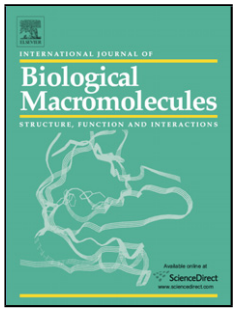

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Polysaccharide with antioxidant, α-amylase inhibitory and ACE inhibitory activities from *Momordica charantia*

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Highlights

- Novel functional polysaccharide was extracted from *Momordica charantia*
- It is an acidic heteropolysaccharide with a molecular weight of ~92 kDa,
- It mainly comprises of glucose, galactose and galaturonic acid
- High emulsifying, foaming and thickening capacities were observed
- It exhibited α-amylase and angiotensin converting enzyme inhibitory activities

Abstract

Functional polysaccharide was isolated from *Momordica charantia*, with a yield of 36% (w/w). *M. charantia* bioactive polysaccharide (MCBP) was an acidic and branched heteropolysaccharide with a molecular weight of 92 kDa. Fourier transform infrared spectroscopic analysis indicated that MCBP was a pectin-like polysaccharide with an esterification degree of 53% and it contains numerous monosaccharides, predominantly glucose, galactose, and galaturonic acid. The results also showed that MCBP exhibited free radical scavenging activity (31.9%), ferric reducing antioxidant power (0.95 mM), α-amylase inhibition (89.1%), and angiotensin-converting enzyme inhibition (94.1%). In the terms of functionality, MCBP showed a lower water-holding capacity but higher in oil-holding capacity, emulsifying activity and foaming capacity compared to citrus pectin. Scanning electron microscopy images demonstrated that MCBP formed gels with a porous structure, and flow analysis showed that the gel solution exhibited pseudoplastic shear-thinning behaviour. These findings indicated that MCBP is a promising functional macromolecular carbohydrate for the food and nutraceutical industries.

Keywords: enzyme inhibitory; functional polysaccharide; *Momordica charantia*

1 Introduction

M. charantia is a tropical plant and it is commonly found in South America, East Africa, and Asia. *M. charantia* is widely cultivated for culinary use and medicinal purposes. For examples, the leaves and the fruits of *M. charantia* have been traditionally used as medicine to cure diabetes and constipation, as well as to relieve mild inflammation, cough, and respiratory and skin diseases [1]. *M. charantia* also exhibited antidiabetic [2], antioxidant [3], antiviral [4], anticancer, immunomodulatory [5], and anthelmintic activities [6]. These therapeutic characteristics were attributed to biologically active chemicals (i.e., small molecules) which naturally present in *M. charantia*. Nevertheless, polysaccharides from *M. charantia*, which may also account for the pharmacological actions has not been studied. Therefore, macromolecular chabohydrates derived from this material must be characterized.

Polysaccharides, which are organic macromolecules found in animals, plants and microorganisms, have shown to exhibit pharmacological properties. Plant polysaccharides, such as pectin, xylan, β-glucan, are promising functional agents in nutraceutical and food industries and they have gained increased attention from consumers because of their natural origins. Scholars have extensively investigated the applications of plant polysaccharides as novel natural carbohydrates with biological and functional properties in many industries. Scientific studies had revealed that plant polysaccharides exhibited diverse therapeutic properties, such as antioxidant, antimicrobial, anticancer, antihypertensive and antidiabetic activities [7-11]. Plant polysaccharides are also regarded as promising functional food ingredients, such as additives, thickeners, emulsifiers, stabilizers, fat replacers, dietary fibers and gelling agents in the food industry [12]. It was also reported that the polysaccharide from *M. charantia* exhibited immunomodulatory activity [13,14]. Two polysaccharides fractions were found by Deng et al.

[13]. The researchers reported that fraction 1, with a molecular weight of 85.5 kDa is mainly composed of glucose and galactose, whereas fraction 2 had a higher molecular weight (441 kDa) and mainly composed of glucose, mannose and galactose. On the other hand, Panda et al. [14] reported that the water extracted polysaccharide from *M. charantia* was composed of D-galatose and D-methyl galaturonate in a molar ratio of 1:4. Apart from that, polysaccharides from *M. charantia* were also reported to have potential in the neuroprotective effects against cerebral ischemia/reperfusion injury through scavenging superoxide, nitric oxide and peroxynitrite oxide [15]. Due to low activity found in this polysaccharide, Liu et al. [16] modified the polysaccharide and they found that the sulfated polysaccharide exhibited higher hydroxyl radical scavenging activity. Therefore, polysaccharides derived from *M. charantia* can be explored as a potential functional hydrocolloid. The previous research was reported using water extraction whereas our study was using citric acid and the effects of extraction parameters were also investigated. The acid extraction method has been commercially employed in the industry owing to the higher extraction efficiency. Acidic condition is not only able to solubilize water-soluble polysaccharides, it is also able to dissolve protopectin through hydrolysis since insoluble protopectin has to be undergone some hydrolysis degradation before it can be isolated. Preliminary showed that the yield of water extraction was relatively low (7.3-8.8%) after 24 hours of extraction compared to acid extraction. Therefore, acidic condition was used in this study. Subsequently, we had characterized the polysaccharide according to its physico-chemical properties (i.e. pH, carbohydrate content, protein content, uronic acid content, protein content, total phenolic content, monosaccharide composition, molecular weight, functional group and solubility), biological activities (i.e. free radical scavenging activity, ferric reducing antioxidant power, α-amylase inhibitory activity and angiotensin converting enzyme inhibitory activity) and

functional properties (i.e. water-holding capacity, oil-holding capacity, foaming capacity, foaming stability, emulsifying capacity, emulsion stability and rheological properties).

2 Materials and methods

2.1 Materials and chemicals

M. charantia fruits were purchased from local markets in Penang, Malaysia. After the seeds of *M. charantia* were removed, the fruits were cut into small pieces and lyophilized. The lyophilized samples were ground into powder, sieved (30-mesh), and stored at 4 °C prior to extraction. Citrus pectin (CP, CAS number: 9000-69-5), which was used as comparison, was purchase from Sigma–Aldrich, Malaysia. All other chemicals used in this study were of analytical grade and purchased from Sigma–Aldrich, Malaysia.

2.2 Extraction of bioactive polysaccharide from *M. charantia*

M. charantia bioactive polysaccharide (MCBP) was extracted according to methods of Gan et al. [17] with some modifications. Briefly, 1 g of the lyophilized *M. charantia* powder was added to 40 mL of 0.1 M citrate–phosphate buffer at different pH values (i.e. pH 2.0-4.5). The suspension was homogenized and incubated in a shaker (IKA, USA) with constant shaking at 250 rpm. Different extraction temperatures (i.e. 30-80 ˚C) and times (i.e. 0.5-3.0 h) were studied. After incubation, the slurries were filtered through a muslin cloth. The filtrates were then precipitated using 3 volumes of 95% (v/v) ethanol for 1 h. The precipitates were separated from the aqueous phase by using a muslin cloth. The polysaccharides were then lyophilized, ground into powder, and stored in a desiccator at room temperature prior to analysis. When the single

factor experiments were conducted, the pH, temperature and time were kept constant at pH 3, 50 °C and 1.5 h, respectively. The extraction yield of MCBP was calculated as follows:

$$\%\text{Yield (w/w)} = (m_p/m_o) \times 100 \quad (1)$$

where m_p is the weight of lyophilized polysaccharide and m_o is the weight of sample.

2.3 Characterization of MCBP

2.3.1 Physicochemical properties of MCBP

2.3.1.1 Determination of pH, total carbohydrates, uronic acid, protein and total phenolic contents

The pH of MCBP was determined as described by Biswajit et al. [18] using a digital pH meter (Mettler Toledo, USA). Total carbohydrate and uronic acid contents of MCBP were determined using the phenol–sulfuric acid [19] and *m*-hydroxydiphenyl methods [20], respectively. Protein content was determined through the Bradford method [21], whereas total phenolic content (TPC) was measured using the method as described by Singleton and Rossi [22].

2.3.1.2 Determination of monosaccharide composition

Monosaccharide composition was determined according to the method of Lv et al. [23] using a high-performance liquid chromatography (HPLC) system that equipped with a UV detector. After hydrolysis using 3 M trifluoroacetic acid, the sample was derivatized with 3-methyl-1-phenyl-2-pyrazoline-5-one (PMP) in methanol. The HPLC system was operated under the following condition: Zorbax SB-C18 reversed-phase column (250 mm × 4.6 mm ID, 5 μm, Agilent, USA) was used and the column temperature was 25 ˚C. Acetonitrile (ACN) was used as mobile phase A, whereas 3.3 mM KH_2PO_4 containing 3.9 mM tris-acetate–EDTA buffer and 10% ACN was used as mobile phase B. The gradient of the mobile phase was as follows: 0–4 min: 94%

B; 4–9 min: from 94% to 88% B; and 9–20 min: 88% B. The flow rate was set at 1.0 mL/min, and the injection volume was 20 μL. The UV detector was set at 250 nm.

2.3.1.3 Determination of molecular weight

The molecular weight of MCBP was examined using a gel permeation chromatography system that equipped with Viscotek Model TDA 305 Triple Detector Array, which consists of detectors for refractive index, light scattering, and viscosity (Malvern, UK). A CLM 3021 column (A6000M, 300 mm × 7.8 mm ID, 13 μm, Malvern, UK) was used. The elution was conducted using 0.1 M $NaNO_3$ containing 0.3% (w/v) NaN_3 to prevent bacterial growth.

2.3.1.4 Fourier Transform Infrared (FTIR) analysis

The FTIR analysis was conducted using AVATAR 360 FTIR (Nicolet, USA) within the range of 400–4000 cm^{-1}. Degree of esterification (DE) of the samples was calculated using the following equation:

$$DE\ (\%) = A_1/(A_1 + A2) \times 100 \quad (2)$$

where A_1 is the peak area at wavenumbers between 1760–1730 cm^{-1}, which represents esterified carboxylic groups, and A_2 is the peak area at wavenumbers between 1600–1650 cm^{-1}, which represents unesterified carboxylic groups [24].

2.3.1.5 Solubility of MCBP in water and alkali solution

The assessment technique described by Fishman et al. [25] was applied to examine the solubility of MCBP in water, whereas solubility test in alkaline solution was conducted as described by Joslyn [26]. A 0.5% (w/v) sample was dispersed in cold water/NaOH. In order to

test the MCBP in hot water/NaOH, the same sample was heated at 90 °C for 15 min, and the changes of MCBP solution after heating were observed.

2.3.2 Bioactivities of MCBP

2.3.2.1 Free radical scavenging and ferric reducing antioxidant power (FRAP) assays

Antioxidant activity of MCBP was evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay as described by Chen et al. [27]. MCBP solution (100 μL, 10 mg/mL) was mixed with 1 mL of 0.1 mM DPPH in methanol and then incubated at 50 °C for 1 h in the dark. Absorbance of the resulting supernatant was determined at 517 nm tusing a UV-Vis spectrophotometer (Spectramax M5, Molecular Devices, USA). A blank sample was prepared using deionized water. The activity was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = (A_o - A_p)/A_o \times 100 \quad (3)$$

where A_p is the absorbance of the mixture of MCBP and DPPH and A_o is the absorbance of the mixture of blank sample and DPPH. Gallic acid was used as positive control.

The FRAP assay was conducted using the method of Benzie and Strain [28]. FRAP reagent (600 μL) was mixed with 8.1 μL of MCBP sample (10 mg/mL). The mixture was then incubated at 37 °C for 1 h, and absorbance at 593 nm was determined. Gallic acid was used as positive control.

2.3.2.2 α-Amylase inhibitory assay

In vitro α-amylase inhibitory assay was conducted as described by Apostolidis et al. [29]. A 100 μL of α-amylase solution and MCBP solution (10 mg/mL) were mixed and incubated at 25 °C for 10 min. The starch solution (100 μL) was then added and incubated for another 10 min.

Subsequently, 200 µL of 3,5-dinitrosalicylic acid was added and heated in boiling water for 5 min. The mixture was then cooled to room temperature and diluted with 3 mL of deionized water. Absorbance was determined at 540 nm by using a UV-Vis spectrophotometer. The activity was calculated as follows:

$$\% \text{ inhibition} = [A_{pos} - (A_s - A_{sb})]/(A_{pos} - A_{neg}) \times 100 \quad (4)$$

where A_{pos} is the absorbance of the positive control, A_{neg} is the absorbance of the negative control, A_s is the absorbance of the α-amylase and MCBP solustion, and A_{sb} is the absorbance of the blank solution.

2.3.2.3 Angiotension-converting enzyme (ACE) inhibitory activity

ACE inhibitory activity of MCBP was determined using the method described by Cheung and Cushman [30]. MCBP solution (50 µL, 10 mg/mL) was mixed with 50 µL of ACE solution (50 mU/mL) and incubated at 37 °C for 10 min. HHL (150 µL) was then added and incubated for another 30 min. The reaction was terminated by adding 500 µL of 1 M HCl. Hippuric acid formed was subsequently extracted using ethyl acetate. Absorbance of the resulting solution was determined at 228 nm by using a UV-Vis spectrophotometer. The activity was calculated using the following equation:

$$\% \text{ inhibition} = [A_{pos} - (A_s - A_{sb})]/(A_{pos} - A_{neg}) \times 100 \quad (5)$$

where A_{pos} is the absorbance of the positive control, A_{neg} is the absorbance of the negative control, A_s is the absorbance of the ACE and MCBP solution, and A_{sb} is the blank solution.

2.3.3 Functional properties of MCBP

2.3.3.1 Water-holding capacity (WHC) and oil-holding capacity (OHC)

WHC and OHC of MCBP were determined using the method established by Bencini [31]. MCBP (1.0 g) was mixed with 30 mL of deionized water (ρ=0.997 g/mL) or 20 mL of palm oil (ρ=0.8875 g/mL) at room temperature for 1 h. WHC or OHC was calculated using the following equation:

$$\text{WHC or OHC} = (V_1 - V_2)/W_s \times \rho \quad (6)$$

where V_1 is the initial water/oil volume (mL), V_2 is the supernatant volume after centrifugation (mL), ρ is the density of water/oil (g/mL), and Ws is the sample weight (g).

2.3.3.2 Emulsifying activity (EA) and emulsion stability (ES)

Emulsification properties were studied according to the procedure of Sathe et al. [32]. Sample (6 mL, 1% or 2% (w/v)) was added to palm oil (6 mL) and homogenized for 2 min to prepare an emulsion. The emulsion was then centrifuged at 3360 rpm for 5 min. The volume of the emulsified layer was measured, and %EA was calculated as follows:

$$\%\text{EA} = V_e / V_T \times 100 \quad (7)$$

Where V_e is the volume of emulsified layer and V_T is the total volume.

For ES test, the prepared emulsion was heated at 80 °C for 30 min and cooled to room temperature. The emulsion was then centrifuged at 3360 rpm for 5 min. The remaining emulsified layer was recorded, and %ES was calculated as follows:

$$\%\text{ES} = V_r/V_e \times 100 \quad (8)$$

Where V_r is the remaining volume of emulsified layer and V_e is the volume of emulsified layer.

2.3.3.3 Foaming capacity (FC) and foam stability (FS)

FC was determined according to the protocol of Mitchell [33]. Sample solution (3 mL, 1% (w/v)) was blended using a TH-02 homogenizer (OMNI International, USA) at 30,000 rpm for 3 min. The volume after homogenization was immediately recorded. FC was calculated using the following equation:

$$\%FC = (V_1 - V_o)/V_o \times 100 \quad (9)$$

Where V_1 is the volume of sample after homogenization and V_o is the volume of sample before homogenization.

The foam was left uninterrupted at ambient temperature to test FS. The total volume after 1 h was measured (V_r). FS was calculated using the following equation:

$$\%FS = (V_r - V_o)/(V_1 - V_o) \times 100 \quad (10)$$

2.3.3.4 Rheological analysis

Polysaccharide solutions at a concentration of 0.5% (w/w) were prepared at pH 2 by adding 0.1 M citric acid solution containing 65% (w/w) sugar. An AR 2000EX rheometer (TA Instrument, UK) with a parallel plate geometry (diameter of 40 mm) and a gap setting of 1 mm was used. The samples were first loaded onto the rheometer and allowed to equilibrate at 25 °C for 5 min. In the flow analysis, shear stress (Pa) and viscosities (Pa s) were determined over a range of shear rate (0.01–1000 s^{-1}). Strain sweep was conducted with stress measurements ranging from 0.001–10,000 Pa at a constant frequency and temperature of 1 Hz and 25 °C, respectively. Frequency sweep test was performed under frequencies ranging from 0.01–100 Hz at a constant temperature of 25 °C and 1% strain. Temperature sweep was performed at temperatures ranging from 5 to 95 °C at a constant frequency of 1 Hz and 1% strain.

2.4 Statistical analysis

Statistical analyses were performed using SPSS version 20 (IBM, USA). All tests were performed in 3 replicates. In the single factor experiment, ANOVA was performed and the means of the results were compared using Duncan's multiple-range test ($p<0.05$). Student's *t*-test was used to compare the means between two samples (MCBP and CP), with significance set at $p<0.05$.

3 Results and discussion

3.1 Effect of pH, temperature, and time on extraction yield

The highest polysaccharide yield (24.1%) was obtained at pH 2, and the yield significantly ($p<0.05$) decreased from pH 2.0 to 3.0 (Fig. 1). These results may be attributed to acid hydrolysis of the bond linkages between polysaccharide and cell wall constituents and then releases the polysaccharides into the extraction buffer [34, 35], thereby, increased the extraction yield.

The polysaccharide yield did not significantly ($p>0.05$) change when the temperature increased from 30 to 50 °C. However, the yield significantly ($p<0.05$) increased when the temperature increased from 50 to 80 °C and reached the maximum yield (up to 30%) at 80 °C. This increasing trend of yield with increasing temperature was related to the solubility of polysaccharides. During extraction, the heat enhanced the rupture of bonds between the polysaccharides and the cell wall. These soluble polysaccharides were then released from the cell wall network and solubilized in the extraction solution [34, 35].

The polysaccharide yield significantly ($p<0.05$) increased when the extraction time increased from 0.5 to 2 h and reached the maximum (12.0%) at 2 h. Extended extraction period

facilitated polysaccharide production as the time required to completely release polysaccharides within the buffer solution was attained; during this period, the liquid entered the dried *M. charantia* powder, dissolved polysaccharides, and then diffused from *M. charantia* cell wall [34]. The yield slightly decreased when incubation was prolonged to 2.5 h and did not significantly ($p>0.05$) change when the extraction time was further extended to 3 h. This result could be due to thermal degradation of polysaccharides after a prolonged extraction [35, 36].

Based on the single-factor experiment results, pH 2.0, temperature of 80 °C, and extraction time of 2 hours were considered the most suitable extraction condition to obtain the highest polysaccharide yield.

3.2 Physicochemical properties

3.2.1 Morphology of MCBP

Figs. 2(a) and 2(b) show the morphology of MCBP, and Fig. 2(a) presents an irregular structure similar to that of a honeycomb; this structure featured a compact and a porous network of polysaccharide gel with an average pore diameter of 135–150 μm. Fig. 2(b) illustrates that MCBP was arranged into a continuous sheet-like network connected to thin films with smooth surfaces.

3.2.2 Extraction yield, pH, total carbohydrate content, uronic acid content, protein content and TPC

Extraction yield, pH, total carbohydrate content, uronic acid content, protein content, and TPC results were summarized in Table 1. The MCBP extraction yield was 36% (w/w) under the selected extraction condition (i.e., pH 2, 80 °C, and extraction time of 2 h). The total

carbohydrate content of MCBP was 45.4%, whereas the uronic acid content was 22.1%. The MCBP solution gave low pH value (i.e. pH 3.4) and this finding indicated that MCBP was an acidic complex carbohydrate. There was a trace amount of TPC (0.47%) observed in MCBP. It was suggested that the physically and/or covalently binding of phenols to MCBP could contribute to the pharmacological effects. Meanwhile, the protein content of MCBP was very low (0.71%), which indicated a high purity of the extracted polysaccharide.

3.2.3 Monosaccharide composition

The monosaccharide components of MCBP were listed in Table 1. MCBP was found to be mainly composed of glucose, galactose, and GalA, with molar ratio of 0.383, 0.306 and 0.145, respectively. Other monosaccharides were also found in MCBP, including mannose, rhamnose, xylose and arabinose. These findings indicated that MCBP was a heteropolysaccharide and predominantly contained glucose and galactose as neutral sugars. The high contents of galactose and arabinose could be due to the presence of rhamnogalacuronan (RG I), which rhamnose functions as the branch point for galactose and arabinose [34].

3.2.4 Molecular weight and intrinsic viscosity of MCBP

Table 2 presents the weight average–molecular weight (M_w), number average–molecular weight (M_n), polydispersity (M_w/M_n), and intrinsic viscosity (IV) of MCBP. These macromolecular features of MCBP were compared with CP. The M_w of MCBP (92 kDa) was higher than that of CP, which indicated that MCBP presented a higher polymerization degree. Polydispersity index (M_w/M_n), an indicator of the degree of homogeneity, is a measurement of the degree of molecular weight distribution in a given polymer [37]. MCBP showed a higher

polydispersity index than CP, which indicated that MCBP exhibited broader molecular weight distribution. This finding could be attributed to the existence of various monosaccharide constituents in MCBP [38]. IV is another parameter that determines the molecular structure of a polymer. The IV of MCBP (1.06 dL/g) was lower than that of CP (2.89 dL/g). A low IV value indicated that MCBP gave a spherical conformation, whereas CP exhibited a linear rod chain conformation, which can extend and occupy high volumes, resulting in lower density (i.e., IV is inversely proportional to molecular density). This assumption was in agreement with the result reported by Yapo and Koffi [39]. Furthermore, low IV values of MCBP could be due to chain branching caused by the presence of high neutral sugar compositions; this branches attached to the MCBP backbone as side chains [40]. Highly branched globular molecules exhibited a compact structure (i.e., smaller volume), resulting in high polysaccharide density and low IV.

3.2.5 FTIR analysis

FTIR was performed to elucidate structural information of MCBP in terms of functional group and group orientation. The IR spectra of MCBP and CP were shown in Fig. 3. As the IR spectrum of MCBP presented a similar transmittance pattern to that of CP, MCBP could be a pectin-like polysaccharide.

A broad band at the region of 3200–3600 cm^{-1} indicated the stretching vibration of the hydroxyl group (O-H). The peaks around 3424–3427 cm^{-1} corresponds to the flexural vibration frequency of the intra- and inter-molecular hydrogen bonds of the polygalacturonic chain [41]. The small band at 2850–2950 cm^{-1} represented C-H absorption, which included CH, CH_2, and CH_3 stretching and bending vibrations. This peak could be due to O-CH_3 stretching caused by the presence of methyl ester groups on the polygalacturonic acid chains. A small band at 2362.3 and 2363.1 cm^{-1} for MCBP and CP, respectively, implied the existence of aliphatic C-H bond

[42]. These C-H bond frequencies could be attributed to the presence of the methyl ester group (OCH_3). In addition, two peaks between 1760–1730 cm^{-1} and 1650–1600 cm^{-1} were characteristics of the ester carbonyl group (C=O) and unesterified carboxylate ions (COO^-), respectively [43,44]. The band area of the esterified carboxylic groups (1760–1730 cm^{-1}) increased with increasing DE, whereas the peak area of the unesterified carboxylate groups (1650–1600 cm^{-1}) decreased. The %DE values of MCBP and CP were 53% and 64%, respectively. MCBP showed an extra peak at 1541 cm^{-1}, which could be typically related to amide II. This extra peak may suggest that MCBP was slightly contaminated with protein [45]. The distinct bands of MCBP and CP at around 1018, 1105, and 1150 cm^{-1} were typical fingerprint regions (800–1200 cm^{-1}) of the pectin [46]. The prominent bands between 1000 and 1300 cm^{-1} could be attributed to C-O stretching in C-O-C- and C-O-H-linked bonds. These bands could be due to the presence of pyranose ring in polysaccharides linked to one another via C-O-C glycosidic linkages or due to C-O-H vibration [10]. Weak peaks between 1300 and 1500 cm^{-1} were referenced to OH bending, CH and CH_3 deformation, and C-O-C stretching frequencies. Minor peaks at 500–800 cm^{-1} corresponded to C-C=O, O-C=O, and O-C-O bending vibrations [47]. Other minor bands at 750–860 cm^{-1} in both MCBP and CP spectra corresponded to equatorial anomeric hydrogen, which could be anticipated as α-glycosidic linkages [48,49].

3.2.6 Solubility

The solubility of MCBP in cold or hot water, as well as its solubility in cold or hot alkaline solution, were presented in Appendix 1. MCBP was partially soluble in cold water and swelled upon water absorption. On the other hand, MCBP was soluble in hot water and the solution viscosity decreased after heating. MCBP solution became yellow after mixing with cold

NaOH, and white suspension was formed upon heating. Begum et al. [50] reported a similar result in pectin extracted from jackfruit and commercial pectin. These findings confirmed that MCBP was a pectin-like polysaccharide.

3.3 Bioactivities

DPPH free radical scavenging activity is commonly used to investigate the antioxidant activity of polysaccharides. The underlying mechanism could be the presence of hydroxyl groups in polysaccharides, which function as hydrogen donator to scavenge DPPH free radicals and, therefore, neutralize the effect of oxidative stress [10]. MCBP showed 31.9% DPPH free radical scavenging activity at concentration of 10 mg/mL. Meanwhile, FRAP assay is used to determine the antioxidant ability of polysaccharides by utilizing the electron-donating capacity of the antioxidant to reduce Fe^{3+} to Fe^{2+} [51]. The result showed that MCBP exhibited a ferric ion reducing power of 0.951 mM at concentration of 10 mg/mL.

The result also demonstrated the effect of MCBP on suppression of α-amylase activity, which can slow down the degradation of carbohydrates/starch, thereby reducing glucose levels in the body [9]. The inhibition capacity of MCBP was observed to be dose dependent, and the activity increased with increasing the MCBP concentration. The concentration of MCBP that retarded 50% of α-amylase activity (IC_{50} value) was 6.69 mg/mL. As shown in Appendix 2, MCBP (10 mg/mL) exhibited excellent α-amylase inhibition activity with the highest value of 89.1%. Subramanian et al. [52] reported that acarbose (as comparison standard) demonstrated a maximum α-amylase$_{inh}$ of 50.1% at the same concentration. This finding revealed that MCBP exhibited higher α-amylase inhibitory activity than typical anti-hyperglycemic drugs and thus could be a promising natural α-amylase inhibitor. This result could be explained by the ability of

MCBP to function as a competitive inhibitor to starch, which reversibly bound to α-amylase catalytic site; therefore reduced the hydrolysis of starch. Hsu et al. [53] confirmed that high percentages of galactose and glucose in polysaccharides were linearly correlated with anti-hyperglycemic effect. Hence, the high anti-hyperglycemic capacity of MCBP was due to the high amounts of galactose and glucose, as reported in Table 1.

ACE catalyses the production of angiotensin II by converting angiotensin I in the renin–angiotensin system and angiotensin II is a vasoconstrictor that responsible to induce high blood pressure [54]. The result showed that MCBP exhibited a higher ACE inhibitory activity (94.1%) compared to other polysaccharide sources, such as almond (79.5%), pistachio (81.8%), and chickpea (87.8%) [11, 55]. Although the ACE inhibitory mechanism of polysaccharides remains unknown, the inhibitory activity of MCBP was presumed to be due to the presence of GalA, which could be ionized when MCBP was dissolved in water and then released hydrogen ions. This process created an acidic environment, which was not suitable for ACE because its optimum pH was pH 8.3. Under acidic condition, ACE would be denatured and therefore lose its activity. This assumption could be supported by the result reported in Table 1, in which the pH of MCBP pH was 3.40.

3.4 Functional properties

3.4.1 WHC, OHC, FC, FS, EA, and ES of MCBP

The functional properties of MCBP are important in food applications, therefore, WHC, OHC, FC, FS, EA, and ES of MCBP must be evaluated (Table 3). In this study, CP was used as a comparison.

WHC is important in the food industry because it can increase bulk volume, inhibit food syneresis, as well as modify viscosity and texture to obtain desirable characteristics [56]. The WHC of CP (7.0 g/g) was significantly ($p<0.05$) higher than that of MCBP (4.7 g/g), which could be due to the polar effect of α-D-GalA in CP that was found in a high amount (96.9%) [17]; this effect occurs because GalA residues with COOH functional groups can form hydrogen bonds with water. As such, low GalA amounts in MCBP (22.1 %) may result in a lower WHC.

OHC is another crucial functional property in formulating food products, particularly high-fat products, which confer foods with mouth feel and greasy sensation. Table 3 illustrates that MCBP showed a significantly ($p<0.05$) higher OHC than CP, which could be due to the existence of methyl ester groups; these groups are responsible for the hydrophobicity and surface porosity of MCBP.

EA is the capacity of an ingredient to disperse in an emulsion and reduce interfacial tension between oil and water in order to stabilize oil–water emulsion [57]. This functional property is important in food products because an emulsifier can prevent the separation of two or more immiscible phases during long storage. Table 3 shows that MCBP exhibited significantly ($p<0.05$) higher EA compared to CP, regardless of concentration. The low EA of CP could be due to the highly polar nature of this polysaccharide. With this hydrophilic characteristic, CP could not be completely adsorbed on the oil droplet/hydrophobic surface. In addition, concentration of polysaccharide showed significant ($p<0.05$) effect. Higher concentration would result in a higher EA because of the presence of high amounts of polysaccharide that can completely saturate the oil–water interface, thus resulting in higher surface coverage [58].

ES indicates the extent at which the polymer can confer a protective barrier at the interface even under heat treatment [59]. Table 3 shows that the ES of MCBP was significantly

($p<0.05$) higher than that of CP and it was more than 100% (i.e. formation of emulsified layer volume after heat treatment was higher than the emulsified layer volume at room temperature). This phenomenon was rarely observed in other studies because the emulsifying ability normally declines or remains after the heat treatment. Wu et al. [59] reported that polysaccharide molecules could form a gel-like film that covers oil droplets after heating, and this film prevents the molecules to coalesce. As such, MCBP could form a gel-like structure after heat treatment and therefore enhanced the emulsifying capacity. It is supported by data shown in section 3.4.2, where the G″ of MCBP was enhanced in the heating process. The superior EA of MCBP might be due to its highly branched hydrophobic structure that can be completely adsorbed on the oil surface, whereas the polar carboxylic functional group of MCBP solubilized on the water surface. Therefore, MCBP could act as an active agent at the oil-water interface that enhance the emulsifying property. On the other hand, the high molecular weight of MCBP contributes to the superior ES because it ensures the viscoelasticity of the film formed in the interface and therefore enhance the ES. Therefore, MCBP could be used as emulsifier and stabilizer in the food industry.

The results on FC and FS were similar to EA and ES, which could be due to the function of surface active ingredients at the gas–liquid interface in stabilizing this two-phase system. Foam is commonly used in the food industry to provide an aerated structure to food products and to modify the viscosity at the air–liquid interface [60]. Table 3 shows the FC and FS of MCBP and CP. The FC of MCBP (191.4%) was significantly ($p<0.05$) higher than CP (116.3%). In addition, MCBP exhibited superior FC and was comparable to other proteins (125% to 250%) as a foaming agent [61]. FS is described as the ability of foam to maintain its property at a certain period. No significant ($p>0.05$) difference was found between MCBP (87.9 %) and CP (85.3%).

These results suggested that MCBP increased viscosity of the solution and generated a network to stabilize the interfacial film of gas–liquid [55]. These high FC and FS values suggested that MCBP could be potentially used in food products, such as milkshakes, ice cream and marshmallows.

3.4.2 Rheological properties of MCBP

As shown in Fig. 4(a), MCBP and CP gels exhibited a similar flow behaviour, i.e. pseudoplastic characteristic. This characteristic was due to the rearrangement of polysaccharide molecule conformation, in which the junction zones were ruptured and caused molecule disaggregation as a result of shearing [62]. Meanwhile, MCBP and CP solutions also exhibited shear-reversible behaviour. The viscosities were reduced asymptotically when the gel networks were disturbed by shearing, whereas the polysaccharides can regenerate their structure and regain viscosity when shear rate was gradually decreased [63]. In comparison with CP, MCBP showed lower apparent viscosity, which could be due to the highly branched spherical conformation of MCBP; this MCBP conformation could hinder polysaccharide chain entanglement compared to the linear rod-chain conformation of CP. Moreover, the gelling ability of high-methoxyl (HM) pectin could be due to hydrogen bonding and hydrophobic interaction. In pectin, GalA residues, which composed of COOH groups, could form hydrogen bonds with other pectin molecules and thus facilitate gelation. CP contained higher GalA contents than MCBP and therefore exhibited higher tendency to form gels via the intermolecular hydrogen bonding between the COOH and OH groups [64]. In addition, hydrophobic interaction mainly occurred between methyl ester groups in pectin at reduced water activity. As such, high numbers of methyl ester groups in CP can promote gelation. Furthermore, the higher gelling capacity of CP

could be due to its higher WHC because the polymer network can hold water molecules tightly and maintain their structure in the 3D gel network.

Oscillatory parameters (i.e., strain, frequency, and temperature sweep tests) of MCBP and CP were measured to obtain detailed information about the rheological characteristics of these polysaccharides. Storage modulus (G′) and loss modulus (G″) were plotted against shear strain (Fig. 4(b)) to obtain LVR, which is defined as the linear response of dynamic G′ and G″ with increasing strain amplitude [65]. Fig. 4(b) shows that the LVRs of MCBP and CP gels were within the range of 0.03–10% and 0.003–20% strain, respectively. CP gel presented higher storage modulus (G′) and longer LVR than MCBP gel, which indicated that the CP gel was more elastic and would not be easily broken down upon deformation. A cross-over of moduli was also observed, in which G″ was same as G′ with increasing strain. The percent strain value where G″ = G′ for MCBP and CP was 20% and 80%, respectively. This cross-over point represented the disruption and changes in the gel-like structures of MCBP and CP into a liquid-like behaviour because of increasing strain. The viscous liquid formation was accompanied by the sharp increase in the phase shift (degree δ) for MCBP and CP.

The dynamic frequency sweep values of MCBP and CP gels were shown in Fig. 4(c). The moduli of MCBP and CP gels were frequency dependent, which indicated the formation of a weak gel. Fig. 4(d) shows the rheological properties of MCBP and CP with increasing temperature. MCBP exhibited a cross-over of the modulus (G′ = G’’) at 18 °C, which indicated the gelling temperature of MCBP, which changed the sample from the viscous-like characteristic to the elastic solid-like behaviour [63]. The moduli of MCBP gel increased as the temperature increased to higher than 40 °C and then decreased when the temperature increased beyond 70 °C. In general, G′ and G″ of polysaccharide gels would decrease with increasing temperature [66,67].

The gelation of HM pectin involves various intermolecular interactions, including hydrogen bonding and hydrophobic interaction between methyl ester groups; in addition, hydrophobic interactions could be strengthened by increasing temperature [68]. MCBP gelation was predicted to be mainly due to hydrophobic interactions, rather than hydrogen bonding, because MCBP contained lower amounts of uronic acids than CP. This peculiar behavior of MCBP gel was presumably caused by the enhanced hydrophobic interaction at high temperatures, which resulted in better gelling behavior. Meanwhile, the moduli (G′ and G′) of CP gel slightly decreased as the temperature increased from 5 °C to 90 °C and G′ was higher than G″ within the testing temperature range. This finding indicated the presence of high gel strength and the temperature tolerance of CP gel [66]. Although CP gel was classified as HM pectin, the increased moduli at increased temperatures were not observed because CP gelation was primarily due to the hydrogen bonding. As the hydrophobic interactions between the methyl ester groups in CP gel was probably used to stabilize the gel, the moduli of CP gel only slightly reduced under the testing temperature range.

4 Conclusion

In this study, polysaccharide was successfully extracted from *M. charantia*. pH, temperature, and extraction time significantly affected the extraction yield. The highest yield of 36% (w/w) was obtained under the extraction condition of pH 2, 80 °C, and extraction time of 2 h. The extracted polysaccharide is an acidic heteropolysaccharide, with a molecular weight of approximately 92 kDa, and contains numerous monosaccharides, predominantly glucose, galactose, and galaturonic acid. MCBP showed potentials in α-amylase and ACE inhibitory activities, in

addition to antioxidant properties, and it demonstrated higher functional properties, such as OHC, EA, ES, FC, and FS compared to CP. Moreover, MCBP could be used as a thickening agent in food because of its pseudoplastic shear-thinning behavior. Therefore, the polysaccharide extracted from *M. charantia* could be explored as a promising natural pharmacological macromolecular carbohydrate or as a functional food ingredient.

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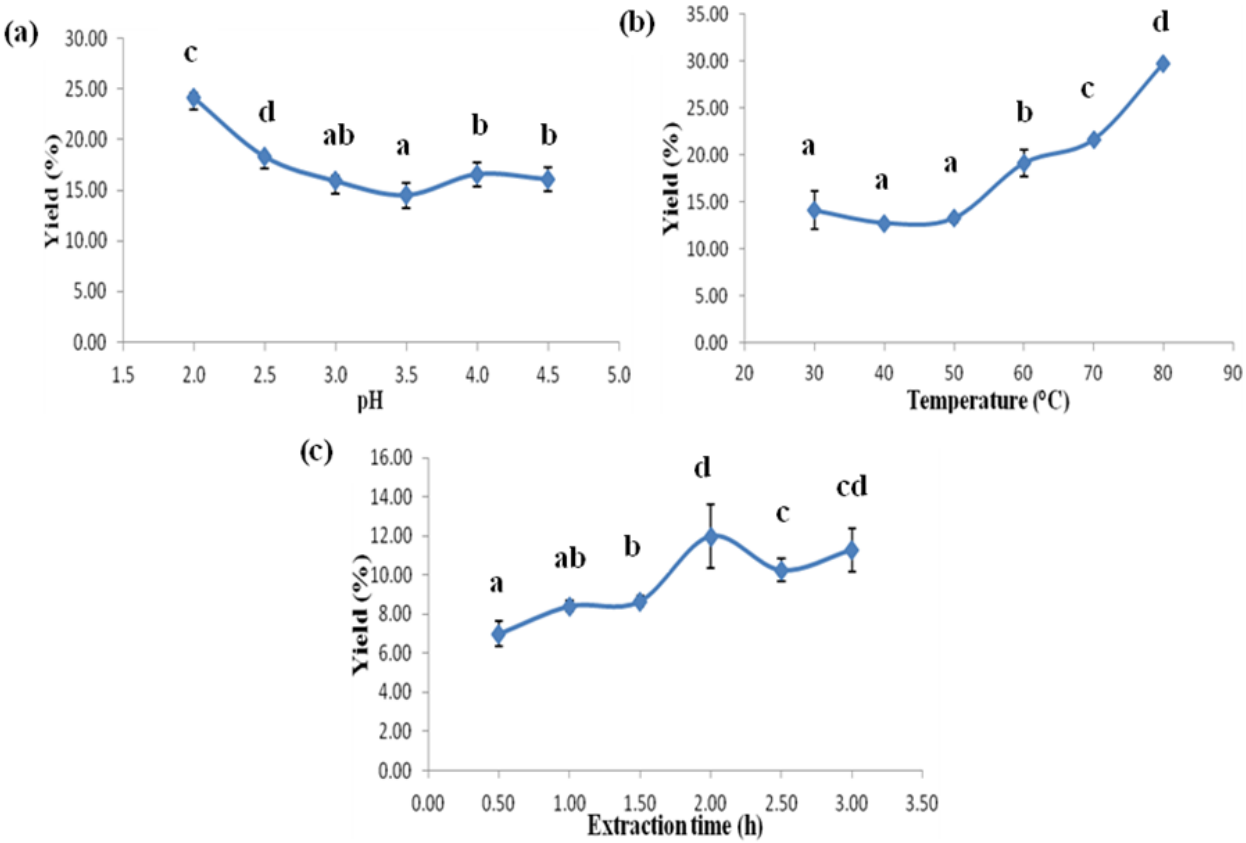

Figure 1 Effects of each factor on the extraction yield of polysaccharide (%): (a) pH; (b) Temperature (°C); (c) Extraction time (h). Error bars indicated the standard deviation of triplicates.

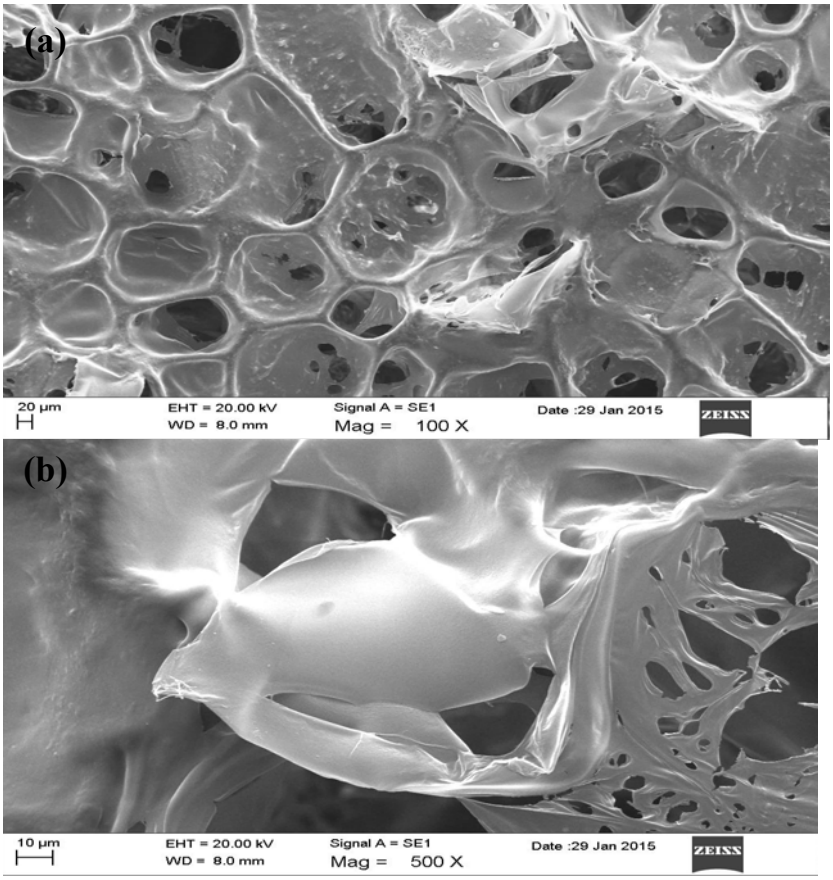

Figure 2 Scanning electron micrographs of (a) lyophilized MCBP gel at 100× magnification; (b) thin film gel network of MCBP gel at 500× magnification

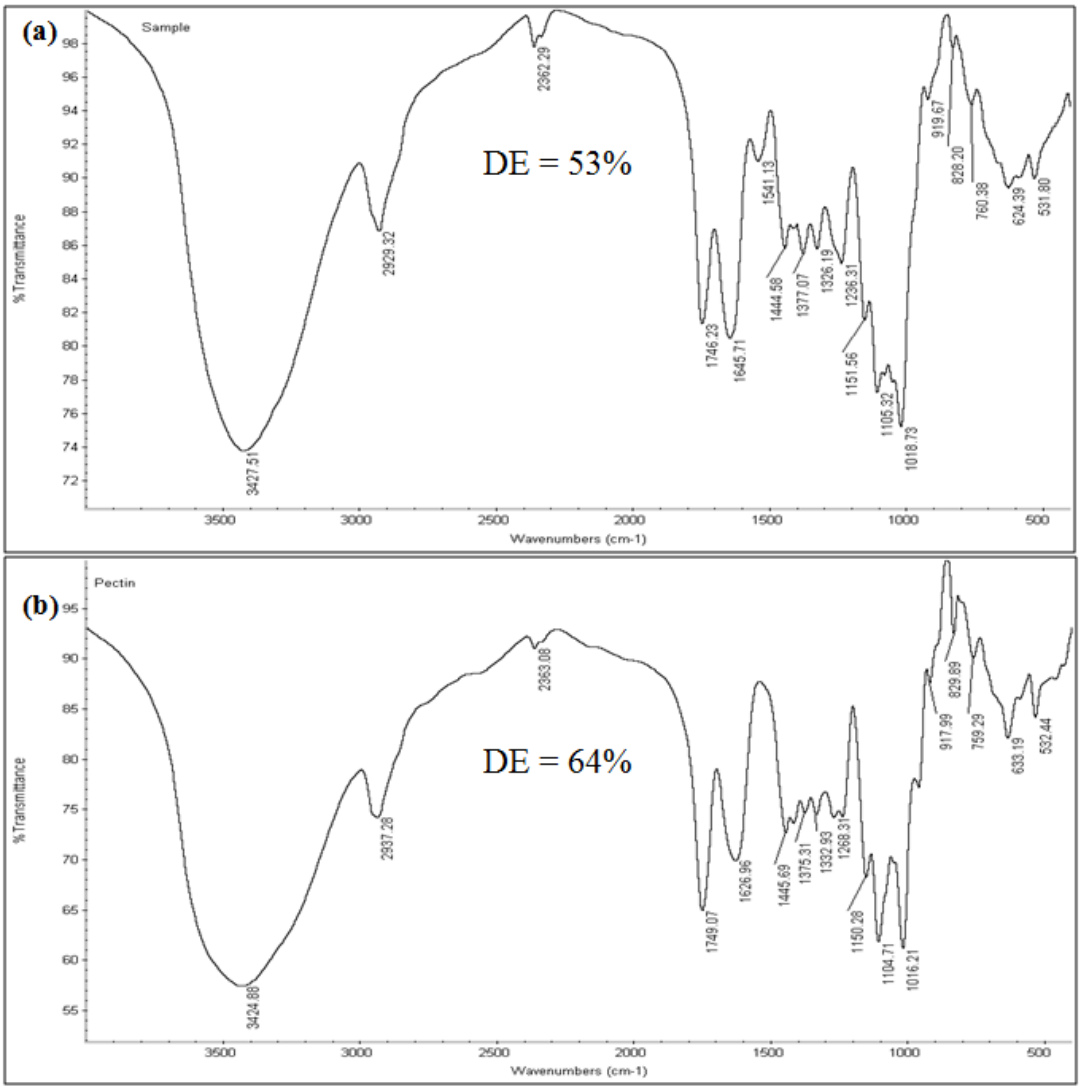

Figure 3 IR spectra of (a) MCBP; and (b) CP

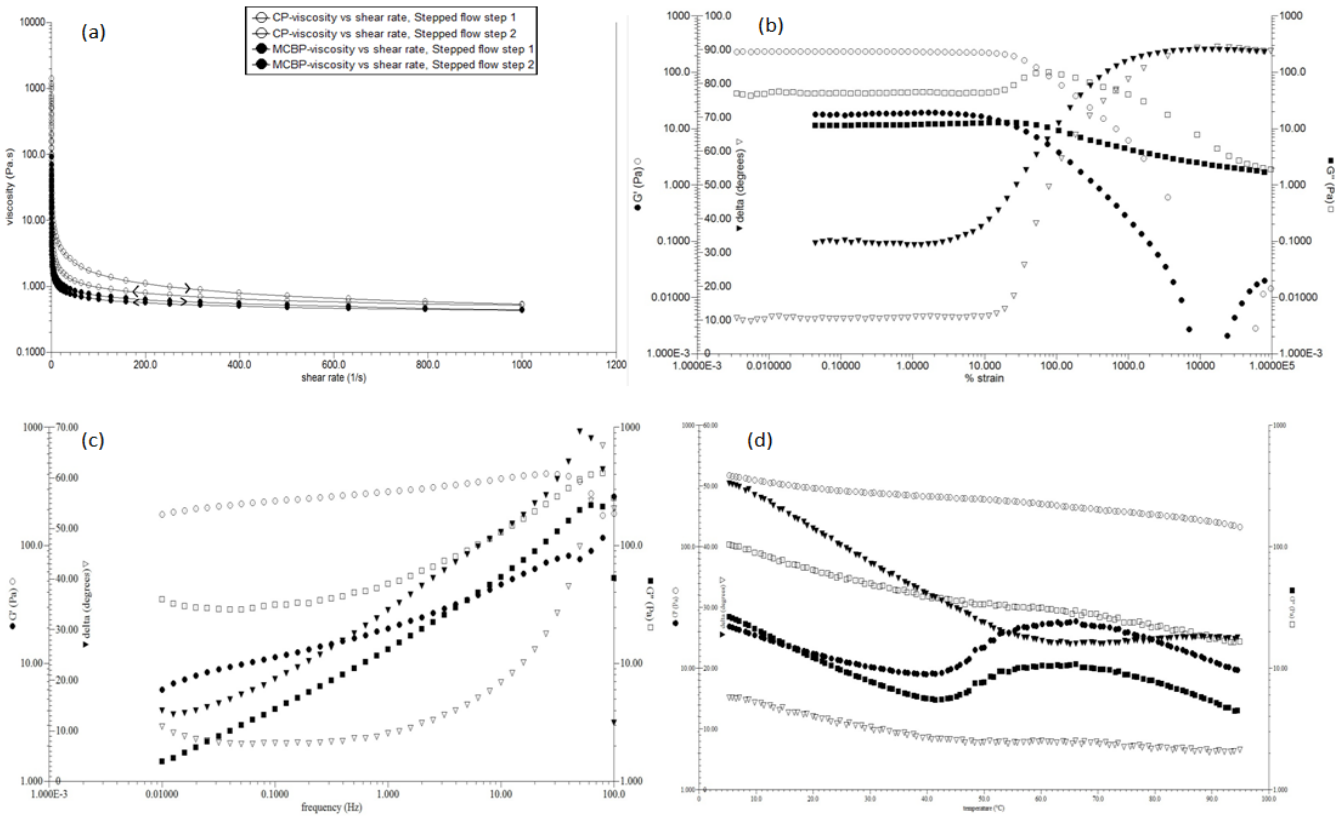

Figure 4 Rheological properties of CP (white) and MCBP (black) gel solution: (a) viscosity (η, Pa.s) dependence curve on shear rate (1/s); (b) dynamic strain sweep test; (c) dynamic frequency sweep test; and (d) dynamic temperature sweep test.

Table 1 Extraction yield, pH, chemical contents and monosaccharide composition of MCBP

Yield (%, w/w)		36.0 ± 0.7
pH		3.40 ± 0.01
Carbohydrates content (% maltose equivalent)		45.4 ± 0.9
Uronic acids content (% GalA equivalent)		22.1 ± 2.6
Proteins content (% BSA equivalent)		0.71 ± 0.13
TPC (% Gallic acid equivalent)		0.47 ± 0.01
Monosaccharides (Molar ratio)	**Glucuronic acid**	ND
	Mannose	0.010 ± 0.001
	Galacturonic acid	0.145 ± 0.003
	Ribose	Trace
	Rhamnose	0.021 ± 0.000
	Glucose	0.383 ± 0.004
	Galactose	0.306 ± 0.002
	Xylose	0.049 ± 0.000
	Arabinose	0.086 ± 0.000
	Fucose	Trace

Data represented means ± standard deviation (n = 3). ND = not detected

Table 2 Number average (Mn), weight average (Mw), polydispersity (Mw/Mn) and intrinsic viscosity (IV) of MCBP

Sample	**M_n (Da)**	**M_w (Da)**	**Polydispersity (M_w/M_n)**	**IV (dL/g)**
MCBP	28,541	91,919	3.22	1.06
CP	20,701	55,894	2.70	2.89

Table 3 Functional properties of MCBP

Functional properties		**MCBP**	**CP**
WHC (g water held/g sample)		4.7 ± 0.1^a	7.0 ± 0.6^b
OHC (g oil held/g sample)		3.1 ± 0.3^a	2.3 ± 0.0^b
FC (%)		191.4 ± 10.2^a	116.3 ± 14.2^b
FS (%)		87.9 ± 5.1^a	85.3 ± 2.8^a
EA (%)	1% (w/v)	44.5 ± 2.9^a	5.4 ± 1.7^b
	2% (w/v)	66.4 ± 1.3^b	13.3 ± 2.9^a
ES (%)	1% (w/v)	131.0 ± 8.8^b	64.1 ± 3.3^a
	2% (w/v)	105.5 ± 2.7^b	47.3 ± 2.7^a

Data represented means ± standard deviation (n = 3). Mean values that did not share a letter within the same row in the table above were significantly different ($P < 0.05$) according to t-test.