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Molecular structure, chemical properties and biological activities of Pinto bean pod polysaccharide

Fazlina Kamarudin, Chee-Yuen Gan*

Analytical Biochemistry Research Centre, 11800 USM, Penang, Malaysia

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ABSTRACT

Pinto bean pod polysaccharide (PBPP) was successfully extracted with yield of 38.5 g/100 g and the PBPP gave total carbohydrate and uronic acid contents of 286.2 mg maltose equivalent/g and 374.3 mg Gal/g, respectively. The M_w of PBPP was 270.6 kDa with intrinsic viscosity of 0.262 dm^3/g, which composed of mannose (2.5%), galacturonic acid (15.0%), rhamnose (4.0%), glucose (9.0%), galactose (62.2%), xylose (2.9%) and arabinose (4.3%) with trace amount of ribose and fucose. The result suggested that PBPP has a spherical conformation with a highly branched structure. Fourier Transform Infrared analysis showed that PBPP has a similar structure as commercial pectin with an esterification degree of 59.9%, whereas scanning electron microscopy study showed that the crude polysaccharide formed a thin layer of film that was made of multiple micro strands of fibre. PBPP exhibited substantial free radical scavenging activity (7.7%), metal reducing capability (2.04 mmol/dm^3) and α-amylase inhibitory activity (97.6%) at a total amount of 1 mg. PBPP also exhibited high water- and oil-holding capacities (3.6 g/g and 2.8 g/g, respectively). At a low concentration, PBPP exhibited emulsifying activity of 39.6% with stability of 38.6%. Apart from that, PBPP was able to show thickening capability at low concentration (0.005 kg/dm^3).

1. Introduction

Pinto bean (*Phaseolus vulgaris* cv. Pinto) is a variety of common beans, which is highly consumed and commercialised worldwide. It was originated in Latin America more than 7000 years ago, and it has been rapidly cultivated throughout the world. According to the Food and Agriculture Organization of the United Nations, the top five dry bean producers are India, Brazil, Myanmar, China and the United States of America, which produced approximately 3.3, 3.0, 2.1, 1.5 and 1.2 million tonnes of dry bean per year, respectively, from the year 1993 to 2014 (http://faostat3.fao.org/browse/Q/QC/E). To our surprise, the leading class of dry common beans produced in the United States is Pinto bean at approximately 42% of the total production (http://www.ers.usda.gov/topics/crops/vegetables-pulses/dry-beans.aspx).

Bean consumption has been reported to minimize risk of chronic diseases, such as diabetes [1], coronary heart disease [2], colon cancer [3], prostate and breast cancer [4,5]. This could be due to the presence of polysaccharides. Previous studies also showed that the bean has the potential in producing functional protein and bioactive peptides [6,7]. However, Pinto bean is purchased as canned pre-cooked or plastic packaged dry beans. The pods are usually removed and discarded. The efforts to study the effects of extraction processes on polysaccharide as well as their molecular structure, chemical properties and biological activities have not yet been performed. Therefore, the pod of Pinto bean was chosen as a source of macromolecule carbohydrate and its potentials, as an alternative macromolecule for commercial uses, need to be evaluated.

Due to the structural features (e.g. degree of substitutions, steric configuration, linkages of monosaccharides and substitutes, and molar mass and its distribution), vegetable polysaccharides play a critical role in their physical properties, such as thickening, gelling, and stabilizing ability [8]. These properties have extended their applications in food and biomedical industries. Modern pharmacological research has identified that polysaccharide as one of the major active components in herbs, which is responsible for various pharmacological activities, such as antioxidant, antiviral, immunostimulatory, antitumour, antifatigue, radioprotection and hepatoprotection activities [9–13]. Clinical studies also suggested that moderate or high intake of dietary fibre, such as cellulose, hemicellulose and lignin, can effectively reduce the risks for developing diseases like diabetes, cardiovascular disease, hypertension, hypercholesterolemia, hyperlipidemia, obesity, and colorectal cancer [14–19]. Sulfated polysaccharides from algae were also reported

* Corresponding author at: Analytical Biochemistry Research Centre, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia.
E-mail address: mattgan81@gmail.com (C.-Y. Gan).

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to possess a higher anticoagulant activity than those of heparin [20] whereas galactoglucomannans and pectins from woody materials have also been reported to exhibit immunostimulating and free radicals scavenging activities [21,22]. Several studies have reported the possibility of using waste products as polysaccharide sources. To date, polysaccharides have been extracted from several under utilised biomasses, such as mango waste, sunflower head residues, sugar beet, soy hull and sweet potato residues [23–25]. Nonetheless, not all sources are suitable for commercial uses. The sources as well as their extraction and purification methodologies may affect the structure and composition of the produced pectin. Therefore, a continuous search for suitable pectin is aggressively performed by researchers.

The main objective of this study was to explore the potential of Pinto bean pod as an alternative source of polysaccharide. The specific objectives of the study were to extract polysaccharide from the pods of Pinto bean using various extraction parameters; and to evaluate the molecular structure, chemical properties as well as the biological activities of the extracted macromolecule carbohydrate.

2. Materials and methods

2.1. Materials

Fresh Pinto bean pods were collected from different markets (~10 kg from each market) in Penang. The pods were cleaned with deionised water and were kept frozen at −20 °C. They were subsequently freeze-dried. The lyophilised pods were ground into fine powder using a blender, sieved (30 Mesh) and stored at 4 °C prior to extraction. All chemicals used in this study were of analytical grade (Sigma-Aldrich, USA).

2.2. Crude polysaccharide extraction

Briefly, 6 g of lyophilised pod powders were added in 300 mL of 0.1 mol/dm^3 citrate-phosphate buffer at different pH values (i.e. pH 2, pH 4 and pH 6) using solid to buffer ratio of 1:50. The mixture was then incubated at different extraction durations (i.e. 1 h, 3 h and 5 h) in an incubator shaker (IKA KS 4000i Control, Staufen, Germany) which constantly shaking at 250 rpm at different temperature settings (i.e. 50 °C, 60 °C and 70 °C). The resulting slurries were then filtered through a muslin cloth (2 layers) to remove solid particles. Thereafter, three volumes of ethanol were added to one volume of extract and incubated at 4 °C for 5 h to precipitate polysaccharide. The precipitates obtained were filtered through a muslin cloth and then washed with ethanol to remove the small molecular weight molecules in the extract. The crude polysaccharide (PBPP) was then lyophilised and stored in a desiccator prior to analysis. The PBPP yield was expressed in g/100 g (w/w, dry basis).

2.3. Scanning electron microscopic (SEM) analysis

Samples were mounted onto SEM specimen stub with a double-sided tape and coated with gold using a Polaron SC 515 Sputter Coater (Fisons Instruments, UK). Subsequently, the samples were photographed using Leo Supra 50VP Field Emission Scanning Electron Microscope equipped with Oxford INCA 400 energy dispersive X-ray Microanalysis system (Oxford Instruments Analytical, UK).

2.4. Protein content, lipid content, total carbohydrate content and uronic acid content determinations

Protein content of PBPP was determined using Bradford method [26], lipid content was determined using Soxhlet method [27] and total carbohydrate content was determined using the method as described by Dubois et al. [28]. Sample (75 μL, 1 mg/mL) was mixed with 75 μL of 0.05 kg/dm^3 phenol solution on an ice bed and sulphuric acid (375 μL) was then added. The mixtures were incubated at 80 °C for 30 min and absorbance was measured at 495 nm using a spectrophotometer (Spectamax M5, Moleular Devices, USA). Maltose was used as a standard.

Uronic acid content was determined using the method as described by Blumenkrantz and Asboe-Hansen [29]. Crude polysaccharide (250 μL) at concentration of 1 mg/mL was mixed with 1.5 mL of 0.0125 mol/dm^3 sulphuric acid/sodium tetraborate solution on an ice bed and then heated at 100 °C for 5 min m-Hydroxydiphenyl (25 μL) was then added and the absorbance was measured after 5 min at 520 nm using a spectrophotometer (Spectamax M5, Moleular Devices, USA).

2.5. Functional groups and degree of esterification (DE) determinations

FTIR spectra of crude pectic-polysaccharide (powder form) were recorded from 650 to 4000 cm^{-1} using Cary 670 FTIR spectrometer with an attenuated total reflectance (ATR) system (Agilent Technologies, CA, USA). The spectra were analysed using Agilent Resolutions Pro software. DE was determined using the following equation:

$$DE = \frac{A_{1730}}{A_{1730} + A_{1600}} \times 100 \quad (1)$$

where A_{1730} was defined as the area of the band at 1730 cm^{-1} and A_{1600} was defined as the area of the band at 1600 cm^{-1} [30].

2.6. Determination of molecular weights

The molecular weight of PBPP was examined using a gel permeation chromatography (GPC) equipped with Viscotek Model TDA 305 Triple Detector Array incorporated Refractive index, Light scattering and viscosity detectors (Malvern, UK). CLM 3021 column (A6000 M, 300 × 7.8 mm, 13 μm beads size, Malvern, UK) was used. A 100 μL of sample (0.01 kg/dm^3) in 0.1 mol/dm^3 $NaNO_3$) was injected and the flow rate and temperature were maintained at 1.0 mL/min and 30 °C, respectively. The elution was carried out with 0.1 mol/dm^3 $NaNO_3$ containing 0.003 kg/dm^3 NaN_3 to prevent bacteria growth. Polyethylene Oxide (18670 Da) was used as working calibration standard. A second standard, dextran (65333 Da) was applied to verify the calibration accuracy with high level of confidence. The molecular weight of the sample was determined by comparing with calibration curve. The chromatogram obtained was analyzed using the OmniSEC software.

2.7. Determination of monosaccharide composition

Monosaccharides composition was determined using the method of Lv et al. [31] using a high performance liquid chromatography (HPLC) system equipped with an UV detector. Sample (10 mg) was hydrolyze using 3 mol/dm^3 trifluoroacetic acid (1 mL) at 95 °C for 8 h, vacuum dried and re-dissolved in 1 mL of deionized water. Sample (100 μL) was then added with 3-methyl-1-phenyl-2-pyrazoline-5-one (PMP, 200 μL) and 0.3 mol/dm^3 NaOH (300 μL) followed by incubation at 70 °C for 1 h. HCl (300 μL, 0.3 mol/dm^3) was then added and the resulting solution was extracted with 1 mL of chloroform for 3 times. The aqueous layer was collected and filtered through a 0.45 μm membrane prior to HPLC analysis. The HPLC system was prepared as the following condition: Zorbax SB-C18 reversed-phase column (250 × 4.6 mm, 5 μm, Agilent, USA). Mobile phase: (A) Acetonitrile; (B) 3.3 mmol/dm^3 KH_2PO_4-3.9 mmol/dm^3 Tris-acetate-EDTA buffer containing 0.10 kg/dm^3 ACN. The gradient: 0–4 min: 94% B; 4–9 min: from 94% to 88%B;

9–20 min: 88% B. The flow rate was set at 1.0 mL/min and the injection volume of 20 μL. The UV detector was set at 250 nm. A mixed standard, which composed of glucoronic acid, mannose, galacturonic acid, ribose, rhamnose, glucose, galactose, xylose, arabinose and fucose were prepared. The result was expressed as a molar percentage.

2.8. Total phenolic content and antioxidant activity determinations

In the total phenolic content (TPC) analysis, sample (1 mg) was mixed with 1.8 mL of Folin-Ciocalteau reagent and incubated at room temperature for 5 min. Sodium bicarbonate (1.2 mL, 0.075 kg/dm^3) was then added and incubated for 60 min at room temperature. The absorbance was measured at 765 nm using a spectrophotometer (Spectamax M5, Moleular Devices, USA) and the result was expressed as mg Gallic acid equivalents (GAE)/100 g extracts [32].

In the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity determination, sample (1 mg) was added to 500 μL of ethanolic DPPH solutions (0.1 mmol/dm^3) and incubated in the dark for 30 min at 30 °C. The discoloration was measured at 517 nm using a spectrophotometer (Spectamax M5, Moleular Devices, USA) and the result was expressed as %/mg extract [33].

In the ferric reducing/antioxidant power (FRAP) analysis, the FRAP reagent (400 μL) was pre-warmed at 37 °C for 30 min and then added to 1 mg of sample. The reaction mixtures were incubated for 1 h at 37 °C and the absorbance at 593 nm was measured using a spectrophotometer (Spectamax M5, Moleular Devices, USA). The result was expressed as mole of $FeSO_4$/g of extracts [34].

2.9. α-*Amylase* inhibitory activity

α-Amylase solution (100 μL, 1 mg/mL) and PBPP solution (1 mg/mL) were mixed and incubated at 25 °C for 10 min. Starch solution (100 μL) in 0.02 mol/dm^3 sodium phosphate containing 6.0 mmol/dm^3 sodium chloride at pH 6.9 was then added into the mixture, followed by incubation at 25 °C for 10 min. Dinitrosalicylic acid (200 μL) was subsequently added into the mixture in order to terminate the reaction and heated in boiling water for 5 min. The mixture was cooled to room temperature and diluted with 3 mL of deionized water. The absorbance was measured at 540 nm using UV–vis spectrophotometer (Spectramax M5, Molecular Devices, USA). Sample blank (mixture of PBPP solution and buffer solution), negative control (only buffer solution), positive control (mixture of α-amylase and buffer solution) were prepared and analyzed using the same procedure. The α-amylase inhibitory activity was calculated as follows:

$$\%inhibiton = \frac{\left[\left(A_{pos} - (A_s - A_{sb})\right)\right]}{\left(A_{pos} - A_{neg}\right)} \times 100 \quad (2)$$

where A_{pos} was the absorbance of positive control, A_{neg} was the absorbance of negative control, A_s was the absorbance mixture of α-amylase solution and PBPP solution, and A_{sb} was the sample blank [35].

2.10. Functional properties determination

Water- and oil-holding capacity (WHC and OHC) were determined using the method described by Chau et al. [36]. Sample (0.1 g) and 10 mL of distilled water or corn oil were added and incubated for 5 h at room temperature. Subsequently, the mixture was centrifuged at 5000*g* for 10 min. WHC and OHC were expressed as g/g extract. Corn oil density is 0.92 g/ml.

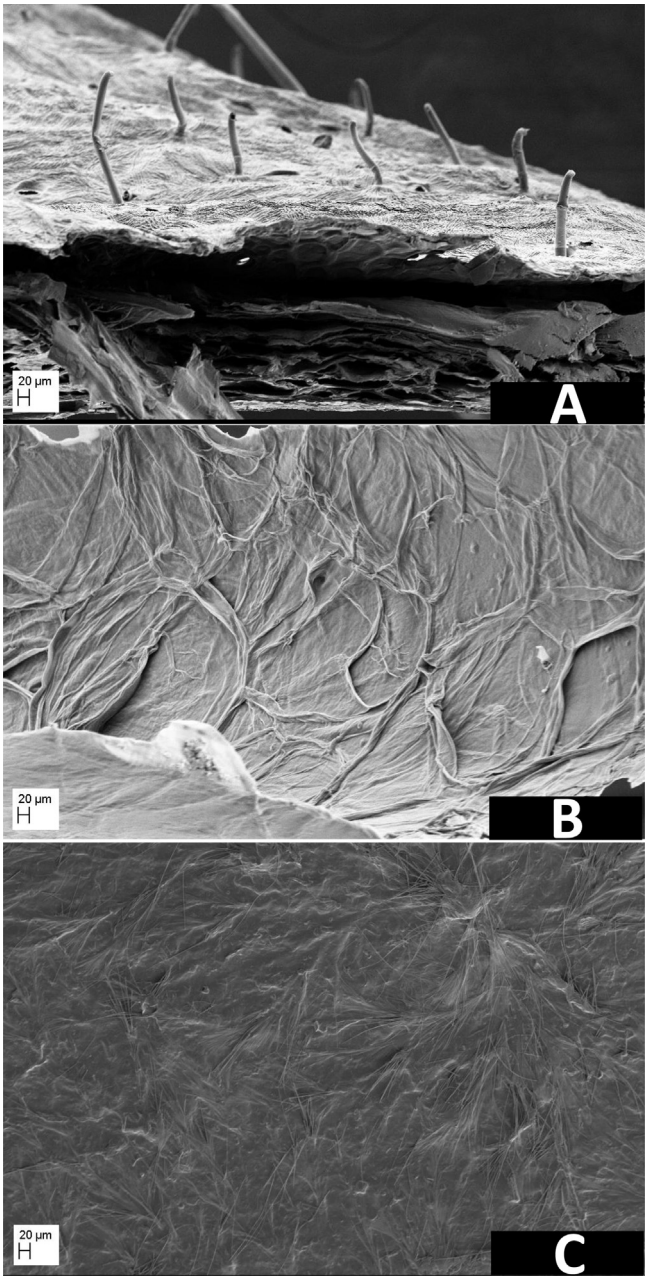

Fig. 1. Morphology of (A) outer layer and midsection of Pinto bean pod, (B) inner layer of the paint bean pod and (C) PBPP.

Emulsifying activity (EA) and emulsion stability (ES) were determined according to Betancur-Ancona et al. [37]. Sample (10 mL, 0.01 kg/dm^3) was homogenized (TH-02, Omni) at 35,000 rpm for 2 min. Corn oil (10 mL) was then added and homogenized for 2 min. The emulsion was centrifuged (Multifuge X1R, Thermo Scientific, USA) at 1200*g* for 5 min and the emulsion volume measured. EA and ES were expressed in percentage.

The thickening capacity was measured based on the viscosity of the sample (0.005 kg/dm^3) at different pH conditions using a rheometer (AR2000ex, TA Instruments, USA) with shear rate ranging from 0.01 to 1000 s^{-1} in 5 min, as described previously [38].

2.11. Statistical analysis

The experiment was first conducted using a factorial design in order to study the suitable condition for the extraction. Results

Table 1
PBPP yields (g/100 g) at different extraction conditions.

Buffer pH (pH)	Temperature (°C)	Extraction duration (h)		
		1	3	5
2	50	20.8 ± 0.3^{b}	27.1 ± 0.5^{bc}	28.2 ± 0.9^{bc}
	60	27.5 ± 0.5^{bc}	33.7 ± 0.3^{c}	35.7 ± 3.0^{c}
	70	29.1 ± 0.6^{bc}	38.5 ± 1.2^{cd}	35.8 ± 0.8^{c}
4	50	15.2 ± 2.4^{a}	22.7 ± 3.8^{b}	20.0 ± 1.0^{b}
	60	24.0 ± 2.3^{b}	26.9 ± 1.9^{b}	29.1 ± 1.5^{bc}
	70	24.4 ± 0.9^{b}	32.1 ± 3.3^{c}	34.0 ± 0.4^{c}
6	50	28.7 ± 0.9^{bc}	30.8 ± 0.8^{c}	21.4 ± 3.7^{b}
	60	25.5 ± 0.9^{b}	33.7 ± 5.9^{c}	33.8 ± 4.3^{c}
	70	26.6 ± 0.7^{b}	32.1 ± 1.9^{c}	33.8 ± 2.4^{c}

Note: Comparison within the column and row in Table with the data written as mean. (n = 3). Means within the same column not followed by the same letter are significantly different at $p < 0.01$ level of significance, according to Duncan's Multiple-Range Test.

Table 2
Protein, lipid, carbohydrate and uronic acid contents of PBPP.

Sample	Protein content (g/100 g)	Lipid content (g/100 g)	Carbohydrate content (mg maltose equivalent/g)	Uronic acid content (mg Gal equivalent/g)
PBPP	0.2 ± 0.0	0.0 ± 0.0	286.2 ± 11.3	374.3 ± 67.3

were expressed as mean values ± standard deviation of three separated determinations. Comparison of means was performed using one-way analysis of variance (ANOVA). When comparing the functional properties of the PBPP with commercial pectin, *t*-test was used with significant level of $p < 0.05$ (SPSS for Windows, Version 19.0, SPSS Institute Inc., Cary NC).

3. Results and discussion

3.1. Microscopic examination of Pinto bean pod

Fig. 1A shows the morphology of the outer layer and the midsection of Pinto bean pod. The presence of micro-filaments on the surface of the outer layer was observed, whereas multiple layers of fibre were observed in the midsection. It was interesting to note that the surface of the inner layer (Fig. 1B) shows the presence of thin film, which could be consisted of cellulose, hemicellulose or pectin. Therefore, Pinto bean pod was suggested to be a good source for the extraction of macromolecule carbohydrate.

3.2. Effect of pH, temperature and extraction time in extraction yield

Results showed that the PBPP yield was depending on the extraction condition (Table 1). According to ANOVA, pH, temperature and extraction time were found significantly ($p < 0.0001$) affecting the yield values. In general, the extraction condition at pH 2 resulted in higher extraction yields. It could be due to the presence of large amounts of hydrogen ions stimulated the hydrolysis of polysaccharide by breaking the glycosidic bonds between polysaccharide molecules and cell wall constituent at low pH, and thus permitting dissolution of polysaccharide into extracting solvent [39]. In terms of the effect of time, 3 h of extraction period sufficiently gave a high yield of ~38.5% at a temperature of 70 °C. The result also showed that higher temperature was preferable in this case of study. It was suggested that a substantial time (i.e. 3 h) and a higher temperature were required for extraction because the high extraction temperature could enhance the rate of extraction by increasing the solubility of polysaccharide into the extracting medium. Increase in temperature would also result in the increase of the diffusion coefficient, which will improve the rate of diffusion [40], whereas a substantial extraction duration may allow the buffer solution to penetrate the dried pods, to dissolve the macromolecules and then to diffuse the macromolecules out of the pod. Such phenomenon demonstrated an existence of interaction between pH, temperature and time, where ANOVA showed that there was a significant ($p < 0.05$) interaction between pH and temperature as well as between pH and time ($p < 0.01$) in affecting the extraction yield. In this case of study, extraction condition at pH 2, temperature of 70 °C and extraction time of 3 h, was selected as the most suitable condition for extracting PBPP because this condition performed better (i.e. gave the highest yield) from the economic point of view, although minor alteration of the structure of the PBPP might occur. It should also be noted that the extraction yields obtained were found to be higher than that of pectin from citrus peels (16.1 g/100 g) and apple pomace (14 g/100 g) [41,42]. Therefore, it was suggested that Pinto bean pod is a good source of macromolecule carbohydrate.

3.3. Morphology and chemical compositions of PBPP

Fig. 1C shows the morphology of PBPP and it was found that this extracted polysaccharide was similar to the thin layer found in the inner layer of the pod (Fig. 1B). It could also be observed that this thin layer of film was made of multiple micro strands of fibre. Table 2 shows the protein, lipid, carbohydrate and uronic contents of PBPP. It was found that PBPP contained very low amounts of protein (0.2 g/100 g) and lipid (0.0 g/100 g) with high amounts of carbohydrate (286.2 mg maltose equivalent/g) and uronic acid (374.3 mg Gal equivalent/g), suggesting that high purity of the polysaccharide was successfully extracted from Pinto bean pod. The result also suggested that this extracted polysaccharide could be a type of pectic-polysaccharide and therefore, FTIR analysis was conducted and then the IR spectra of PBPP was compared with the structural information of commercial pectin.

3.4. Functional groups and esterification degree (DE) of PBPP

The structural information of PBPP was confirmed based on FTIR spectra by comparing the fingerprint region (800–1400 cm^{-1}) (Fig. 2). Result showed that the spectrum was similar to the commercial pectin IR spectra, as reported previously, where the peaks at 757, 830, 890, 917, 973, 1019, 1050, 1072, 1094, 1143, 1231, 1330, 1368, 1413 and 1440 cm^{-1} were found, which corresponding to C—H_3 deformation, C—O—C stretching, O—H bending, C—H deformation, O—C=O bending, O—C—O bending as well as C—O=O bending in the pectin chain [43]. In addition, a broad band of the OH stretching (2500–3600 cm^{-1}), which is due to the inter/intra-

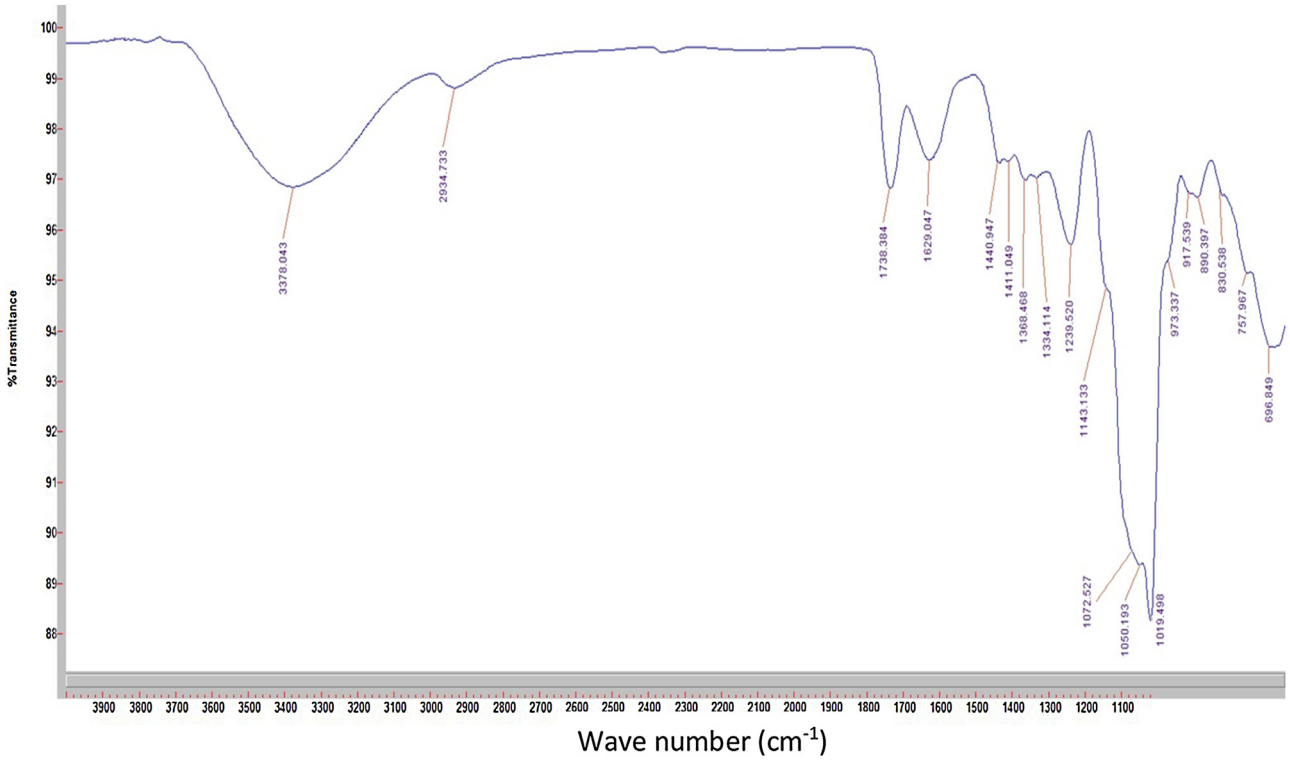

Fig. 2. FTIR spectra of PBPP.

Table 3
Number average molecular weight (M_n), weight average molecular weight (M_w), polydispersitty index (PDI), and intrinsic viscosity (IV) of PBPP.

Property	PBPP	Commercial pectin
M_n (kDa)	148.7	20.7
M_w (kDa)	270.6	55.9
PDI	1.82	2.70
IV (dm^3/g)	0.262	0.289

molecular hydrogen bonding of the galacturonic acid backbone and the presence of methyl ester group (2750–2950 cm^{-1}) were also found [44]. The peaks of 1730 and 1620 cm^{-1}, which represented the esterified and free carboxyl groups, respectively, were found and the ratio between these peaks represented the degree of esterification. By comparing the citrus peel pectin (~68%) and apple pomace pectin (~69%) [45,46], PBPP gave a lower DE value (59.9%). Hence, it could be anticipated that the functional properties would be different from those in the commercial markets.

3.5. Molecular weight and monosaccharide composition of PBPP

Table 3 shows that PBPP gave values for number average molecular weight (M_n), weight average molecular weight (M_w), polydispersity index (PDI), and intrinsic viscosity (IV) of 148.7 kDa, 270.6 kDa, 1.819, and 0.262 dm^3/g, respectively. These results indicated that PBPP had a higher degree of polymerization with a narrower molecular weight distribution compared to commercial pectin. It could also be found that PBPP had a lower IV, suggesting that this polysaccharide might have a more spherical conformation comparing to commercial citrus pectin which has a linear rod conformation [47]. This could be due to the presence of high amount of neutral sugars that acted as the branches, which attached to the PBPP backbone. The chromatogram in Fig. 3 shows that the PBPP composed of mannose, galacturonic acid, rhamnose, glucose, galactose, xylose and arabinose with molar ratio of 1.0: 6.1: 1.6: 3.6: 25.0: 1.2: 1.7 with trace amount of ribose and fucose. Their corresponding mole percentages were 2.5%: 15.0%: 4.0%: 9.0%: 62.2%: 2.9%: 4.3%, respectively. It was noted that galactose was the predominant monosaccharide in PBPP. Therefore, it was confirmed that PBPP was a highly branched heteropolysaccharide.

3.6. TPC, antioxidant activities and α-amylase inhibitory activity of PBPP

It was interesting to note that the extracted PBPP contained approximately 10× higher TPC compared to apple pomace (8.56 mg GAE/100 g) but ~50% less than citrus peel (169.6 mg GAE/100 g) [48,49]. Results also showed that PBPP exhibited relatively low DPPH free radical scavenging activity (7.7%/mg), however, high FRAP (2.0 mmol/dm^3 $FeSO_4$/mg) and high α-amylase inhibitory activity (97.6%/mg) were observed (Table 4). The IC_{50} for α-amylase inhibitory activity was ~0.46 mg. This finding revealed that PBPP exhibited high α-amylase inhibitory activity compared to a typical anti-hyperglycemic drug (i.e. acarbose). The antioxidative activities could be closely related to its phenolic components because they are able to effectively donate electrons to free radicals. Apart from that, it was recently reported that these components are also categorized as one of the largest group of the critical digestive enzyme inhibitors and they were widely used as functional foods [50]. This inhibition activity was due to the phenolic components could strongly interact with proteins and inhibit the α-amylase by forming complexes with the enzyme and by changing their conformations [51]. Further study showed that these small and low polar phenolic compounds could easily interact with the hydrophobic residues of the active sites of α-amylase, thus caused the inhibition of its activity [52]. However, the content of polyphenols in PBPP is relatively low (approximately 0.8 μg of TPC was used in the antioxidative activity determination and 0.08 μg of TPC in α-amylase inhibitory activity), therefore, it was strongly believed that the activities were mainly contributed by the polysaccharide. As

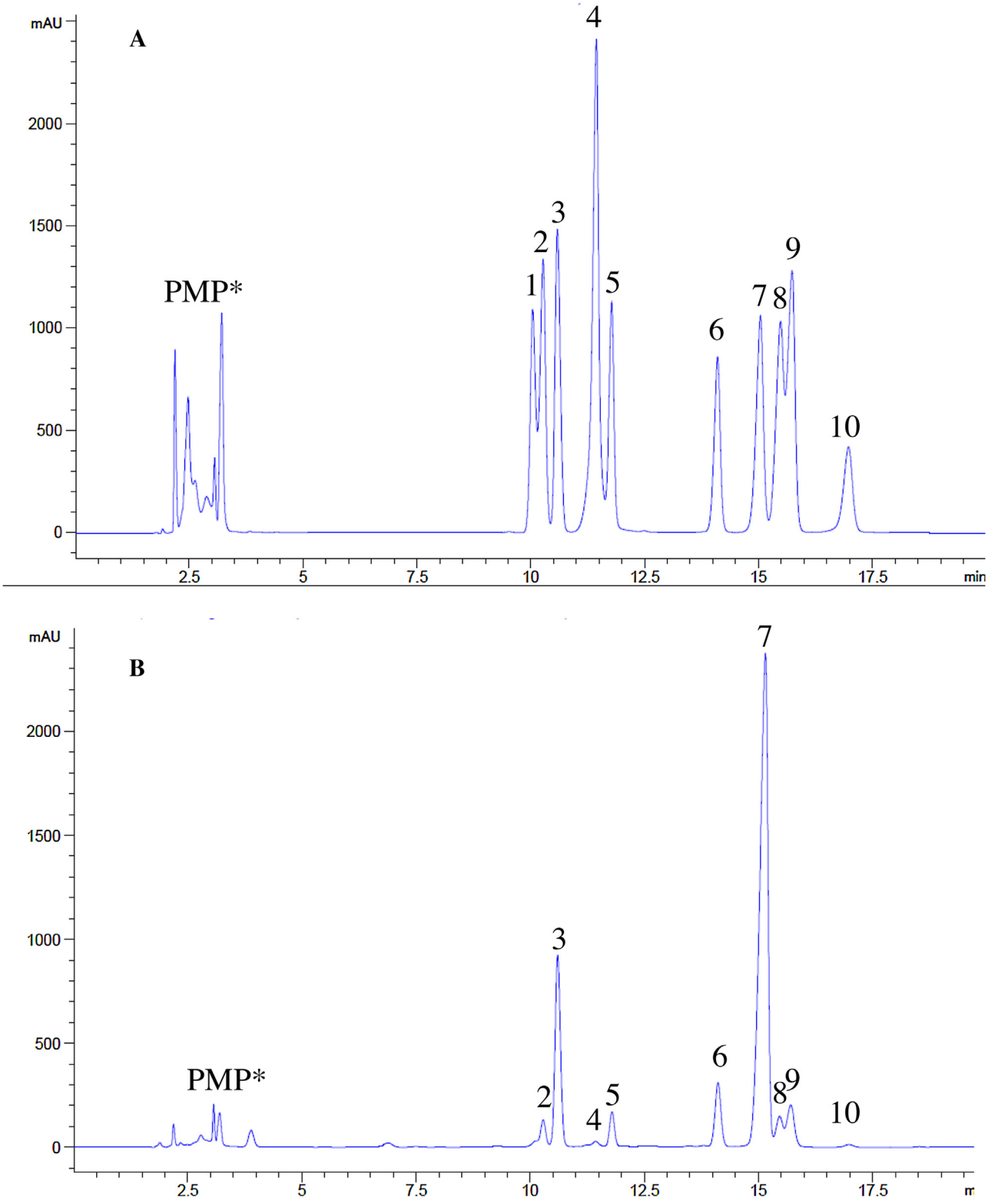

Fig. 3. Chromatogram of: (A) mixed standard composed of (1) glucoronic acid, (2) mannose, (3) galacturonic acid, (4) ribose, (5) rhamnose, (6) glucose, (7) galactose, (8) xylose, (9) arabinose and (10) fucose, and (B) PBPP. *PMP = 3-methyl-1-phenyl-2-pyrazoline-5-one.

aforementioned that PBPP is an acidic heteropolysaccharide, it was suggested that PBPP have the capability in donating electrons or hydrogen atoms, which was the mechanism of these attributes. It was also suggested that PBPP altered the pH condition of the α-amylase hydrolysis environment, thus the enzyme could be denatured and lose its activity. Hsu et al. [53] also reported that high percentage of galactose polysaccharides were linearly correlated with anti-hyperglycemic effect. Thus, it could be used as a promising natural antioxidant and as a α-amylase inhibitor.

Table 4
TPC, DPPH free radical scavenging activity, FRAP and α-amylase inhibitory activity of PBPP.

TPC (mg GAE/100 g)	DPPH free radical scavenging activity (%/mg)	FRAP (mmol/dm^3 $FeSO_4$/mg)	α-amylase inhibitory activity (%/mg)
80.6 ± 14.2	7.7 ± 0.6	2.0 ± 0.0	97.6 ± 6.7

Table 5
Functional characteristics of PBPP.

Functional properties		PBPP	Commercial pectin
WHC (g of water/g of extracts)		3.6 ± 0.9[a]	7.0 ± 0.6[b]
OHC (g of oil/g of extract)		2.8 ± 0.8[a]	2.3 ± 0.5[a]
Emulsifying activity (%)		39.6 ± 0.9[b]	5.4 ± 1.7[a]
Emulsion stability (%)		38.6 ± 0.3[a]	64.1 ± 3.3[b]
Viscosity (Pa.s)	pH 2	0.72 ± 0.31[a]	15.25 ± 1.12[b]
	pH 4.5	2.72 ± 0.06[a]	2.51 ± 0.26[a]
	pH 6.5	1.54 ± 0.06[b]	0.32 ± 0.17[a]

Note: The data were written as mean ± SD. (n = 3). Means within the same column not followed by the same letter are significantly different at $p < 0.01$ level of significance, according to *t*-test.

3.7. Functional properties

Table 5 shows that PBPP obtained a lower WHC (3.6 g/g) compared to commercial pectin (7.0 g/g). Galacturonic acid content in PBPP could be the cause of the low WHC [38]. It was reported that carboxyl groups in galacturonic acid were responsible to form hydrogen bonds with water molecules in order to bind them tightly. At such low amounts of galacturonic acid in PBPP (15%, mole percentage) may result in a lower WHC. This property is important in the food industry due to its ability to increase bulk volume, to inhibit food syneresis, as well as to modify food texture.

The result showed that OHC for both PBPP and commercial pectin were similar (2.3–2.8 g/g). Therefore, PBPP can be used to formulate food products, particularly high-fat products, which confer foods with mouth feel and greasy sensation. Although the methyl ester content, which was responsible for the hydrophobicity and surface porosity of pectin, was higher (~68%) in commercial pectin compared to PBPP (59.9%), it was suggested that the highly branched structure of PBPP contributed to the hydrophobicity property and the surface exposure to the oil molecules. Therefore, a similar OHC was observed.

In terms of emulsifying activity, PBPP apparently showed a higher activity (39.6%) compared to commercial pectin (5.4%). This could be due to the balance between the hydrophobicity and hydrophilicity properties of the polysaccharide. The branched structure (hydrophobic component) of PBPP could be adsorbed onto the oil surface, whereas the carboxylic group (hydrophilic component) could be solubilized in the aqueous phase. On the other hand, commercial pectin, which contains high amount of galaturonic acids, was more favourable in the aqueous phase. Therefore, PBPP could be considered as an active agent that has the capacity to stabilize the emulsion by reducing the interfacial tension between oil and water. However, the result showed that the stability of the PBPP emulsion was relatively low (38.6%), which indicated that the interactions between PBPP and oil or water were rather weak and they could be easily disrupted by heat. Once heat treatment was induced, the protective barrier at the interface was ruptured.

In terms of gelling capacity, both PBPP and commercial pectin showed pseudoplastic shear-thinning behaviour. According to Wang and Cui [54], the viscosity of a solution decreases at high shear rate, given that the number of chain entanglements reduces as the chain adjust themselves with the flow direction. Formation of new entanglements cannot counterbalance for those being disentangled, hence shear thinning flow behaviour was observed. Sato et al. [55] also reported that viscosity of polysaccharide was shear rate dependent and the existence of weak structures in pectin molecules can be easily destroyed by the low shear rate.

Apart from that, the viscosity of these samples were depending on the pH condition given. At pH 2, PBPP gave a low viscosity solution (0.72 Pa s) whereas commercial pectin gave a highly viscous solution (15.25 Pa s). However, when pH increased to pH 4.5, both samples showed similar viscosity (~2.5-2.7 Pa s). As the pH increased further (pH 6.5), commercial pectin solution gave a very low viscosity (0.32 Pa s) compared to PBPP (1.54 Pa s).

In general, the viscosity of PBPP solution was lower than commercial pectin. According to Thakur et al. [56], the gel formation of HM pectic-polysaccharide involves a combination of the hydrophobic interactions between the pectin molecules as well as hydrogen bonds between the free carboxyl groups on the pectin molecules.

Therefore, it could be explained that highly branched conformation of PBPP induced a steric hindrance, which interrupted the hydrophobic interactions between pectic chains, whereas the rod shape structure of commercial pectin encourage the hydrophobic bonds. PBPP was therefore depending on the hydrogen bonds formed between the molecules, which was pH dependent. At a mild acidic pH (i.e. pH 4.5), PBPP would tend to ionize and a substantial amount hydrogen bond (or an adequate charge density) was formed, which encouraged the gelation of PBPP. As the pH increased (pH 6.5), the repulsive effect of the negative charge between the molecules was apparent, thus reduced the viscosity of PBPP solution. From the results, it could be observed that gelling behaviour of PBPP primarily involved hydrogen bond compared to commercial pectin where hydrophobic interaction was more profound.

4. Conclusion

PBPP was successfully extracted from the Pinto bean pod. It is a highly branched heteropolysaccharide with a high degree of polymerization. It is composed of multiple monosaccharides, predominantly galactose, and FTIR analysis confirmed that the structure is similar to commercial pectin with a DE of 59.9%. PBPP exhibited substantial antioxidative and α-amylase inhibitory potentials with desired functional properties. It was therefore believed that PBPP could be used as a food additive commercially. To our knowledge, these data were first reported and would provide not only the rationale of utilization of the Pinto bean pod in general, but also a base study in the food and pharmaceutical industries.

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