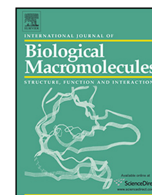
International Journal of Biological Macromolecules 88 (2016) 280–287

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac



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

ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form 29 February 2016
Accepted 1 April 2016
Available online 1 April 2016

Keywords:
α-Amylase inhibitor
Pod
Polysaccharide

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).
© 2016 Elsevier B.V. All rights reserved.

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).

http://dx.doi.org/10.1016/j.ijbiomac.2016.04.003
0141-8130/© 2016 Elsevier B.V. All rights reserved.

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;

Download English Version:

https://daneshyari.com/en/article/1986124

Download Persian Version:

https://daneshyari.com/article/1986124

Daneshyari.com