



Research Article

Molecular basis of the promoting effect of jicama (*Pachyrhizus erosus*) non-starch polysaccharides on *in vitro* digestion of jicama starch

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Abstract: Starch and non-starch polysaccharides (NSPs) are two important nutrients, which cause different blood glucose responses after ingestion. To develop novel starchy-based food with low glycemic index values, the introduction of NSPs into starchy system is one of the promising methods. However, in our previous study, NSPs from jicama root (named as PEP-6P) unexpectedly reduced the digestive resistance of jicama starch (PES). To unravel the underlying mechanisms, PEP-6P was treated by three amylolytic enzyme systems. Results showed that PEP-6P could be rapidly digested by α -amylase, indicating the possible existence of α -(1 $\rightarrow$ 4)-glucosyl bonds. Further structural analysis confirmed this deduction, and a proposed galactoglucan main chain of PEP-6P was deduced based on the methylation-GC/MS and NMR data. Conformation analysis suggested that PEP-6P presented spherical and aggregated structures in water and 0.1M NaNO₃. These findings revealed that the unexpected pro-digestive effect of jicama NSPs on starch is attributable to the presence of α -(1 $\rightarrow$ 4)-glucosyl linkages in the galactoglucan backbone, providing a molecular basis for formulating low-GI jicama-based foods.

Keywords: jicama; galactoglucan; structural analysis; conformation; digestive behavior

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1 Introduction

According to the World Health Organization (WHO), an unhealthy diet and insufficient physical activity are the leading global risks to health. Starch is an important chemical compound in food materials and is the main source of energy for humans. The rapid digestion of starch in diets generally produces high glycemic responses, leading to the development of insulin resistance and fatty liver^[1]. Nowadays, the high incidence of chronic diseases worldwide has caused increasing demands for the development of foods with low glycemic index (GI) values or functional ingredients that could delay intestinal absorption of glucose from a meal^[1]. Dietary fiber is a complex mixture of non-digestible molecules, mostly NSPs. Multiple epidemiological studies have demonstrated that diets with higher contents of dietary fiber significantly reduce the risks of obesity, type II diabetes, cardiovascular disease, liver cancer, colorectal cancer, and premenopausal breast cancer^[2]. Therefore, NSPs are considered as promising candidates to modify functional properties of natural starches and prepare low GI starchy-based food^[3].

Pachyrhizus erosus L., also called jicama, yam bean, or

bengkoang, is a kind of global agricultural product belonging to the family Leguminosae. It is an underutilized crop that is easy to cultivate^[4]. As one of the main constituents (>10%), fiber from jicama roots was reported to effectively prevent excessive blood glucose and body weight increase without affecting food intake in mice fed with a high-sugar diet^[4,5], and modulate immune system activity in male mice^[6]. Therefore, jicama NSPs have great potential, and systematic evaluations on their chemical structure, functional properties, and rheological properties need further investigations.

In our previous work, jicama starch (PES), jicama NSPs (PEP), and its two subfractions (PEP-3P and PEP-6P) were sequentially isolated. Primary structure and some physicochemical properties were determined^[7,8]. The interaction of PES and three NSPs was also analyzed, and results showed that the addition of PEP, PEP-3P, and PEP-6P reduced the resistance to enzymatic digestion of PES^[8]. This finding contradicted the prevailing view that NSPs universally inhibit starch digestion. Herein, we hypothesized that glucosyl residues (the main components) in PEP may link to each other in α -(1 $\rightarrow$ 4) and/or α -(1 $\rightarrow$ 6) forms, which can be digested by

the digestive enzymes. To explore the underlying molecular mechanism, PEP-6P was selected as a representative sample owing to its narrower distribution of molecular weights and simpler monosaccharide composition. The *in vitro* digestive behaviors of PES, PEP-6P, and PES/PEP-6P in three amylolytic enzyme systems were first investigated. Then, detailed structural and conformational analysis of PEP-6P was conducted by means of methylation and GC/MS analysis, NMR spectroscopy, high-performance size-exclusion chromatography (HPSEC), Congo red test, X-ray diffraction (XRD), and so on. The results will promote the understanding of starch and non-starch polysaccharides from jicama and the development of jicama food products for special purposes.

2 Materials and methods

2.1 Materials and reagents

PES, PEP-6P, and their composites (PES/PEP-6P with a mass ratio of 9:1) were prepared in our laboratory according to the previous methods^[8]. Porcine pancreas α -amylase (AMY) with an active unit of 50 U/mg, amyloglucosidase (AMG) with an active unit of 70 U/mg, deuterium oxide (purity, 99.9% D), sodium borodeuteride (98 atom% D), and methyl iodide (MeI) were all produced by Sigma-Aldrich Co. (St. Louis, MO, USA). Trifluoroacetic acid (TFA) of analytical grade was bought from the Aladdin Industrial Corporation (Shanghai, China). Aqueous solutions were prepared with ultrapure water prepared by a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other reagents were products at analytical grade unless otherwise specified.

2.2 *In vitro* digestive behaviors of PES, PEP-6P, and PES/PEP-6P

To explore the reason for the promoting effects of the digestive rate of PES caused by PEP-6P, the *in vitro* digestive behaviors of PES, PEP-6P, and PES/PEP-6P in three enzymatic systems, including the alone AMY system (300 U/mL), the AMY-only system (20 U/mL), and a complex system of AMY and AMG, were conducted. The three samples were digested at 37 °C with constant shaking at 200 rpm according to the reported methods^[8]. Aliquots (100 μ L) were taken at 0, 20, 40, 60, 80, 100, 120, 140, 160, and 180 min into a 2 mL centrifuge tube containing 900 μ L of absolute ethanol to stop the enzymatic reaction. The content of glucose in the solution was determined using the 3,5-dinitrosalicylic acid (DNS) colorimetric method^[8]. The obtained glucose concentrations were then transformed to concentrations of starch digested by multiplying by 162/180 (the molecular weight ratio from anhydrous glucose to glucose). Final starch digestion curves were plotted as percentage of starch digested versus time.

2.3 Modeling of the digestibility data by LOS plot

Referring to the description of Li *et al.*^[9] the typical digestive data were further analyzed using first-order kinetic function (Eq. (1)) and its transformed function, i.e., the logarithm of slope (LOS) plot (Eq. (2)):

$$C_t = C_\infty(1 - e^{-kt}) \quad (1)$$

$$\ln\left(\frac{dC}{dt}\right) = \ln(C_\infty k) - kt \quad (2)$$

where C_t is the percentage of starch digested at a certain time (t),

C_∞ is the estimated percentage of starch, and k is the starch digestion rate coefficient.

2.4 Detection of functional groups by FT-IR spectrum

FT-IR spectrum of PEP-6P was tested using a Nicolet iS50 FT-IR instrument (Thermo Fisher Scientific, Waltham, MA) with a universal attenuated total reflectance diamond crystal sampling accessory. The spectrum was acquired in the range of 500–4,000 cm^{-1} at a resolution of 4 cm^{-1} , averaging 32 scans per spectra against air as the background.

2.5 Methylation combined with GC-MS analysis of linkage types of PEP-6P

Methylation analysis of PEP-6P was conducted according to the method before^[10]. Briefly, the vacuum-dried PEP-6P (*ca.* 3–5 mg) was fully dissolved in 2.0 mL anhydrous DMSO solution. The pre-dried NaOH powder (25–30 mg) was added to the solution and incubated under stirring for 2 h. To methylate the hydroxyl group, iodomethane (0.8–1.0 mL) was added to the above solution drop by drop under the ice bath. After reacting in the dark for 3 h, several drops of deionized water were added to stop the reaction. Dichloromethane was used to extract the methylated polysaccharide, which was then dried and detected by FT-IR spectrum to make sure that the peak of -OH at 3200–3700 cm^{-1} disappeared completely. The methylated sample was hydrolyzed by 0.5 mL of TFA (4 M) at 100 °C for 6 h and then reduced with sodium borodeuteride and acetylated with acetic anhydride. The generated partially methylated alditol acetates (PMAAs) were subjected to a GC-MS apparatus (Thermo Quest Finnigan, San Diego, CA) having an SP-2330 capillary column (Supelco, Bellefonte, PA). The temperature programming condition of GC and the ion trap MS detector were referring to Zhang *et al.*^[11].

2.6 NMR spectroscopy of PEP-6P

Polysaccharides (60 mg) dissolved in D_2O (99.8%) were lyophilized to exchange hydrogen with deuterium three times. The samples were finally dissolved in 1.0 mL D_2O (99.9%) and transferred into a 5-mm nuclear tube. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance NEO 600 MHz Nuclear Magnetic Resonance Spectrometer (Bruker, Rheinstetten, Germany) at around 295 K. Two-dimensional experiments, including the homonuclear $^1\text{H}/^1\text{H}$ correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC), heteronuclear multiple-bond correlation (HMBC), and nuclear overhauser effect spectroscopy (NOESY) using the standard Bruker pulse sequence were also conducted for fine structural analysis.

2.7 Solution properties detected by HPSEC-MALLS-Vis-RID

The high-performance size-exclusion chromatographic system (HPSEC, Wyatt Technology Co., Santa Barbara, CA, USA) was equipped with the same online detectors as previously reported^[12]. was used for the determination of direct molecular weights and calculation of conformation parameters. Samples (1.0 mg/mL) dissolved in the mobile phase were prepared by passing through a 0.22 μm membrane filter before being injected into the chromatographic system. It was tested and analyzed using the previous analytical conditions^[12].

2.8 Congo red test

Congo red test was used to evaluate the helix conformation of

PEP-6P with the method modified from a previous study^[11]. The 50 μL of polysaccharide solution (1 mg/mL) was mixed with an equal volume of Congo red solution (80 $\mu\text{mol/L}$) in a 96 well microporous plate. Then, 1 mol/L NaOH solution was slowly added to the mixture until the final concentration of NaOH reached 0–0.5 mol/L. The mixture was kept at room temperature for 1 h. Congo red solution without polysaccharides was used as a control group. The maximum absorption wavelength was determined by scanning in a range from 400 to 600 nm with a microplate reader (SpectraMax i3x instrument, Molecular Devices, USA).

2.9 Characterization of crystallinity

X-ray diffraction (XRD) of PEP-6P was conducted using an Empyrean PANalytical diffractometer (Netherlands). The scan was performed at 30 kV and 10 mA for a 2θ range of 5–50° with a step size of 0.01. The relative crystallinity was calculated using the Jade 6 software^[13].

2.10 Thermogravimetric (TG) analysis

TG analysis was performed with a thermogravimetric analyzer (STA449C, NETZSCH Corporation, Germany). The dried PEP-6P (10 mg) was placed in the platinum pan of TGA furnace and the assessments were accomplished with a heating rate of 10 °C/min from 20 to 600 °C under nitrogen atmosphere at a flow rate of 20.0 mL/min.

2.11 Microscopic observations

To monitor the morphology of PEP-6P, it was prepared in two

ways and then subjected to a FEI Nova NanoSEM 230 Field Scanning Electron Microscope (Czech Republic) under different magnifications. Firstly, samples were dissolved in ultrapure water at a concentration of 1 mg/mL for further use. On the one hand, sample solutions were diluted to 50 $\mu\text{g/mL}$ in 2.0 mL microtubes and then quickly immersed in liquid nitrogen, followed by freeze-drying. On the other hand, sample solutions were diluted to a concentration of 50 and 5 $\mu\text{g/mL}$ and then deposited on a silicon wafer, left to be adsorbed and subsequently gently dried naturally. The above two kinds of samples were dispersed onto an aluminum stub, and gold coated with an ionic sputter coater. The acceleration voltage was 5 kV, and the magnifications were set at 5,000–100,000 folds under high vacuum conditions.

2.12 Statistical analysis

All experiments were performed at least in triplicates, and the data were expressed as mean values $\pm$ standard deviation. One-way analysis of variance (ANOVA) with Tukey's post-hoc test was performed to compare mean group values. Figures were drawn using Origin software (Version 8.5, OriginLab Corporation, Northampton, United States).

3 Results and discussion

3.1 *In vitro* digestion and kinetics analysis

Fig. 1 displays the digestion plots of digested amount versus time and the corresponding LOS plots curves for PES, PEP-6P, and PES/PEP-6P in the enzymatic systems of AMY, AMG, and their combinations. As shown in Fig. 1A, the hydrolysis rate of

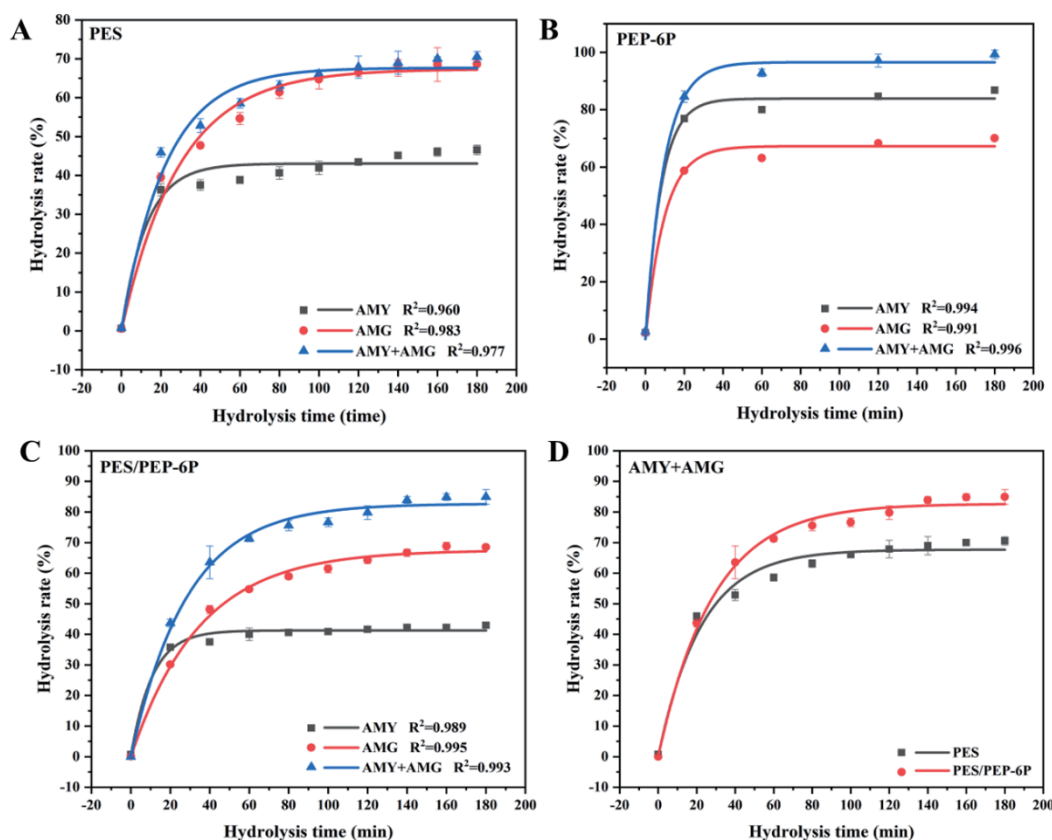


Figure 1 *In vitro* digestion curves (scatter) and first-order kinetic fitting results (curve) of PES (A), PEP-6P (B), PES/PEP-6P (C) under AMY, AMG, and complex enzymes, and a comparison of the results of PES and PES/PEP-6P under complex enzymes hydrolysis (D).

PES at 180 min was 70.53% in the combined enzymatic system, which was higher than that in the alone AMY system (46.57%), but close to that in the AMG system (68.66%). It was to say that AMG played a determinant role during the digestion process of PES, suggesting that there were considerable amounts of α -(1 $\rightarrow$ 6)-glucosyl residues existing in PES. Different from PES, PEP-6P could be quickly hydrolyzed in all three systems (Fig. 1B), and the final hydrolysis rates came to 86.83%, 70.06%, and 99.29% at 180 min for AMY, AMG, and the combined system, respectively. Thus, PEP-6P probably contained large amounts of α -(1 $\rightarrow$ 4)-glucosyl residues, which contributed to the rapid hydrolysis. For the complex of PES and PEP-6P (Fig. 1C), a proportion of 42.92%, 68.50%, and 84.94% starch was degraded by AMY, AMG, and combined enzymes, respectively. In Fig. 1D, the digestive curves of PES and PES/PEP-6P in the complex enzymatic system were compared. It showed that the two samples had no significant differences within the first 20 min. The reason may be that the addition of PEP-6P into PES was not enough to cause obvious changes in the total amounts of glucose in the hydrolysates with 20 min. During 20–120 min, PEP-6P was further hydrolyzed by AMY, leading to the fact that the hydrolysis rates of PES/PEP-6P were always higher than those of PES.

The hydrolysis kinetics related parameters of PES, PEP-6P, and PES/PEP-6P under AMY, AMG, and complex enzymatic systems are listed in Table S1. Comparing to the values of C_{∞} , the addition of PEP-6P inhibited the hydrolysis of PES in the AMY system but promoted the digestion in the AMY and AMG combined system. This result may be explained by the fact that PEP-6P with many α -(1 $\rightarrow$ 4)-glucosyl residues competed with PES for amylase binding sites in the alone AMY system. While in the combined AMY and AMG system, PEP-6P showed slight effects on the enzymatic efficiency of AMG, which played a determinant role during the digestion process of PES as described above.

For the hydrolysis rate constant k , its value of PES/PEP-6P in the AMY systems was much higher than that of PES, but that in the AMY and AMG system was lower. The result also supported the deduction that PEP-6P may contain many α -(1 $\rightarrow$ 4)-glucosyl residues. Additionally, the k value in the combined system was found to be lower than that in the AMY system, which may be owing to the fact that the replacement of PEP-6P for PES at a mass ratio of 10% brought relatively more content of α -(1 $\rightarrow$ 4)-glucosyl residues that are very sensitive to AMY.

3.2 Structural characteristics of PEP-6P

3.2.1 FT-IR spectrum

To confirm the above deduction, it is necessary to conduct fine structure identification of PEP-6P, which contained 94.3% total sugar and 5.5% uronic acid^[8]. Previous analysis of composition of neutral sugar suggested that PEP-6P mainly contained galactose ($4.8 \pm 0.5\%$) and glucose ($80.4 \pm 1.7\%$) with a molar ratio of galactose to glucose at 1:17^[8]. The FT-IR spectrum of PEP-6P was provided in Fig. S1A and it possessed typical peaks of carbohydrates. For example, stretching vibrations of O-H, C-H, C-O groups, and angular twisting of C-H and CH₂ of carbonyl groups were averaged at 3,322, 2,926, 1,626, 1,410, and 1,362 cm⁻¹, respectively. The strong absorption bands at around 1,000, 1,077, and 1,147 cm⁻¹ suggested that it contained C-O-C glycosidic bond and pyranose rings. The peak at 849 cm⁻¹ indicated the presence of α -configurations in PEP-6P^[14].

3.2.2 Results of methylation and GC/MS

The total ion chromatogram of PMAAs of PEP-6P by GC/MS

analysis is presented in Fig. S1B, and typical peaks were marked. Results of methylation analysis were summarized in Table S2. From Fig. S1B, five major methylated sugar residues were found, which were assigned to be Glcp-(1 $\rightarrow$, $\rightarrow$ 4)-Galp-(1 $\rightarrow$, $\rightarrow$ 4)-Glcp-(1 $\rightarrow$, $\rightarrow$ 3,4)-Glcp-(1 $\rightarrow$, and $\rightarrow$ 4,6)-Galp-(1 $\rightarrow$. The corresponding molar ratio was approximately 9.7:1.6:89.0:1.0:4.0. The ratio of proportions of galactosyl residues (5.6%) and glucosyl residues (94.4%) was 1:16.9, which was close to the result of monosaccharide composition. Obviously in Table S2, (1 $\rightarrow$ 4)-linked-glucosyl linkage was the most abundant one, and only glucose was found in terminal residue of the polysaccharide chain. Total amounts of the mono-substituted residues ($\rightarrow$ 3,4)-Glcp-(1 $\rightarrow$ and $\rightarrow$ 4,6)-Galp-(1 $\rightarrow$) were 4.8%, which corresponded to half of the terminal one (9.7%). Therefore, PEP-6P was deduced to have a main chain of (1 $\rightarrow$ 4)-linked-galactoglucan with a few side chains.

3.2.3 1D/2D NMR spectroscopy

3.2.3.1 Identification of sugar residues

The ¹H, ¹³C, and HSQC NMR spectra of PEP-6P were shown in Fig. 2. In Fig. 2A, there were seven major peaks in the anomeric region from 90 to 100 ppm, which were labelled from residue A to residue G with chemical shifts of their anomeric carbons at 99.5, 100.0, 98.5, 104.3, 95.7, 91.8, and 102.5 ppm, respectively. The corresponding signals of anomeric protons of residues A–E were located at 5.32, 5.28, 4.88, 4.55, and 4.56 ppm, respectively. However, those of residue F and residue G were not observed in Fig. 2B, which were further distinguished at 5.14 and 4.44 ppm in Fig. 2C. The long range or short-range correlated signals of H/H or C/H were shown in Fig. 3. Detailed data analysis of main residues was provided as below, and chemical shifts of all glycosyl residues were listed in Table 1 based on NMR data from Figs. 2, 3 and previous reports^[14–16].

Residue A with the most intensive signal was identified as $\rightarrow$ 4)- α -Glcp-(1 $\rightarrow$. Starting from the anomeric proton at 5.32 ppm, the H-1/H-2, H-2/H-3, and H-3/H-4 correlated signals were recognized at 5.32/3.54, 3.54/3.87, and 3.87/3.57 ppm in the COSY spectrum (Fig. 3A). The determination of H-5 at 3.74 ppm was derived from H-1/H-5 correlated signal in NOESY spectrum (Fig. 3D)^[14–17]. Referring to the chemical shifts of H-1–H-5, those of C-2 to C-5 were assigned one by one from the non-anomeric region of the HSQC spectrum (Fig. 3B). Signals of C-6/H-6 and C-6/H-6' were appointed at 60.3/3.75 and 60.3/3.56 ppm. The above assignments were verified afterwards by the signals of intra-residues of H-1/C-5&3&4 (labelled as L1); H-3/C-4 (labelled as L2); H-4/C-6&5&3 (labelled as L3); H-5/C-4&3 (labelled as L4); C-1/H-3&4 (labelled as L5) in the HMBC spectrum (Fig. 3C), H-1/H-4&5&3 (labelled as N1); H-3/H-4&6 (labelled as N5), H-6/H-4 (labelled as N6) in the NOESY spectrum (Fig. 3D and Table S3).

For residue B ($\rightarrow$ 4)-3- α -Glcp-(1 $\rightarrow$), the resonance of H-2 was 3.54 ppm owing to its correlation with H-1 (5.28 ppm) in the COSY spectrum (Fig. 3A). The chemical shift of C-2 at 72.2 ppm was deduced from the HSQC spectrum (Fig. 3B) according to the C-2/H-2 cross-peak at 72.2/3.54 ppm. Similarly, chemical shifts of C/H-3, C/H-4, C/H-5, and C/H-6 of residue B were completely assigned in the COSY and HSQC spectra, respectively. Because of the downfield spin system of both C-4 (76.6 ppm) and C-6 (69.2 ppm), residue B was assigned to $\rightarrow$ 4)-3- α -Glcp-(1 $\rightarrow$ ^[18].

For residue D ($\rightarrow$ 4)- β -Galp-(1 $\rightarrow$), cross-peaks including H-1/H-2 (4.55/3.58) and H-3/H-4 (3.68/4.08) in Fig. 3A, H-1/H-5 (4.55/3.62), H-1/H-4 (4.55/4.08), and H-1/H-3 (4.55/3.68)

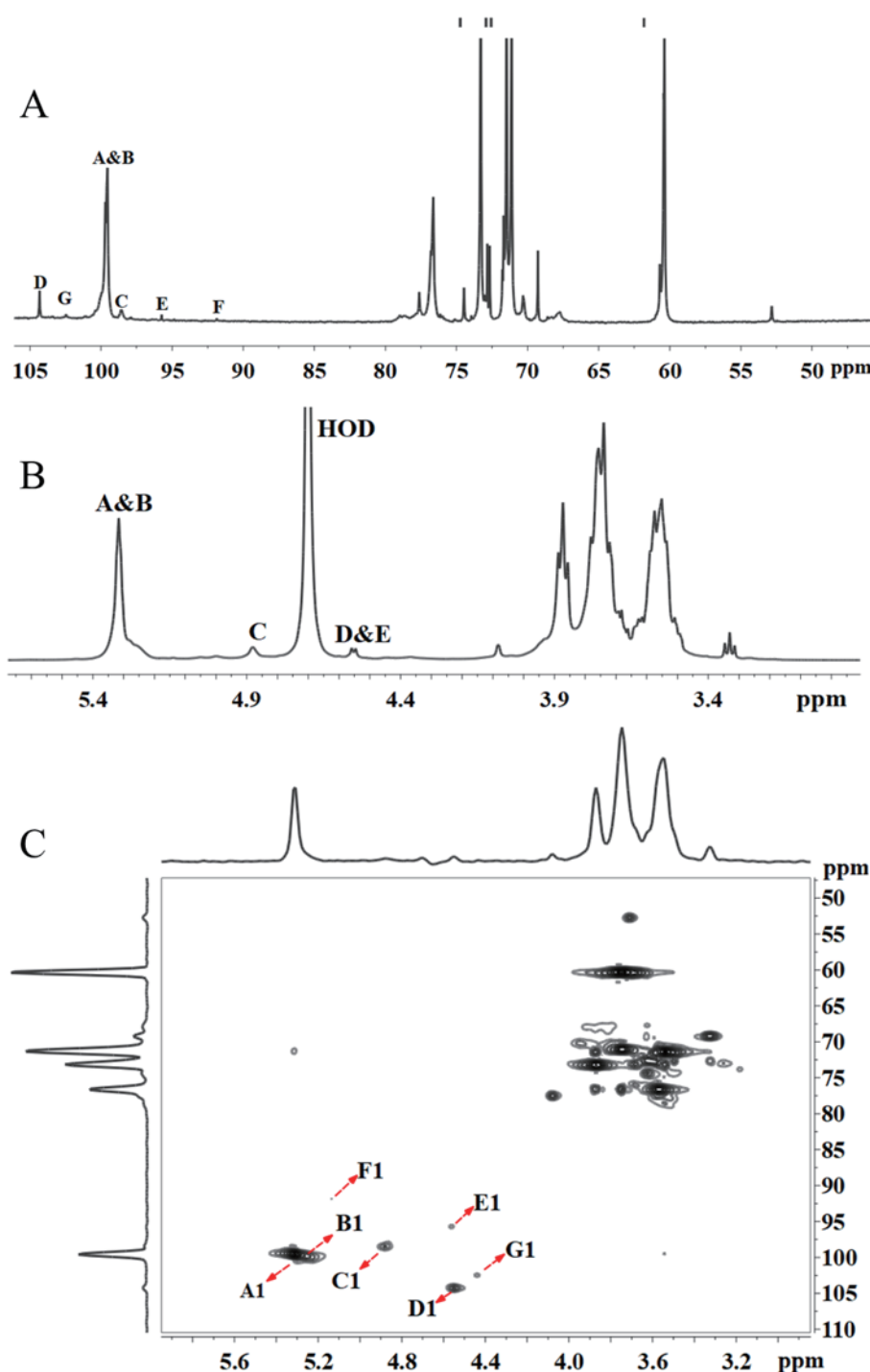


Figure 2 ^{13}C (A), ^1H (B), and HSQC (C) NMR spectra of PEP-6P.

(labelled as N3) in Fig. 3D gave the information of protons^[19]. The signal at 77.4/4.55 ppm labelled as 9 was found in Fig. 3C. The results were applied to assign the chemical shifts of correlated ^{13}C signals in Fig. 3B according to the NMR data reported before^[19-22]. Moreover, residue G ($\rightarrow 4,6$)- β -Galp-(1 $\rightarrow$) was determined based on data from those reports^[16,22-24], which were C-1/H-1 at 102.5/4.44 ppm (Fig. 2C), C-2/H-2 at 71.4/3.43 ppm, C-3/H-3 at 80.2/3.67 ppm, C-4/H-4 at 68.3/4.10 ppm, C-5/H-5 at 73.0/3.90 ppm, and C-6/H-6 (H-6') at 68.0/3.81(3.87) ppm (Fig. 3B). The cross-peaks at 104.3/4.08 ppm (labelled as L6) and 71.4/3.67 ppm (labelled as 11) in Fig. 3C conformed to the above deduction.

Generally, a molecular chain of polysaccharide contains terminal sugar residues at the reducing end and non-reducing end, which correspond to residues E&F and residue C, respectively^[21]. By referring to previous reference^[25], residue C was determined to be α -GlcP-(1 $\rightarrow$ with the C-1/H-1 (98.5/4.88 ppm), C-2/H-2 (72.7/3.50 ppm), C-3/H-3 (73.5/3.62 ppm), C-4/H-4 (72.7/3.33 ppm), C-5/H-5 (73.2/3.94 ppm), C-6/H-6 (60.3/3.84 ppm) in the HSQC spectrum (Fig. 3B). The correlated signals of 72.7/3.50 ppm (C-4/H-2) (labelled as 13), 73.2/3.50 ppm (C-5/H-2) (labelled as 12), 60.3/3.33 ppm (C-6/H-4) and 72.7/3.33 ppm (C-2/H-4) (labelled as L7)) in the HMBC spectrum (Fig. 3B) agreed

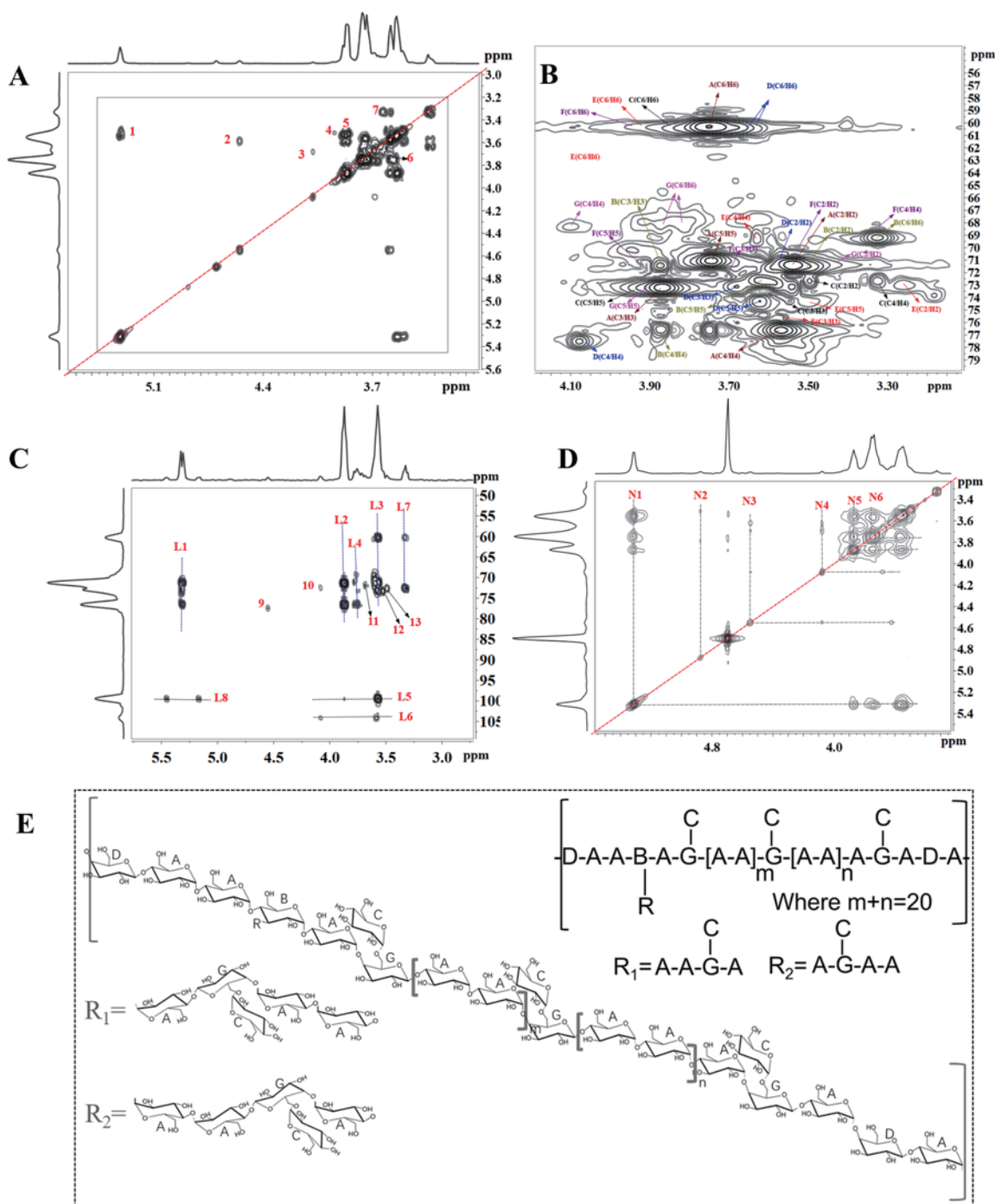


Figure 3 COSY (A), non-anomeric region of HSQC (B), HMBC (C), NOESY (D) spectra, and predicted structure of the repeating unit (E) of PEP-6P.

with the above result. Residue E (β -Glc at reducing end) with the ^1H signals of H-2 (3.26 ppm), H-3 (3.58 ppm), H-4 (3.63 ppm), H-5 (3.49 ppm), H-6 (3.95 ppm) and 6' (3.70 ppm) were detected based on data reported by Cui *et al.*^[21]. The relevant ^{13}C NMR signals of residue E were 73.0, 75.6, 69.2, 74.3, and 60.3 ppm deduced from the HSQC spectrum (Fig. 3B). Similarly, the chemical shifts of carbons and protons of residue F (α -Glc at reducing end) were recognized as C-1/H-1 (91.8/5.14 ppm), C-2/H-2 (69.9/3.52 ppm), C-3/H-3 (72.3/3.71 ppm), C-4/H-5 (68.4/3.33 ppm), C-5/H-5 (71.4/3.91 ppm), and C-6/H-6 (60.3/3.94(3.76) ppm) in the HSQC spectrum (Fig. 2C and Fig. 3B)^[21,26].

3.2.3.2 Linkage sites and sequence among sugar residues

Data of inter- and intra-residues in HMBC and NOESY spectra (Figs. 3C, D) were collected in Table S3. The cross-peaks between H-1 and C-3 & 4 & 5, H-4 and C-6 & 5 & 3, C-1 and H-3 & 4 of residue A in the HMBC spectrum (Fig. 3C) suggested that the backbone of PEP-6P was mainly linked by (1 $\rightarrow$ 4)-O-glycosidic bonds. The inter-residual cross-peaks of H-3 of residue A and C-4 of residue D, C-1 of residue A and H-6 of residue D in HMBC spectrum (Fig. 3C) indicated the presence of $\rightarrow$ 4)- α -Glc-(1 $\rightarrow$ 4)- β -Galp-(1 $\rightarrow$ 4)- α -Glc-(1 $\rightarrow$ unit (-A-D-A-). The dimer of $\rightarrow$ 4)- α -Glc-(1 $\rightarrow$ 3,4)- α -Glc-(1 $\rightarrow$ (-A-B-) was resulted from C-2 of residue B and H-5 of residue A. Likewise, the α -Glc-(1 $\rightarrow$ 4)- β -Galp-(1 $\rightarrow$ and

Table 1 Assignment of chemical shifts of main residues of PEP-6P

Sugar residues	Atoms	Chemical shifts (ppm)					
		1	2	3	4	5	6/6'
Residue A	H	5.32	3.54	3.87	3.57	3.74	3.75/3.56
→4)-α-Glcp-(1→	C	99.5	71.4	73.2	76.6	71.1	60.3
Residue B ^a	H	5.28	3.54	3.87	3.87	3.61	3.39
→4)-3-α-Glcp-(1→	C	100.0	72.2	71.4	76.6	72.7	69.2
Residue C	H	4.88	3.50	3.62	3.33	3.94	3.84
α-Glcp-(1→	C	98.5	72.7	73.5	72.7	73.2	60.3
Residue D	H	4.55	3.58	3.68	4.08	3.62	3.70/3.63
→4)-β-Galp-(1→	C	104.3	71.3	73.1	77.4	74.3	60.4
Residue E	H	4.56	3.26	3.58	3.63	3.49	3.95/3.70
→4)-β-Glcp	C	95.7	73.0	75.6	69.2	74.3	60.3
Residue F	H	5.14	3.52	3.71	3.33	3.91	3.94/3.76
→4)-α-Glcp	C	91.8	69.9	72.3	68.4	71.4	60.3
Residue G	H	4.44	3.43	3.67	4.10	3.90	3.81/3.87
→4,6)-β-Galp-(1→	C	102.5	71.4	80.2	68.3	73.0	68.0

α-Glcp-(1→4,6)-α-Glcp-(1→ units (-C-D- & -C-G-) were deduced from C-2 of C and H-4 of D, H-1 of C and H-6 of G, respectively. Based on the above analysis and the molar ratio of residues from methylation analysis, a proposed repeating unit of PEP-6P was shown in Fig. 3E, which was a linear galactoglucan with a few short side chains. This proposed structure verified the deduction in the *in vitro* enzymatic hydrolysis experiment in section 3.1.

In previous reports, similar galactoglucans containing great amounts of →4)-α-Glcp-(1→ residues have been reported. Those galactoglucans with antioxidant activity, hepatoprotective activity, anti-inflammatory effects, antitumor effects, and immunomodulating effects were isolated from *Russula vinosa* Lindblad^[11], *Pleurotus djamor*^[27], *Poria cocos*^[28], *Fortunella margarita*^[29], *Cistanche deserticola*^[22], and *Castanea mollissima* Blume^[21]. However, the FP40 from *Fortunella margarita* and B86-III from *Antrodia cinnamomea* had →4)-β-Glcp-(1→4)-α-Glcp-(1→4)-α-Galp-(1→^[29] and →4)-β-Glcp-(1→^[30] in the main chain, respectively. Only fractions from *Cistanche deserticola* and *Castanea mollissima* Blume had the same dominant glycosyl residues with PEP-6P in the current study. In detail, galactoglucan (HCPA) from *C. mollissima* Blume fruits contained [→4)-α-D-Glcp-(1→], [→4,6)-α-D-Glcp-(1→], [→4)-β-D-Galp-(1→], and [α-D-Glcp-(1→] motifs in a molar ratio of approximately 6.11:1.08:1.00:1.03^[21]. The CDA-0.05 isolated from *C. deserticola* contained 1,4-linked α-D-Glcp, 1,4,6-linked α-D-Glcp, and 1,4-linked β-D-Galp, with branches of T-linked α-D-Glcp attached at C-6 of 1,4,6-linked α-D-Glcp residues in a molar ratio of 64.3:16.1:3.5:16.1^[22].

3.3 Solution conformation of PEP-6P

3.3.1 Solution conformation analyzed by HPSEC

The elution curves of PEP-6P determined by high performance size-exclusion chromatography system and the logarithmic plots of the molecular weight versus cumulative weight fraction, intrinsic viscosity, and hydrodynamic radius (R_h) were performed in Fig. 4. The mass recovery, weight-average molecular weight

(M_w), number-average molecular weight (M_n), ratio of M_w and M_n , mean square radius of gyration (R_g), and hydrodynamic radius (R_h) of PEP-6P were 80.9%, 47.8 kDa, 24.4 kDa, 1.96, 2.5 nm, and 7.4 nm, respectively, suggesting that it had a relatively high purity with low molecular weights. Fig. 4B showed the changes of cumulative weight fraction with the increase of molecular weights. The values of Log M of PEP-6P were concentrated in the range of 4.39–5.12, which was applied in further data analysis about the relationship of Log M with intrinsic viscosity and R_h . Intrinsic viscosity ($[\eta]$) is a critical parameter that can be used to obtain polysaccharide conformation^[31]. In general, a higher value of intrinsic viscosity relates to a more extended structure at a given chain length. In a specific solvent, the relationship of intrinsic viscosity and viscosity-average molecular weight is given by the Mark-Houwink equation ($[\eta]=KM_v^{\alpha_v}$). The exponent α_v usually lies in the range of 0.5–0.8 for random coil conformation and increases with increasing chain stiffness^[32-33]. The intrinsic viscosity for PEP-6P was 1.37 dL/g, and the α_v values of PEP-6P were 0.35 in the low- M_w fraction (89% cumulative fraction) and 2.15 in the high- M_w region (Fig. 4C), suggesting that PEP-6P exhibited major spherical conformations and minor rigid rod-like conformations in 0.1 M NaNO₃ solution^[32-34]. Polysaccharides with rigid rod-like entities usually have aggregates, while those having spherical conformations may have polymer strands coordinated to rigid rodlike entities in solution^[32]. The sphere-like structure had also been identified in the yeast mannans^[33]. But a contrary result was obtained about scleroglucan, which had a spherical conformation in the high- M_w fraction and a rigid rod-like conformation in the low- M_w fraction, reflected by $\alpha_v = 0.03$ and $\alpha_v = 2.5$, respectively^[32].

To confirm the above result, the double logarithmic relationship between R_h versus M_w was established in Fig. 4D based on the power-law function ($R_h = KM_w^{\alpha_h}$) from literature^[16]. The conformation parameter (α_h) was related to the shape of macromolecules in solution, and the stiffness of polymer chains increased with the increasing α_h value^[35]. The calculated α_h for PEP-6P was 0.43 in the low- M_w fractions and 0.99 in the high- M_w

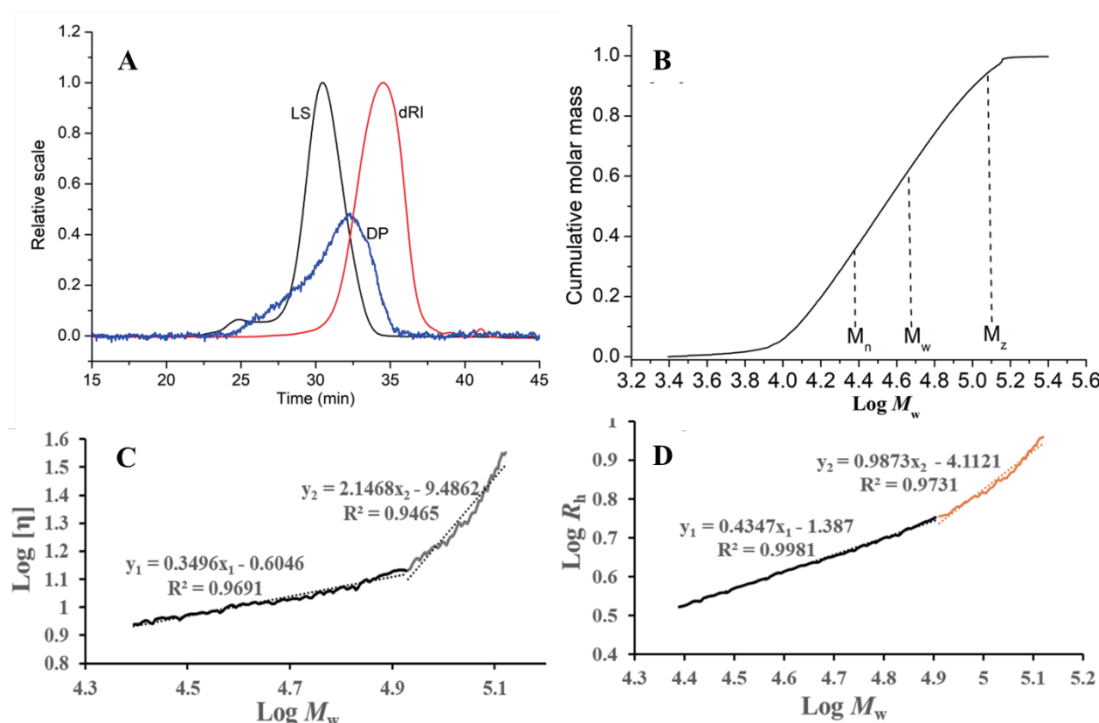


Figure 4 HPSEC elution profiles on three online detectors (A), logarithmic plot of the molecular weight versus cumulative weight fraction (B), intrinsic viscosity (C), and hydrodynamic radius (R_h) (D) of PEP-6P in 0.1 M NaNO_3 solution (containing 0.02% NaN_3).

fractions (Fig. 4D), implying a spherical conformation and aggregates.

3.3.2 helical structure by Congo-red tests

Congo-red analysis is usually used to identify the triple-helical conformation of polysaccharide, which can form a complex with Congo-red, and the maximum absorption wavelength (λ_{max}) will undergo a bathochromic shift in the range of 400–600 nm. The λ_{max} of the complex with triple helical structure could shift to a longer wavelength of 509 nm and decline to 490 nm gradually with the rise of NaOH concentration^[25]. As shown in Fig. 5A, the values of λ_{max} of PEP-6P plus Congo red at different concentrations of NaOH (0–0.5 M) were always higher than those of Congo red at the same concentrations. Therefore, PEP-6P had no triple helix structure, which was consistent with the result of HPSEC.

3.3.3 Crystal structure analysis by XRD

X-ray diffraction (XRD) can investigate the polymer conformation and calculate the helical pitch. However, it requires crystalline solid state and high purity polysaccharide samples to facilitate the elucidation of the preferred molecular structure, which is challenging for most amorphous polysaccharides^[36–37]. The XRD pattern of PEP-6P in Fig. 5B showed a “bun-shaped” curve indicating that PEP-6P had no crystal structure^[38].

3.4 Solid topography of PEP-6P

The SEM images of PEP-6P (Figs. 5C, 5D) showed spherical and flaky structures at the magnifications of 5,000 and 30,000. The small spheres aggregated into a cluster with mean diameters at around 40 nm (Figs. 5E, 5F). A few previous investigations reported linear and spherical morphology structures of polysaccharides. For instance, Dong *et al.*^[39] identified a novel spherical polysaccharide from *Fructus Mori* with diameters at around 43 nm. TM3a from sclerotia of *Pleurotus tuber-regium* in

0.25 M LiCl/DMSO exhibited a globular molecular structure and the average diameter was about 23.0 nm^[34].

3.5 Thermal properties of PEP-6P

Thermogravimetric analysis is applied to study the decomposition pattern and the thermal stability of polymers and DSC is performed to study the endothermal and exothermal changes occurring with the increase of temperature^[40]. As presented in Figs. 5G–5H, PEP-6P exhibited three decomposition patterns. In the first stage of the temperature ramp, a minor mass loss of 7.0% at 102.1 °C happened, which was attributed to desorption of moisture. In the second stage, the main decomposition of the polysaccharides started from 275 °C and ended at 338 °C with a weight loss of 56.7%^[41]. The final residue of PEP-6P was 13.8%. Correspondingly, the DSC thermogram of PEP-6P contained enthalpy changes for the first thermal transition (ΔH_1) and the second thermal transition (ΔH_2) of 378.2 and 37.2 J/g, respectively, which were similar to the results of galactomannan^[41] and *Tremella fuciformis* polysaccharides^[40].

4 Conclusions

In this study, a non-starch polysaccharide fraction (PEP-6P) isolated from jicama roots was structurally characterized to explore the molecular mechanisms underlying the increasing hydrolysis rates of gelatinized jicama starch induced by PEP-6P. PEP-6P was probably a galactoglucan containing 1,4-linked α -D-Glcp, 1,3,4-linked α -D-Glcp, 1,4-linked β -D-Galp, and 1,4,6-linked β -D-Galp. The side chain was composed of T-linked α -D-Glcp attached to O-3 of 1,4-linked β -D-Galp and O-6 of 1,4-linked β -D-Galp. The presence of abundant α -(1 $\rightarrow$ 4)-glucosyl linkages in the galactoglucan backbone explains the unexpected enhancing effect of PEP-6P on starch digestion. It may also be contaminated by some acidic polysaccharide fraction. Conformation analysis suggested that PEP-6P had a narrow distribution of molecular

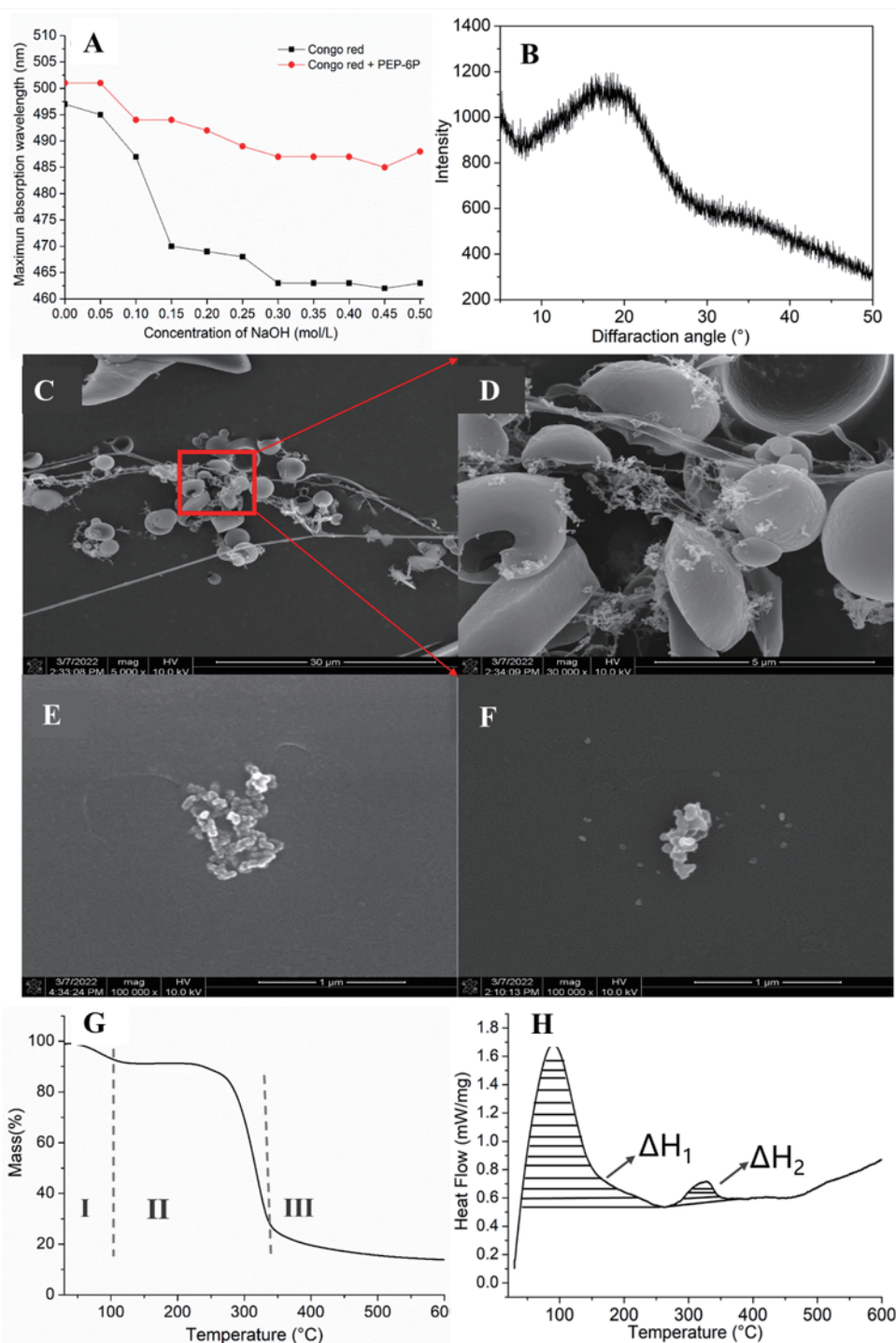


Figure 5 The maximum absorption wavelengths of Congo red-PEP-6P complex and Congo red solution at various NaOH concentrations (A), XRD patterns (B), SEM images of PEP-6P pre-freezed in liquid nitrogen with magnification of 5,000 (C), pre-freezed in liquid nitrogen with magnification of 30,000 (D), naturally dried in silicon wafer at magnification of 100,000 at 50 $\mu\text{g/mL}$ (E), naturally dried in silicon wafer at magnification of 100,000 at 5 $\mu\text{g/mL}$ (F), TG (G) and DSC (H) curves of PEP-6P.

weight and a sphere-like conformation without helical structure. The fine structural data lays foundation for further studies on bioactivity or structure-activity relationship of jicama non-starch polysaccharides.

Electronic Supplementary Material (ESM)

The online version contains supplementary material available at <https://doi.org/10.26599/FMH.2027.9420155>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and/or the Electronic Supplementary Material.

Conflicts of interest

The authors declare that there are no conflicts of interest in this

work.

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Author contribution statement

Xiao-Dan Shi: Conceptualization, experimental design, project administration, validation, writing manuscript. **Yan Wang:** Data curation, validation, experimental design. **Jing-Jing Huang:** Methodology, Data curation, visualization. **Hong-Mei Yin:** Project administration, Resources, Visualization. **Zhi-Jun Wang:** Project administration, writing- review & editing. **Tao Yuan:** Project administration, funding acquisition, writing- review & editing. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

None.

References

- [1] Li, Y., Liang, W., Huang, W., et al. Complexation between burdock holocellulose nanocrystals and corn starch: gelatinization properties, microstructure, and digestibility *in vitro*. *Food & Function*, **2022**, 13: 548–560. <https://doi.org/10.1039/d1fo03418a>
- [2] Waddell, I. S., Orfila, C. Dietary fiber in the prevention of obesity and obesity-related chronic diseases: From epidemiological evidence to potential molecular mechanisms. *Critical Reviews in Food Science and Nutrition*, **2023**, 63: 8752–8767. <https://doi.org/10.1080/10408398.2022.2061909>
- [3] Chen, S., Qin, L., Chen, T., et al. Modification of starch by polysaccharides in pasting, rheology, texture and *in vitro* digestion: A review. *International Journal of Biological Macromolecules*, **2022**, 207: 81–89. <https://doi.org/10.1016/j.ijbiomac.2022.02.170>
- [4] Sarkar, R., Bhowmik, A., Kundu, A., et al. Inulin from *Pachyrhizus erosus* root and its production identification using evolutionary algorithm approach and response surface methodology. *Carbohydrate Polymers*, **2021**, 251: 1–11. <https://doi.org/10.1016/j.carbpol.2020.117042>
- [5] Santoso, P., Maliza, R., Insani, S. J., et al. Preventive effect of jicama (*Pachyrhizus erosus*) fiber against diabetes development in mice fed with high-fat diet. *Journal of Applied Pharmaceutical Science*, **2021**, 11: 137–143. <https://doi.org/10.7324/JAPS.2021.110116>
- [6] Baroroh, H. N., Nugroho, A. E., Lukitaningsih, E., et al. Water-Soluble Fiber from Bengkoang (*Pachyrhizus erosus* (L.) Urban) Tuber Modulates Immune System Activity in Male Mice. *Scientia Pharmaceutica*, **2020**, 88: 1–12. <https://doi.org/10.3390/scipharm88030034>
- [7] Shi, X. D., Huang, J. J., Wang, S. Y., et al. Polysaccharides from *Pachyrhizus erosus* roots: Extraction optimization and functional properties. *Food Chemistry*, **2022**, 382: 132413. <https://doi.org/10.1016/j.foodchem.2022.132413>
- [8] Huang, J., Yu, M., Wang, S., et al. Effects of jicama (*Pachyrhizus erosus* L.) non-starch polysaccharides with different molecular weights on structural and physicochemical properties of jicama starch. *Food Hydrocolloids*, **2023**, 139: 108502. <https://doi.org/10.1016/j.foodhyd.2023.108502>
- [9] Li, X., Yue, X., Huang, Q., et al. Effects of wet-media milling on multi-scale structures and *in vitro* digestion of tapioca starch and the structure-digestion relationship. *Carbohydrate Polymers*, **2022**, 284: 119176. <https://doi.org/10.1016/j.carbpol.2022.119176>
- [10] Shi, X. D., Nie, S. P., Yin, J. Y., et al. Polysaccharide from leaf skin of *Aloe barbadensis* Miller: Part I. extraction, fractionation, physicochemical properties and structural characterization. *Food Hydrocolloids*, **2017**, 73: 176–183. <https://doi.org/10.1016/j.foodhyd.2017.06.039>
- [11] Zhang, H., Li, C., Lai, P., et al. Fractionation, chemical characterization and immunostimulatory activity of beta-glucan and galactoglucan from *Russula vinosa* Lindblad. *Carbohydrate Polymers*, **2021**, 256: 117559. <https://doi.org/10.1016/j.carbpol.2020.117559>
- [12] Shi, X. D., Yin, J. Y., Huang, X. J., et al. Structural and conformational characterization of linear *O*-acetyl-glucmannan purified from gel of *Aloe barbadensis* Miller. *International Journal of Biological Macromolecules*, **2018**, 120: 2373–2380. <https://doi.org/10.1016/j.ijbiomac.2018.09.005>
- [13] Liu, M., Yao, W., Zhu, Y., et al. Characterization, antioxidant and anti-inflammation of mycelia selenium polysaccharides from *Hypsizygus marmoreus* SK-03. *Carbohydrate Polymers*, **2018**, 201: 566–574. <https://doi.org/10.1016/j.carbpol.2018.08.099>
- [14] Wang, J., Li, Z., Yang, X., et al. The antitumor role of a newly discovered α -D-glucan from *Holotrichia diomphalia* Bates as a selective blocker of aldolase A. *Carbohydrate Polymers*, **2021**, 255: 117532. <https://doi.org/10.1016/j.carbpol.2020.117532>
- [15] Shi, X. D., Li, O. Y., Yin, J. Y., et al. Structure identification of α -glucans from *Dictyophora echinovolvata* by methylation and 1D/2D NMR spectroscopy. *Food Chemistry*, **2019**, 271: 338–344. <https://doi.org/10.1016/j.foodchem.2018.07.160>
- [16] Zhang, Z., Guo, L., Yan, A., et al. Fractionation, structure and conformation characterization of polysaccharides from *Anoetochilus roxburghii*. *Carbohydrate Polymers*, **2020**, 231: 115688. <https://doi.org/10.1016/j.carbpol.2019.115688>
- [17] Wang, Y. X., Yin, J. Y., Huang, X. J., et al. Structural characteristics and rheological properties of high viscous glucan from fruit body of *Dictyophora rubrovoluta*. *Food Hydrocolloids*, **2020**, 101: 105514. <https://doi.org/10.1016/j.foodhyd.2019.105514>
- [18] Gangoiti, M. V., Puertas, A. I., Hamet, M. F., et al. *Lactobacillus plantarum* CIDCA 8327: An α -glucan producing-strain isolated from kefir grains. *Carbohydrate Polymers*, **2017**, 170: 52–59. <https://doi.org/10.1016/j.carbpol.2017.04.053>
- [19] Cui, L., Chen, L., Yang, G., et al. Structural characterization and immunomodulatory activity of a heterogalactan from *Panax ginseng* flowers. *Food Research International*, **2021**, 140: 1–10. <https://doi.org/10.1016/j.foodres.2020.109859>
- [20] Chan, M. K., Yu, Y., Wulamu, S., et al. Structural analysis of water-soluble polysaccharides isolated from *Panax notoginseng*. *International Journal of Biological Macromolecules*, **2020**, 155: 376–385. <https://doi.org/10.1016/j.ijbiomac.2020.03.233>
- [21] Cui, H., Li, H., Wang, Y., et al. Structural characterization and biological activity of galactoglucan from *Castanea mollissima* Blume. *Journal of Carbohydrate Chemistry*, **2019**, 38: 398–411. <https://doi.org/10.1080/07328303.2019.1630838>
- [22] Zeng, H., Huang, L., Zhou, L., et al. A galactoglucan isolated from *Cistanche deserticola* Y. C. Ma. and its bioactivity on intestinal bacteria strains. *Carbohydrate Polymers*, **2019**, 223: 1–8. <https://doi.org/10.1016/j.carbpol.2019.115038>
- [23] Capek, P., Matulová, M., Navarini, L., et al. Structural features of an arabinogalactan-protein isolated from instant coffee powder of *Coffea arabica* beans. *Carbohydrate Polymers*, **2010**, 80: 180–185. <https://doi.org/10.1016/j.carbpol.2009.11.016>
- [24] Niu, H., Song, D., Sun, Y. L., et al. Preparation and sulfation of an α -glucan from *Actinidia chinensis* roots and their potential activities.

- International Journal of Biological Macromolecules*, **2016**, 92: 981–987. <https://doi.org/10.1016/j.ijbiomac.2016.07.091>
- [25] Gong, Y., Cao, C. Y., Ai, C. Q., et al. Structural characterization and immunostimulatory activity of a glucan from *Cyclina sinensis*. *International Journal of Biological Macromolecules*, **2020**, 161: 779–786. <https://doi.org/10.1016/j.ijbiomac.2020.06.020>
- [26] Huo, J. Y., Wu, J. H., Sun, B. G., et al. Isolation, purification, structure characterization of a novel glucan from Huangshui, a byproduct of Chinese Baijiu, and its immunomodulatory activity in LPS-stimulated THP-1 cells. *International Journal of Biological Macromolecules*, **2020**, 161: 406–416. <https://doi.org/10.1016/j.ijbiomac.2020.06.028>
- [27] Maity, G. N., Maity, P., Khatua, S., et al. Structural features and antioxidant activity of a new galactoglucan from edible mushroom *Pleurotus djamora*. *International Journal of Biological Macromolecules*, **2021**, 168: 743–749. <https://doi.org/10.1016/j.ijbiomac.2020.11.131>
- [28] Cheng, Y., Xie, Y., Ge, J., et al. Structural characterization and hepatoprotective activity of a galactoglucan from *Poria cocos*. *Carbohydrate Polymers*, **2021**, 263: 117979. <https://doi.org/10.1016/j.carbpol.2021.117979>
- [29] Chen, P., Lei, S., Chen, Y., et al. Structural characterization of a novel galactoglucan from *Fortunella margarita* and its molecular structural change following simulated digestion *in vitro*. *Journal of Functional Foods*, **2020**, 71: 104024. <https://doi.org/10.1016/j.jff.2020.104024>
- [30] Lu, M. K., Lin, T. Y., Hu, C. H., et al. Characterization of a sulfated galactoglucan from *Antrodia cinnamomea* and its anticancer mechanism via TGF beta/FAK/Slug axis suppression. *Carbohydrate Polymers*, **2017**, 167: 229–239. <https://doi.org/10.1016/j.carbpol.2017.02.104>
- [31] Wu, D. T., Deng, Y., Zhao, J., et al. Molecular characterization of branched polysaccharides from *Tremella fuciformis* by asymmetrical flow field-flow fractionation and size exclusion chromatography. *Journal of Separation Science*, **2017**, 40: 4272–4280. <https://doi.org/10.1002/jssc.201700615>
- [32] Eder, S., Zueblin, P., Diener, M., et al. Effect of Polysaccharide Conformation on Ultrafiltration Separation Performance. *Carbohydrate Polymers*, **2021**, 260: 1–11. <https://doi.org/10.1016/j.carbpol.2021.117830>
- [33] Xu, X., Chen, A., Ge, X., et al. Chain conformation and physicochemical properties of polysaccharide (glucuronoxylomannan) from Fruit Bodies of *Tremella fuciformis*. *Carbohydrate Polymers*, **2020**, 245: 116354. <https://doi.org/10.1016/j.carbpol.2020.116354>
- [34] Liu, H. Z., Li, Y. N., Shi, A. M., et al. Rheological characteristics and chain conformation of mannans obtained from *Saccharomyces cerevisiae*. *International Journal of Biological Macromolecules*, **2018**, 107: 2404–2411. <https://doi.org/10.1016/j.ijbiomac.2017.10.126>
- [35] Tao, Y. Z., Zhang, L. N., Yan, F., et al. Chain Conformation of Water-Insoluble Hyperbranched Polysaccharide from Fungus. *Biomacromolecules*, **2007**, 8: 2321–2328. <https://doi.org/10.1021/bm070335+>
- [36] Guo, X., Kang, J., Xu, Z., et al. Triple-helix polysaccharides: Formation mechanisms and analytical methods. *Carbohydrate Polymers*, **2021**, 262: 1–12. <https://doi.org/10.1016/j.carbpol.2021.117962>
- [37] Meng, Y., Lyu, F., Xu, X., et al. Recent Advances in Chain Conformation and Bioactivities of Triple-Helix Polysaccharides. *Biomacromolecules*, **2020**, 21: 1653–1677. <https://doi.org/10.1021/acs.biomac.9b01644>
- [38] Qian, J. Y., Chen, W., Zhang, W. M., et al. Adulteration identification of some fungal polysaccharides with SEM, XRD, IR and optical rotation: A primary approach. *Carbohydrate Polymers*, **2009**, 78: 620–625. <https://doi.org/10.1016/j.carbpol.2009.05.025>
- [39] Dong, Y. H., Chen, C., Huang, Q., et al. Study on a novel spherical polysaccharide from *Fructus Mori* with good antioxidant activity. *Carbohydrate Polymers*, **2020**, 117516. <https://doi.org/10.1016/j.carbpol.2020.117516>
- [40] Lin, C., Tsai, S. Differences in the moisture capacity and thermal stability of *Tremella fuciformis* polysaccharides obtained by various drying processes. *Molecules*, **2019**, 24: 2856. <https://doi.org/10.3390/molecules24152856>
- [41] Cerqueira, M. A., Souza, B. W. S., Simões, J., et al. Structural and thermal characterization of galactomannans from non-conventional sources. *Carbohydrate Polymers*, **2011**, 83: 179–185. <https://doi.org/10.1016/j.carbpol.2010.07.036>