS.

2. Materials and methods

2.1. Materials and reagents

The *Mesona chinensis* Benth was planted in the botanical garden of our laboratory at Lingnan Normal University (110°21′ E,21°16′ N, China). Coomassie brilliant blue G250, Bovine serum albumin ,the standard monosaccharides including mannose, fructose, rhamnose, xylose, glucose, galactose, arabinose, ribose, and fucose were purchased from Sigma-Aldrich (Sigma, USA). Sodium hydrogen carbonate, Glucose, Phenol and Sulfuric acid were purchased from Guangzhou Chemical Reagent Factory(Guangzhou, China). Corn starch was purchased from Dongxi Fanhua Starch Technology Co., Ltd. (Guangxi, China). All chemical reagents were of analytical grade.

2.2. Extraction of Mesona chinensis Benth polysaccharide

The fresh *Mesona chinensis* Benth plants were picked and extracted with 6 times volume of pure water and 1.6% $NaHCO_3$ at 100 °C for 10min. Then the plants and solution were poured into the high-speed blender to break into homogenate which was then filtered through a piece of 200 mesh gauze, and extrused and squeezed repeatedly by hand until the gum was evacuated from the residue. After centrifugation at 4500×*g* for 10min, the supernatant was kept at 4 °C to desalt by dialysis for 48hr. The supernatant polysaccharide solution was named as wet fresh *Mesona chinensis* polysaccharide (WMCP), an aliquot of this WMCP was taken to be air-dried to estimate the concentration and total amount of polysaccharide. There were also other batches of polysaccharide prepared by the dry plant material at room temperature(25°C–37 °C) with storage times of 1 year with the same extract protocol. They were named as dry *Mesona chinensis* polysaccharides (DMCP). The percentage yield of polysaccharide (Y) out of *Mesona chinensis* was counted as,

$$Y\ (\%) = C \times V / W \times 100\% \quad (1)$$

Where "C" was the concentration (mg/mL) of polysaccharide in the sample solution, "V" was the volume (mL) of the sample solution, and "W" was the dried weight (mg) of the sample after fresh plant dehydration.

2.3. Determination of polysaccharide composition

The total sugar content in *Mesona chinensis* polysaccharide was measured at 490 nm by phenol sulfuric acid method, and referred to Dubois et al. (1956) with appropriate modifications of optimization(1.0 mL of phenol, 3.5 mL of sulfuric acid, and 10 min of heating time). The uronic acid in *Mesona chinensis* polysaccharide was estimated at 525 nm by using the sulfuric acid-carbazole method, and referred to Blumenkrantz and Asboe-Hansen (1973) with appropriate modifications of adding 0.025 mol/L Sodium tetraborate in the sulfuric acid. The protein content was measured at 595 nm by spectrophotometry method, and referred to Bradford (1976).

2.4. Analysis of UV–Vis absorption characteristics of polysaccharide

Two micrograms of MCPs were dissolved in 5 mL of pure water for purity assay. Three milliliters of the solution were poured into a 4 mL quartz cuvette and the absorbance of MCPs was measured by UV–visible spectrophotometer UV5200 (METASH, China).

2.5. Aanlysis of molecular weight and monosaccharide composition in polysaccharide

The molecular weight was analyzed by a high-performance gel permeation chromatography (HPGPC) method on a Shimadzu(LC-10A) system with a BRT105-104-102™Linear column (8.0 mm×300 mm) (Tang.et al., 2017). The Dextrans with molecular weights of 5, 11.6, 23.8, 48.6, 80.9, 148, 273, 409.8 and 667.8 kDa were served as the control for a standard curve.

The monosaccharide compositions of WMCP & DMCP were determined by Xie et al. (2013) with minor appropriate modifications. Briefly, five mg sample was hydrolyzed with 2 mL of TFA (2 M) under 120°Cfor 3 h. Then the hydrolyzation was continued at 105°Cfor 4 h. The hydrolysate was finally made up to 50 mL with deionized water. The compositions of WMCP and DMCP were determined by the DionexICS-2500 ion chromatography system (Dionex, USA) coupled with a Dionex Carbopac™ PA20 analytical column (3.0×150 mm).

2.6. Preparation of CS-MCPs

The corresponding weight of MCPs were dissolved enough in ultrapure water with stirring (65 °C, 1 h) to acquire a gradient concentration of MCPs solutions ranging from 0.3% to 2.0%. The prepared samples were stored (4 °C, 12h) for adequate hydration.

CS (4%, w/v) and MCPs (0.3%–2.0%, w/v) were co-dissolved in 100 mL volume by adding the ultrapure water, and continuously heating and stirring for 10 min until boiled. The mixtures were then cooled to 25 °C to form hydrocolloids named as CS-MCPs (WMCP/DMCP). One half of the samples were used directly to measure the thermal, and other physical properties and in vitro digestion. The other half of the samples were used to analyze X-ray, FT-IR and SEM after lyophilization.

2.7. Thermal analysis of CS-MCPs

The thermal transition and melting properties of native starches and the mixed CS-MCPs (WMCP/DMCP) were determined by differential scanning calorimeter (DSC 214 Polyma NETZSCH instruments, Germany) using the protocol of Y. Zhang et al. (2019) CS of 2.5 mg was accurately weighed in an aluminum crucible. Various concentrations of WMCP or DMCP were added as 5 μL solutions to make final concentrations of 0%, 0.3%, 0.6%, 0.9%, 1.2%, 1.5%, w/v, respectively. Then the crucibles were sealed with aluminum lids and kept at 25 °C for 0.5 h to hydrate the mixtures. For the gelling analysis, thermogram was drew up with heating at a rate of 10 °C/min from 30 °C to 100 °C. Indium was used for calibration and the empty pan was used as a control. The To (onset temperature), Tp (peak temperature), Tc (conclusion temperature), and the ΔH (gelatinization enthalpy) obtained by DSC program to feature thermal properties of CS-MCPs mixtures. The measurements were conducted in triplicates and the averages were recorded.

2.8. Freeze-thaw stability analysis of CS-MCPs

The freeze-thaw stability of CS-MCPs was conducted according to Lee et al. (2002). The CS-MCPs gel was weighed (M, g) and kept at −40 °C to freeze in a freezer overnight. The samples were then taken out to thaw at 25 °C, and centrifuged at 4500×*g* for 20 min to weigh the CS-MCPs gel without seeping water (m, g). The water separation rate (W, %) in freeze-thaw stability of CS-MCPs was calculated with reference to the following formula. W (%) =(M-m)/M × 100%.

2.9. Water holding capacity analysis of CS-MCPs

The water holding capacity (WHC) was measured using the method of Wang et al., 2018a. with minor modifications. The freshly prepared CS-MCPs gel s were stored at 4 °C in a refrigerator for 8, 20, 32, 44, 56, 68 and 80 h, respectively, and then centrifuged at 4800×*g* for 20 min. The residue materials (WR, g) were weighed after the supernatants (WS, g) were decanted. WHC (%) of the CS-MCPs were calculated using the equation. (Charoenrein, et al., 2011). WHC (%) = WR/(WS + WR) × 100%.

2.10. Gel strength and hardness analysis of CS-MCPs

CS-MCPs gels of 8 mL were prepared for the RVA analysis and kept into 20 m tube at 4 °C for 12 h. Afterwards, the tubes were inverted for 2min to estimate the strength of the CS/MCPs gels. No flow behavior was observed during the invention of samples, indicating that it reached the critical gel concentration Ccrit (Wang et al., 2018b).

Hardness tests of CS-MCPs gels was carried out according to Wang et al. (2018b). with slight modifications. The analysis was conducted using a texture analyzer (TCS-PRO, England) coupled with a P/0.5 probe. The test parameters were as follows: the trigger type was automatic, and the speed before, during and after the test was 2 mm/s, and the inspection distance was 1.50 cm, and the trigger force was 25 N. Each sample was performed in triplicates.

2.11. Solubility and swelling power analysis of CS-MCPs

Swelling power and solubility were performed using the protocols of Xie et al. (2019). The starch (4%, w/v) was mixed with different gradient concentration of MCPs in a volume of 50 mL in centrifuge tubes. All mixed samples were incubated at 95 °C for 0.5 h (M). The gelatinized samples were then cooled down to 25 °C on the bench, followed by centrifugation at 4500×*g* for 30 min. The supernatant and precipitate mass were collected and dried at 105 °C, respectively (namely m_1 and m_2, respectively). The solubility of CS-MCPs (Sol) and the swelling power (Swe) were calculated as followed. Sol (%) = (m_1/M) × 100%, Swe (g/g) = m_2/M (1-Sol).

2.12. X-ray diffraction (XRD) analysis of CS-MCPs

All CS-MCPs gels samples for XRD analysis were freeze-dried in a freeze dryer (Labconco Co., USA), cut into thin slices and used in X-ray diffraction tests. The samples were analyzed by an X-ray diffractometer (X'pert Pro, PANalytical, Netherlands) with the 2θ (°) range of 8°–45° (Liu et al., 2019a). The area of crystalline region (Ac) and amorphous region (Aa) of each sample were calculated by MDIJADE6.0 software. The relative crystallinity (RC) of samples were calculated according to the equation. RC (%) =(Ac/(Ac + Aa)) × 100%.

2.13. Short-range ordered structure analysis of CS-MCPs

FT-IR spectra of the samples were analyzed using a Fourier transform infrared spectrophotometer (FT-IR, Nicolet 6700, Thermo Fisher Scientific, USA) following the procedures of Y.T. Zhang et al. (2019). The samples were dried with a freeze dryer (Labconco Co., USA), and then mixed with 150 mg KBr and ground to powder. The mixture was pressed into round tablets and scanned the range of 4000−400 cm^{-1} at 25 °C. The absorbance heights at 1050 cm^{-1}, 1022 cm^{-1} and 995 cm^{-1} were recorded.

2.14. Scanning electron microscopy analysis of CS-MCPs

The microstructure of CS/MCPs were observed by using an SEM system (VEGA3 SBH, PHILIPS, Netherlands). The dried samples were cut into 1 mm slices and sprayed with gold using Ion coating instrument (EIKOIB-5) with the parameters of the acceleration voltage of 5.0 kV and ion coating time of 120 s.

2.15. In vitro digestibility analysis of CS-MCPs

The digestibility properties were analyzed following the procedures described by Englyst et al. (1992). Firstly, dried CS of 200 mg was gelatinized in 5 mL of pure water at 95°Cfor 20min with and without MCPs solutions (5 mL double distilled water was used as a blank control), and rapidly cooled to 25 °C to prevent the retrogradation of CS. Then, a 30 mL of NaAc buffer (0.1 M, pH 5.2 ± 0.02) was added to each sample solution, and equilibrated at 37 °C for 10 min. Afterwards, ten mL of combined enzyme solution (3000 BPU/g pancreatin and 140AGU/mL amyl glucosidase) was added to the sample solution. Finally, each sample was incubated in a shaking water bath at 37 °C. The sample solutions of 2 mL were pipetted out at 20, 40, 60, 90, 120 and 180 min, and mixed with 3 vol ethanol to terminate enzymatic reaction. The supernatant was collected after 4500×*g* for 8 min, the glucose content was measured by the 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959). The percentage of hydrolyzed starch was calculated by multiplying a coefficient factor of 0.9 (the glucose content of starch). The contents of glucose in the sample before hydrolysis, after 20 min and after 120 min were G0, G20 and G120, respectively. Hydrolysis rate and the content of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were estimated. Hydrolysis rate (%) = (Content of hydrolyzed glucose × 0.9)/TS × 100%, RDS (%) =((G20-G0) × 0.9/TS) × 100%, SDS (%) = ((G120 -G20) × 0.9/TS) × 100%, RS (%) = (1-RDS-SDS) × 100%.

2.16. Statistics analysis

In the tables or figures, the data from the experiments in triplicate were presented as mean ± standard deviation (SD) with different lowercase letters to indicate SD at the 0.05 level. The data were analyzed by Origin Pro 8.0 software (Stat-Ease Inc., Minneapolis, MN, USA). Analysis of variance (ANOVA) was performed using Duncan's test ($p < 0.05$), and IBM SPSS 26.0 software (SPSS Inc., Chicago, IL, USA) was used to evaluate the significant difference between the mean values.

3. Results and discussion

3.1. Chemical characteristic analysis of MCPs

The extraction yields and the chemical components of the WMCP and DMCP were described in Table 1. The prepared yields of the WMCP and DMCP were 75.2% and 39.9% using the same extraction protocol respectively. Such high content indicated polysaccharides were the most abundant ingredients of all biomass components in the leaves of *Mesona*

Table 1
Chemical composition, molecular weight and monosaccharide composition of two polysaccharides.

Samples	WMCP	DMCP
Yield (%)	75.20	39.90
Chemical composition (Weight percentage)		
Total sugar (%)	67.13 ± 1.23	60.69 ± 2.12
Uronic acid (%)	25.03 ± 0.28	24.88 ± 1.11
Protein (%)	0.48 ± 0.02	0.54 ± 0.03
Molecular weight (Weight-average, Da)		
	47.36 kDa	42. 98 kDa
Monosaccharide composition (Amount ratios)		
Rha	0.117	0.42
Ara	0.173	0.30
Gal	0.232	0.221
Glu	0.032	0.031
Xyl	0.096	0.066
GalA	0.350	0.345

chinensis Benth. Both results of this study were significantly higher than the yield of 0.84% with dry plant materials reported by Chen et al. (2022). The main reason for this phenomenon was that the gum was removed from the residue by repeatedly squeezing the gauze bag wrapped with homogenate by hand. The viscosity of polysaccharide and other substances was relatively high, and colloid could not easily pass through 50 mesh gauze when homogenate was filtered by other methods. In addition, it was easy to destroy the cells from the fresh plant materials and the polysaccharide molecules had a complete molecular weight, so WMCP had the highest extraction rates. However, the content of total sugar and uronic acid in WMCP was slightly higher than that in DMCP, while the content of protein was lower(Fig. S1 A & B). The reason might be that the content of DMCP would change during the storage of raw materials. Or different batches of raw materials lead to different sampling ingredient. In addition, polysaccharides from different sources interact with each other in the precipitation process (Olawuyi et al., 2020). The content of protein was also used as an index to indicate the purity of polysaccharide. The little differences in two kinds of polysaccharide contents and purity might cause by growth condition and development phase of the crude and raw materials.

As shown in Table 1, the purity of WMCP and DMCP was 92.13% and 85.57%, respectively. Meanwhile the protein contents in WMCP and DMCP were 0.48% and 0.54%, respectively, as determined by using BCA protein detection kit, indicating that only small amounts of protein and nucleic acid were present as contaminants. The average Mw of the main fractions of WMCP and DMCP were 47.36 kDa and 42.98 kDa, respectively(Fig. S1 A & B). Obviously, the molecular weight of the polysaccharide from *Mesona chinensis* Benth were degrading gradually during storage. HPAEC-PAD was a common technique used for analyzing the monosaccharide composition of polysaccharide via comparisons of retention times with standards (Xie et al., 2016). As shown in Table 1, both WMCP and DMCP had the same six types of monosaccharides, rhamnose, arabinose, galactose, glucose, xylose, and galacturonic acid. These results showed that MCPs was an acidic heteropolysaccharide. Furthermore, galactose and galacturonic acid were the main monosaccharides of MCPs, accounting for about 60% molar ratio of all six monosaccharide compositions. However, the monosaccharide contents and ratios differed a little between the two samples, partially because long termed storage caused a little structural disruption to DMCP. Chen et al. (2022).reported that MCP consisted of seven monosaccharide, with an extra fructose, which was discrepant with our data, possibly for the raw material origins, at different growth stage or growth conditions (Yan et al., 2019). Whereas, the higher contents of galacturonic acid and galactose of MCP were consistent with other reports of MCP (Tang et al., 2017). Surprisingly, this character also existed in other hydrocolloid polysaccharide such as agarose and guar. Agarose was a heteropolysaccharide derived from certain red seaweed. The main components of agarose are D-galactose and 3,6-anhydro-L-galactopyranose. Guar gum was a neutral polysaccharide extracted from a legume plant seeds, consisting of a mannose chain backbone with single galactose side units that occur on almost two out of every three mannose units.

3.2. Thermal properties analysis of MCPs

The thermal parameters and the corresponding thermal curves of the CS-MCPs gelatinization were determined by differential scanning calorimetry (DSC), as shown in Table 2 and Fig. S2 A & B, respectively. From the Fig. S2 A & B, all the samples displayed one single endothermic peak mainly because of the disruption of crystal structures after the gelatinization of starch granules. Gelatinized reaction of starch granules occurs when heated. And the structure of starch molecules was changed from highly ordered to disordered state with the thermal transition (Liu et al., 2018). The onset (To), peak (Tp), and concluding (Tc) temperatures obviously characterized the thermal transition process. From the Table 2, all three kinds of temperatures of CS-MCPs in this study were less than those of CS alone, which could be due to the addition of MCPs, thus causing gelatinization to proceed at a lower temperature. The addition of MCP decreased the To, Tp, and Tc of CS, indicating that MCPs could promote the gelatinization of starch-MCPs gels. These phenomena were mainly caused by the competition between MCP and starch for H_2O, thereby elevating the activity of H_2O. The increase in the activity of H_2O made itself easy to penetrate the starch. Therefore, the number of hydrogen bonds between water and starch was increasingly formed. However, the hydrogen bonds among starch molecules were accelerated to be disrupted, and the swelling of starch was released, thus reducing the required energy for gelatinization. In addition, the T_o and T_p of CS-DMCP were also less than those of CS alone, while Tc fluctuated irregularly between the range of CS-DMCPs and CS control. The ΔH value mainly featured the loss of double helix and the melting of crystals. The change of ΔH value was closely related to the amount of crystal regions and the interactions among crystal molecules (Fu et al., 2013). The addition of MCPs reduced the ΔH values of CS, and the ΔH decreased with increased MCPs concentration. Compared with the control groups, the ΔH of WMCP and DMCP decreased by 11.52% and 24.06% with 1.5% concentration, respectively. The decrease of ΔH might be due to the double helix structural disruption of the crystalline and amorphous distribution of the starch molecules. It might also be resulted from the partial gelatinization of amylose and amylopectin molecules that were unstable during heating. Our results demonstrated that the addition of MCPs had a profound impact on starch structure and gelatinization.

Table 2
Thermal parameters of CS-WMCP systems and CS-DMCP systems.

CS-WMCP systems				
Samples	T_0(°C)	T_P(°C)	T_C(°C)	ΔH(J/g)
0 %	53.89 ± 0.17 ^{a}	67.82 ± 0.04 ^{a}	78.35 ± 0.65 ^{a}	30.184
0.3 %	51.66 ± 0.27 ^{a}	67.23 ± 0.22 ^{b}	76.05 ± 0.99 ^{ab}	29.118
0.6 %	51.74 ± 0.09 ^{a}	66.09 ± 0.08 ^{c}	75.16 ± 0.89 ^{b}	28.362
0.9 %	51.34 ± 0.32 ^{b}	64.32 ± 0.50 ^{d}	74.89 ± 0.74 ^{a}	25.302
1.2 %	52.46 ± 2.88 ^{a}	63.49 ± 0.26 ^{e}	74.34 ± 2.17 ^{b}	21.285
1.5 %	46.84 ± 0.88 ^{c}	58.51 ± 0.21 ^{f}	69.77 ± 1.78 ^{c}	18.661
CS-DMCP systems				
0 %	53.97 ± 0.74 ^{a}	65.85 ± 0.74 ^{a}	78.93 ± 1.23 ^{a}	29.971
0.3 %	52.91 ± 0.98 ^{ab}	63.99 ± 0.47 ^{bc}	79.51 ± 1.08 ^{a}	16.648
0.6 %	50.23 ± 0.45 ^{d}	64.75 ± 0.2 ^{b}	72.83 ± 0.41 ^{b}	15.259
0.9 %	52.24 ± 0.88 ^{bc}	63.78 ± 0.28 ^{cd}	79.39 ± 1.13 ^{a}	14.091
1.2 %	50.77 ± 0.83 ^{cd}	63.95 ± 0.57 ^{bc}	78.43 ± 1.19 ^{a}	9.169
1.5 %	51.09 ± 1.04 ^{cd}	63.04 ± 0.23 ^{d}	79.19 ± 1.09 ^{a}	5.914

Note: CS –WMCP represents the gel systems between corn starch and fresh wet *Mesona chinensis* polysaccharides. CS –DMCP represents the gel systems between corn starch and dried *Mesona chinensis* polysaccharides. Different letters indicate significant differences between different samples ($P < 0.05$) (the same below).

3.3. Freeze-thaw stability analysis of CS-MCPs

The freeze-thaw stability of the compounded system of MCPs-CS was shown in Fig. S3. It showed that the water separation rate of native CS after freezing and thawing was 40.65 ± 0.04%, and the water separation rate when adding MCPs showed a downward trend, to 7.68% and 16.15% in the end for CS-WMCP and CS-DMCP, respectively, indicated that the water retention capacity of the single gel system of CS was poor, and that the MCPs could reduce the retrogradation of CS and improved freeze-thaw stability. When the polysaccharide concentration exceeded 0.9%, the water separation rate increases slightly, indicating that the inhibitory effect of starch retrogradation stopped increase with the concentration of MCPs higher than 0.9%. However, WMCP has better inhibitory effect at high concentration than DMCP.

3.4. Water holding capacity analysis of CS-MCPs

Water holding capacity of hydrocolloid affects the process of starch retrogradation, which was the arrangement and reaggregation of CS molecules from amorphous to ordered crystalline forms, thus causing the enhancement of hardness and syneresis (Fu et al., 2013; Luo et al., 2021). Water holding capacity of hydrocolloid profoundly impacted the flavor, texture, shelf life and digestible extent of food hydrocolloid products (Patel et al., 2017; Wang et al., 2015). High WHC indicated low syneresis after centrifugation. The retrogradation of CS-WMCP and CS-DMCP were determined in Fig. S4. From the Fig. S4, WHC of all samples decrease with increase of storage time. However, MCPs addition significantly reduced the syneresis of the hydrocolloid system in this study. The low concentration of 0.3% had the best WHC for WMCP and the 1.5% high concentration for DMCP. Our data showed that hydrocolloids improved the WHC of CS in aqueous phase. Similar results were reported in the guar/xanthan-starch mixture in the report of Nawab et al. (2016). These studies showed that MCP could effectively improve the WHC of CS-MCP gels and inhibited starch retrogradation, which was contrary to the study of Luo et al. (2020). In addition, Starch retrogradation could be impacted by both internal factors such as the structural differences of starch molecules, and external factors including storage conditions and additives, such as sucrose, salts and other non-starch polysaccharides (Xu, et al., 2013; Yoshimura et al., 1999).

3.5. Gel strength and hardness analysis of CS-MCPs

The texture characteristics of CS-WMCP system and CS-DMCP system were shown in Table 3. CS–WMCP had higher gel strength and better textural properties than CS-DMCP. Additionally, compared with gelatinized starch as control, adding MCPs enhanced the hardness, springiness, cohesiveness, gumminess, and chewiness. Furthermore, the values of textural parameters became higher with increasing concentrations of MCPs. This was possibly because MCPs addition enhanced the interaction among starch and polysaccharide and H_2O through secondary bonds such as hydrogen bonds and intermolecular rearrangement of amylose. Finally, sodium carbonate as an edible alkali was also added in the CS-MCPs in our study, the molecular force between sodium carbonate, MCPs and CS was changed. Ren et al. (2020) reported that alkali increased the dynamic modulus of sweet potato starch-MCP gels by increasing its textural parameters, such as hardness and elasticity. Liu et al., 2019a demonstrated that adding small amounts of alkali improved the textural properties of CS-MCPs. The results of this study were in accordance with the results of Liu et al., 2019a.

3.6. Solubility and swelling power analysis of CS-MCPs

The structural properties of starch were tightly associated to solubility, swelling power, crystallinity and short-range order (Zheng et al., 2019). Swelling force and solubility characterize the water absorption properties and water holding capacity of starch during gelatinization (Wang et al., 2017). With the increase of temperature, the movement of starch molecules were accelerated and the solubility was increased continuously. The structure of the starch crystalline region became loose and easy break. Meanwhile, the exposed polar groups of starch combine with water molecules to form interactions, resulting in the expansion of starch granules. Fig. 1 showed that the solubility and swelling power of CS gels was 1.25% and 8.56 g/g at 95 °C, respectively. Both values were obviously and coordinately increased after adding MCPs, indicating that MCPs had significant effects on these properties, and that MCPs could enhance the swelling power of starch granules and leaching of amylose. The values reached the peak at 1.2% CS-WMCP and 0.9% CS-DMCP, respectively. However, the solubility of CS-WMCP increased more significantly than that of CS-DMCP with the higher concentration of gels. It implied that WMCP had higher water absorption capacities than DMCP. Hence, the fresh WMCP might be given priority to the food industry.

3.7. FT-IR and short-range order analysis of CS-MCPs

The Fourier transform-infrared (FT-IR) of CS-MCPs were as shown in Fig. 2 A & B. Compared with the CS as the blank reference, there wasn't new absorption peaks observed in the compounded samples containing different concentrations of MCPs, indicating that CS didn't covalently interact to MCPs. All the FT-IR spectra presented a wide and strong absorption peak ranged from 3200 cm^{-1}–3700 cm^{-1}. The peak was from the vibration stretching of O–H groups between polysaccharide molecules. The one at 2925 cm^{-1} was from the stretch vibrating peak of CH, whereas the one at 2362 cm^{-1} was the bending vibration absorption peak resulted from glycosidic linkage (Zhou et al., 2019). The total shape of the FT-IR spectrum in this study did not change after adding MCPs, but the relative height and position of the absorption peak shifted to a small extent. The red-shifting or blue-shifting appeared, suggesting that the hydrogen bonds between the MCP and CS in the mixture was increased. Hence, the starch molecules showed good compatibility with the polysaccharide.

FT-IR spectrum could uncover the effects of MCPs on the three dimensional conformation of helical structures of CS chain. The 900–1200 cm^{-1}deconvoluted spectra was shown in Fig. 2 C&D. The absorption ratios of 1050/1022 cm^{-1} and 1022/995 cm^{-1} were presented in Table 4. The ratio of the integrated peak area at 1050/1022 cm^{-1} and 1022/995 cm^{-1} could reveal the internal changes in degree of

Table 3
Textural properties of CS-WMCP systems and CS-DMCP systems.

CS-WMCP systems						
Samples	Hardness	Springiness	Adhesiveness	Gumminess	Chewiness	Cohesiveness
0 %	0.2 ± 0.16 ^{c}	0.66 ± 0.21 ^{b}	0.05 ± 0.02 ^{a}	0.07 ± 0.03 ^{c}	0.04 ± 0.02 ^{b}	0.36 ± 0.02 ^{c}
0.3 %	1.74 ± 0.31 ^{b}	2.41 ± 0.62 ^{a}	0.06 ± 0.01 ^{a}	0.67 ± 0.15 ^{b}	1.81 ± 0.62 ^{b}	0.39 ± 0.01 ^{b}
0.6 %	3.46 ± 1.41 ^{a}	3.46 ± 1.35 ^{a}	0.11 ± 0.07 ^{a}	1.23 ± 0.24 ^{b}	4.31 ± 2.97 ^{ab}	0.42 ± 0.02 ^{b}
0.9 %	4.62 ± 0.68 ^{a}	3.82 ± 1.25 ^{a}	0.15 ± 0.07 ^{a}	1.73 ± 0.03 ^{a}	5.29 ± 0.96 ^{ab}	0.41 ± 0.01 ^{b}
1.2 %	4.91 ± 1.68 ^{a}	4.20 ± 1.53 ^{a}	0.14 ± 0.11 ^{a}	1.91 ± 0.42 ^{a}	7.16 ± 4.71 ^{a}	0.42 ± 0.02 ^{b}
1.5 %	5.71 ± 1.34 ^{a}	4.44 ± 1.66 ^{a}	0.16 ± 0.04 ^{a}	2.17 ± 0.64 ^{a}	8.68 ± 1.97 ^{a}	0.46 ± 0.02 ^{a}
CS-DMCP systems						
0 %	0.19 ± 0.021 ^{c}	0.46 ± 0.17 ^{d}	0.05 ± 0.02 ^{a}	0.069 ± 0.02 ^{d}	0.04 ± 0.04 ^{c}	0.27 ± 0.07 ^{b}
0.3 %	0.272 ± 0.04 ^{bc}	0.91 ± 0.15 ^{b}	0.09 ± 0.04 ^{b}	0.07 ± 0.02 ^{d}	0.06 ± 0.01 ^{c}	0.28 ± 0.04 ^{b}
0.6 %	0.43 ± 0.109 ^{bc}	1.33 ± 0.11 ^{b}	0.08 ± 0.02 ^{b}	0.11 ± 0.07 ^{c}	0.12 ± 0.09 ^{c}	0.22 ± 0.04 ^{c}
0.9 %	0.68 ± 0.07 ^{b}	2.01 ± 0.41 ^{b}	0.06 ± 0.02 ^{b}	0.23 ± 0.04 ^{c}	0.44 ± 0.05 ^{c}	0.34 ± 0.05 ^{b}
1.2 %	1.45 ± 0.42 ^{a}	3.12 ± 0.39 ^{a}	0.16 ± 0.05 ^{b}	0.43 ± 0.09 ^{b}	1.54 ± 0.24 ^{b}	0.35 ± 0.05 ^{b}
1.5 %	1.86 ± 0.33 ^{a}	3.47 ± 1.27 ^{a}	0.18 ± 0.04 ^{b}	0.77 ± 0.14 ^{a}	2.47 ± 0.43 ^{a}	0.46 ± 0.05 ^{a}

Results are presented as mean ± SD(n = 3). Values in the same column with different letters are different significantly ($p < 0.05$).

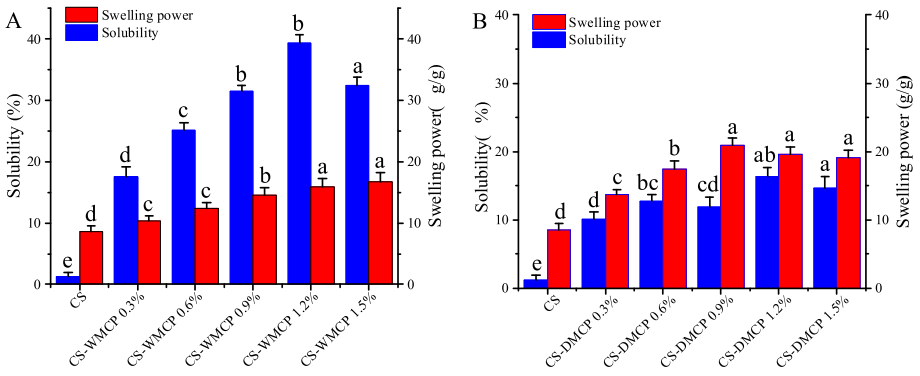

Fig. 1. The solubility and the swelling power of CS-WMCP(A) and CS-DMCP(B).

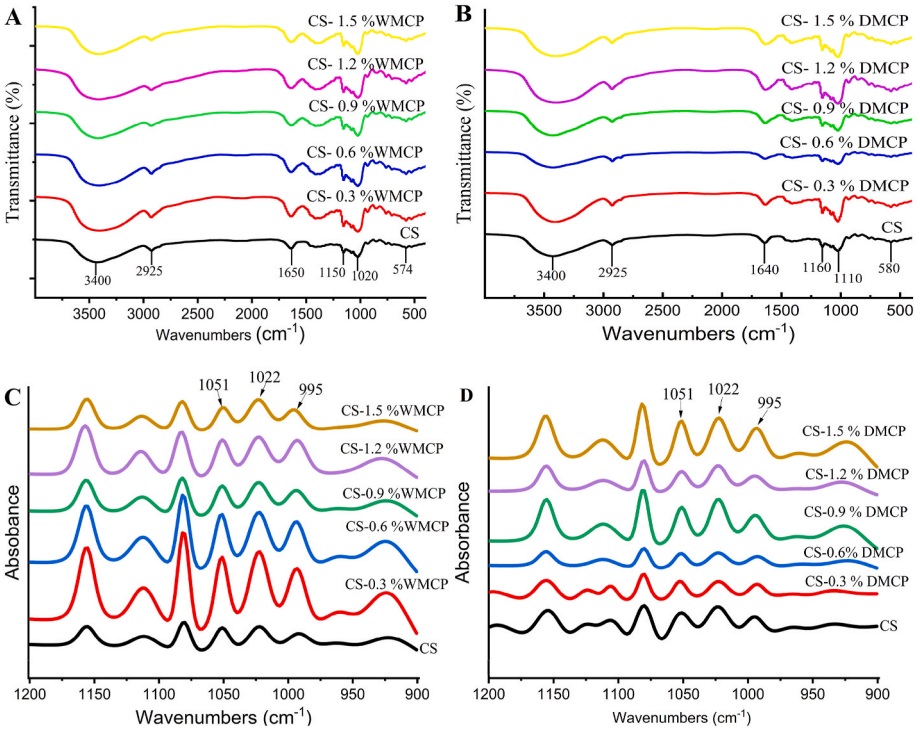

Fig. 2. FT-IR spectra of CS-WMCP (A) and CS-DMCP (B), and short-range order analysis of CS-WMCP (C) and CS-DMCP (D).

Table 4
Short-range ordered structure of CS-WMCP and CS-DMCP.

Samples	(1050/1022)cm^{-1}	(1022/995)cm^{-1}
CS	1.04	1.22
CS-0.3 % WMCP	0.908	1.36
CS-0.6 % WMCP	0.84	1.38
CS-0.9 % WMCP	0.79	1.51
CS-1.2 % WMCP	0. 72	1.69
CS-1.5 % WMCP	0.66	1.79
CS-0.3 % DMCP	0.95	1.25
CS-0.6 % DMCP	0.94	1.34
CS-0.9 % DMCP	0.84	1.39
CS-1.2 % DMCP	0.77	1.63
CS-1.5 % DMCP	0.76	1.67

order (DO) and degree of double helix (DD), respectively (Zhou et al., 2019). From the Table 4 and Fig. 2 C&D, the IR ratios of 1050/1022 cm^{-1} and 1022/995 cm^{-1} of CS were 1.04 and 1.22, respectively. DO gradually increased and DD gradually decreased with the addition and increase of concentration of MCP, indicating that MCP could inhibit more ordered gel structure and starch retrogradation. The changed values of DO and DD of CS/MCPs, indicate that WMCP and DMCP promoted the formation of an ordered structure of CS, and the density of double helices became increasingly compact in the region near the surfaces of starch granule upon reorientation. The results of short-range order in this study were in consistence with those of rheological experiments.

3.8. X-ray diffraction and SEM analysis of CS-MCPs

The X-ray diffraction patterns were as shown in Fig. 3 A & B. Native CS had an A-type patte rn with strong diffraction peaks at 15.27° and 23.01° and double peaks at approximately 17.15°and 18.17° (Zhu et al., 2017). The relative crystallinity (RC) of native CS was 36.82% as calculated by JADE6.3 software. The diffraction peak shifted to the right at 19.83°, and became lower and wider in the CS-MCPs than in the native CS. Meanwhile, the peak strength of CS-MCPs was becoming weaker with increasing concentrations of MCPs. It is indicated that the adding MCPs had significant effects on the crystal structure of gelatinized starch, which was in consistence with Liu et al., 2019a. and Liu

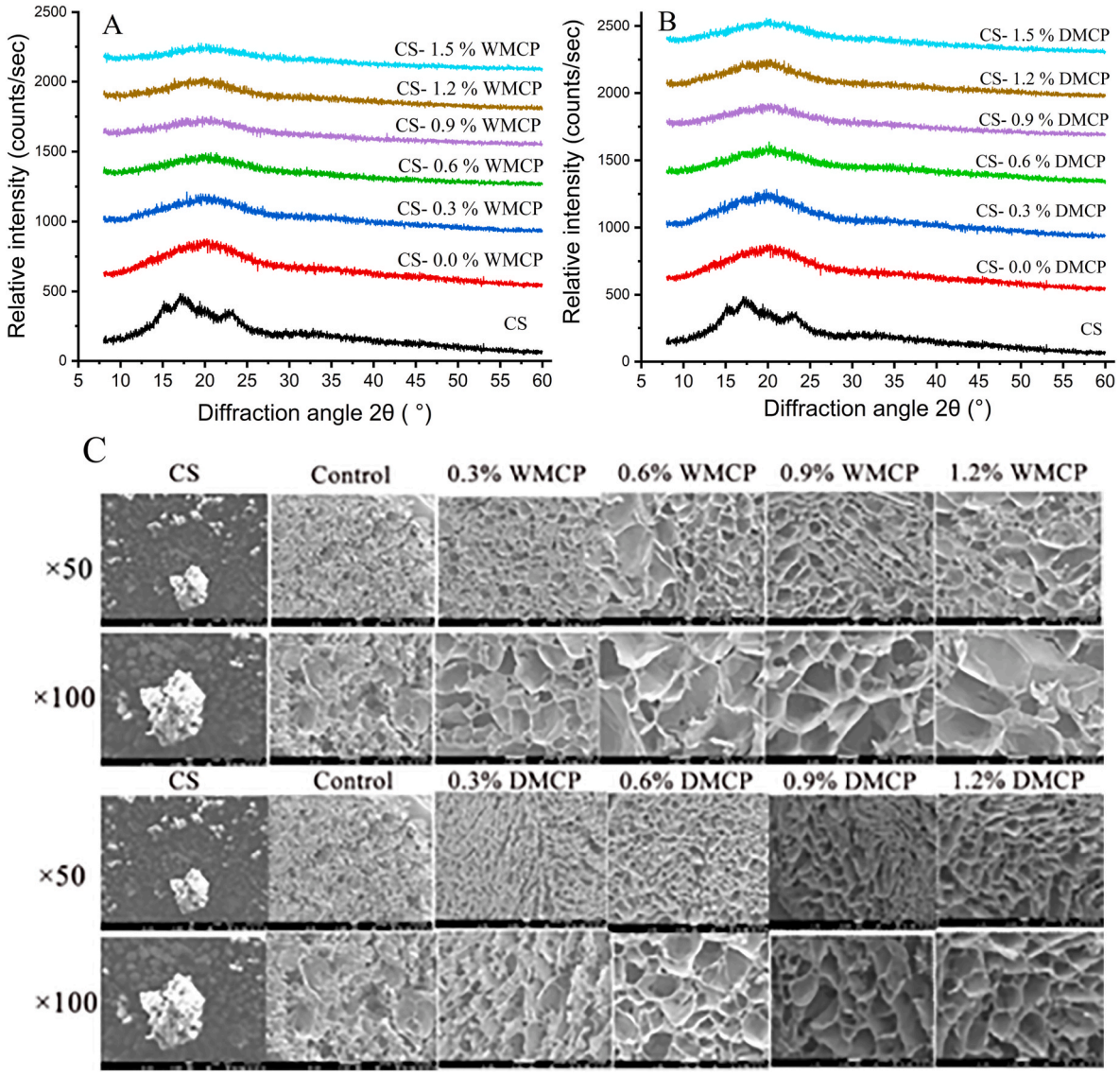

Fig. 3. X-ray diffraction spectra of CS-WCP (A)and CS-DMCP(B), SEM micrographs of native CS and CS/MCPs. CS: native corn starch, control: gelatinized CS, the upper panel: CS/WMCP at different concentrations (0.3, 0.4, 0.9,1.2%), the lower panel: CS/DMCP at different concentrations (0.3, 0.4, 0.9,1.2%).

et al., 2019b. The different MCP concentrations had no effects on peak shapes in this study. It was obvious that the crystallinity of samples was slightly decreased with the increase of gradient MCP concentration. Similar results were reported for pullulan on the crystallinity of Tapioca starch (Sheng et al., 2018). This phenomenon was because WMCP and DMCP inhibit the long-term retrogradation of CS. WMCP had better inhibition effects than DMCP, which helped extend the shelf life of CS-MCPs food.

From the Fig. 3 C, the SEM images showed that native CS granules were globular and spherical with small numbers of irregular cracks on the surface. Such surface characteristics might be related to mechanical damage in the process of preparation. The surface structure of gelatinized CS consisted of some amorphous cells with different sizes and obviously collapsed cell walls. Whereas, CS-WMCP/DMCPs all showed a honeycomb-like structure of which the cells were orderly and tightly connective to one other. Such uniform multi-pore structure featured high water holding capacity and good tastes with smooth surface and elastic texture of jelly food. All the microstructures of Starch-based gel showed honeycomb like structure previously reported for MCP-tapioca starch (Xiao et al., 2020) and konjac glucomannan-rice starch hydrocolloid (Charoenrein et al., 2011). Additionally, the honeycomb structure was becoming more ordered, compact and thick to the cell wall with higher concentrations of 0.3–0.9% MCPs, and less with 1.2% MCPs. It indicates that 0.9% MCPs was the best concentration for industrial production. Meanwhile, CS-WMCP had a better honeycomb-like structure than CS-DMCP at concentrations of 0.3–0.9% MCPs.

3.9. *In vitro digestibility analysis of CS-MCPs*

Starch was a main source of energy for human's diet, and its digestion rate was closely linked to the pathway of human nutrition and health (Chen et al., 2019). The rate of hydrolysis of starch was in tightly relation to several factors, including amylose level, molecular weight, crystal structure and sample purity(Yuris et al., 2019). The hydrolysis rate curves of CS/MCPs in this study simulated the digestive process of the gastrointestine were showed in Fig. 4 A&B. It showed the in vitro hydrolysis rate curve of CS-MCPs within the whole 180 min. The hydrolysis rate of all samples fastly increased in the first 20 min. The rate became relatively flat between 20 and 120 min. It stayed essentially unchanged after 120 min. Therefore, all samples showed faster hydrolysis rates and higher degrees of hydrolysis, particularly in the 0–120 min as a main and long digestive time. Furthermore, adding MCPs had

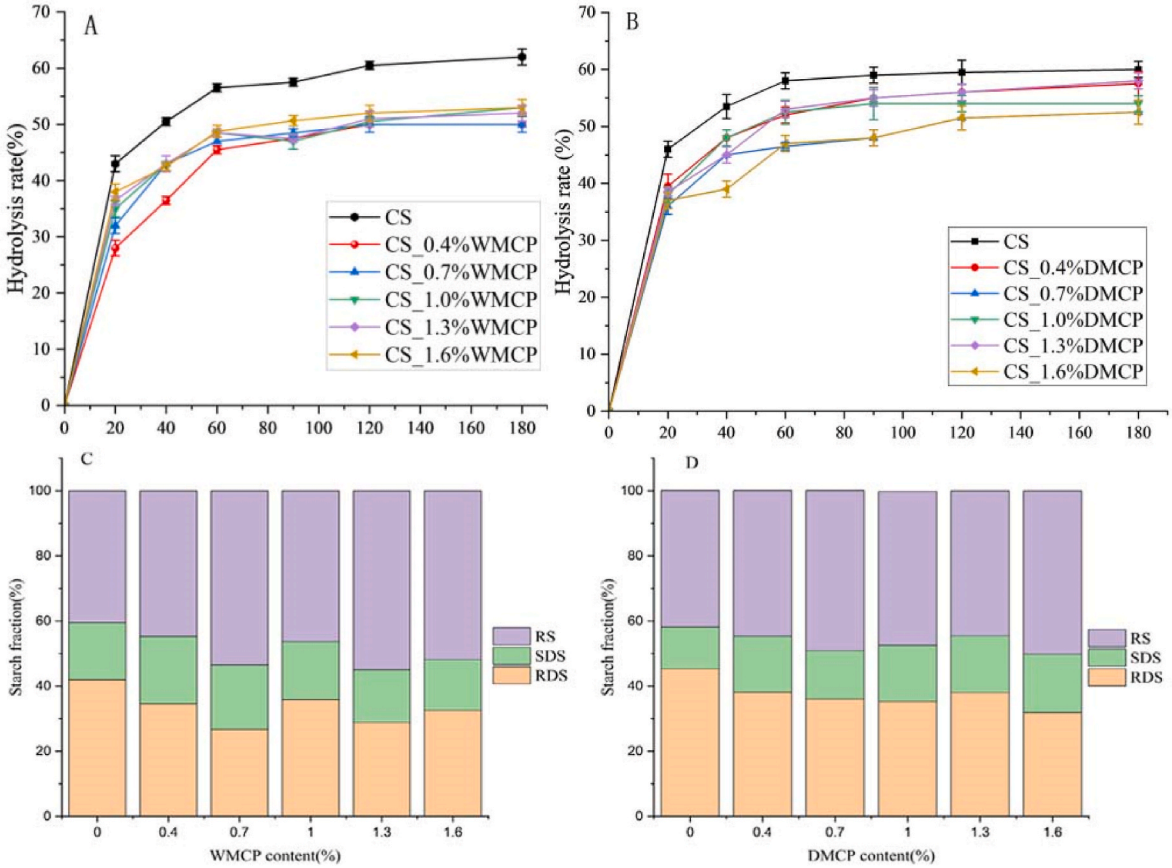

Fig. 4. Digestibility in vitro rate curve of CS-WMCP (A) and CS-DMCP (B), diagram of starch digestion fraction of CS-WMCP (C) and CS-DMCP (D).

the similar increasing trend of hydrolysis rate curve to gelatinized CS, but it was a little lower than CS at the checked time point, in addition, CS-WMCP had a lower hygrolysis rate than CS-DMCP. This indicated that adding MCPs perhaps inhibited digestive hydrolysis of starch, and adding WMCP had better inhition effect than DMCP. The condition might be due to the higher textural parameters and swelling power of CS/MCPs than that of only CS. The higher values did not contribute to the increase of enzymatic digestibility, hence resulting in decreased accessibility of starch molecules to digestive enzymes (Xu et al., 2020). Thus, adding MCP, especially WMCP, is more beneficial to the diet of the patients with obesity and diabetes today.

The starch fraction of starch-MCP gels were showed in Fig. 4 C&D. The addition of WMCP and DMCP significantly increased the resistant starch (RS) and decreased the rapidly digestible starch (RDS) contents. Furthermore, the content of RS enhanced more with more MCPs addition at lower concentration of 0–0.7%, when compared with the control. There was a fluctuation in RS contents in the MCP concentration range of >0.7%, indicating that MCPs can inhibit CS digestibility and can help to retard the occurrence of high level of postprandial blood glucose (Yuris et al., 2019; X.W. Zhang et al., 2019). From these results, it could be concluded that the interactional forces between the linear chain and branched chain of gelatinized starch molecules was altered with the adding MCPs, and the leaching and resetting of amylose and short amylopectin were changed. The addition of MCP disturbed the intramolecular interaction of amylose-amylopectin and the steric conformation of CS-MCPs, and a portion of RDS was converted to RS. Our results in this study were in accord with those of Luo et al. (2021). Our results showed that MCPs could keep the balance of postprandial blood glucose and insulin levels. The concentration of MCPs of 0.7% was the best concentration when mixed with 4% CS, moreover, WMCP had better inhition effect of CS digestibility than DMCP at 0.7% concentration, which is more conducive to the diet of the people of obesity and diabetes today.

4. Conclusion

The pasting gel strength, hardness, swelling power and solubility, water holding capacity and freeze thaw stability of CS increased after adding two kinds of MCPs. It demonstrated that MCPs could improve textural property and inhibit retrogradation of CS. The adding MCPs also decreased the To, Tp, and Tc of CS, indicating that MCPs can promote the gelatinization of starch-MCPs gels. The consequences of rheological tests and FT-IR spectroscopic analysis elucidated that the elasticity and ordered extent of CS enhanced after mixing with MCPs. SEM showed MCPs reduced the pore size of cells and enhanced the thicknesses of the cell walls. The above results indicated that MCPs affected the physical structure and chemical properties of CS. MCPs could stop the swelling of CS molecule and leaching of amylose, and helped CS to form an ordered hydrocolloid, however, MCPs could increase the hardness and strength of the CS-MCPs by enhancing the stiffness of CS molecules. In addition, in the digestibility in vitro experiment, the RS and SDS content of CS was elevated after adding MCPs, whereas the RDS content was reduced. Therefore, it was concluded that CS, together with either WMCP or DMCP, and generated an order and fine gel network, which could effectively alter the digestive properties of CS. In addition, WMCP had a higher yield and purity and higher advantage with CS to form gels than DMCP, therefore, WMCP was chosen preferentially to meet the requirements of different gel-like starch in the food industry.

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CRediT authorship contribution statement

Shengjian Ma: Data curation, Writing – review & editing, Project administration, and, Funding acquisition. **Yijun Liu:** Writing – review & editing. **Wei Dong:** Writing – original draft, Methodology, Conceptualization. **Wenxin Ma:** Writing – original draft, Resources. **Yanxia Li:** Writing – original draft, Methodology, Conceptualization, and, Visualization. **Hao Luo:** Software, and, Validation, All authors have read and agreed to the published version of the manuscript.

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.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100665.

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