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Physicochemical properties and *in vitro* antioxidant activities of polysaccharide from *Artocarpus heterophyllus* Lam. pulp

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ABSTRACT

A water-soluble polysaccharide from *Artocarpus heterophyllus* Lam. (jackfruit) pulp (JFP-Ps) was purified and its physicochemical properties were investigated. The *in vitro* antioxidant activities of JFP-Ps was evaluated by measuring DPPH• and •OH radicals scavenging activities, as well as reducing power. The results showed that JFP-Ps contained 79.12% of total sugar, 5.83% of protein, 15.65% of uronic acid, and 15 kinds of amino acids with high levels of Asp, Glu, Val, Leu and Lys. JFP-Ps was mainly composed of Rha, Ara, Gal, Glc, Xyl and GalA, with an average molecular weight of 1668 kDa. FT-IR results showed the bands at the range of 1200–850 cm^{-1} suggested the presence of carbohydrates in JFP-Ps. The results of antioxidant activities showed that JFP-Ps exhibited strong DPPH• and •OH radical scavenging activities, with a relatively lower reducing power, suggesting that JFP-Ps can be exploited as effective natural antioxidant applications in medical and food industries.

1. Introduction

Artocarpus heterophyllus Lam. (jackfruit) is a kind of large tropical tree which belong to the family Moraceae (Baliga, Shivashankara, Haniadka, Dsouza, & Bhat, 2011). They are native to India and grown abundantly over several tropical and sub-tropical countries, especially in many parts of Southeast Asia (Saxena, Bawa, Raju, & Yahia, 2011), In China, jackfruit are widely cultivated in Hainan, Guangdong, Guangxi, Yunnan and Fujian Provinces. A previous study found that jackfruit contained lots of phytonutrients, for example, every 100 g of ripe fruit pulp contained 16.0–25.4 g carbohydrate, 1.2–1.9 g protein and 0.1–0.4 g fat (Swami, Thakor, Haldankar, & Kalse, 2012), indicating that jackfruit are good sources of these essential nutrients.

Current research is directed towards finding natural antioxidants of plant origin as nutraceuticals to enhance health benefits (Shanmugapriya, Saravana, Payal, Mohammed, & Binnie, 2011). Jackfruit has been used as a traditional folk medicine in South-East Asia, Indonesia, western part of Java and India (Jagtap & Bapat, 2010). Numerous studies found that jackfruit exerted many biological activities, including anti-bacterial, anti-diabetic, anti-inflammatory, antioxidant and anti-helmintics activities (Shanmugapriya et al., 2011). Biworo, Tanjung, Iskandar, Suhartono (2015) reported that jackfruit extract exhibited antioxidant and antidiabetic activities by decreasing the formation of reactive oxygen species (ROS). Ethyl acetate extracts of jackfruit fruits were found to inhibit lipopolysaccharide (LPS)-induced production of nitro oxide (NO), prostaglandin E2 (PGE2), and reactive oxygen species (ROS) in RAW264.7 cells, suggesting that fruits of jackfruit may be beneficial for the treatment or prevention of inflammation-mediated diseases (Fang, Hsu, & Yen, 2008).

Polysaccharides are widely distributed in plants and have recently received considerable attention because of their potential biological activities including anti-tumor (Jin, 2012), immune-regulation (Wang, Huang, Sun, & Pan, 2015), antioxidant activity (Samavati & Manoochehrizade, 2013), hypoglycemic effect (Zhu et al., 2013) and antibacterial effect (Nie, Zhang, Li, & Xie, 2013). Few studies focused on the polysaccharides of jackfruit pulp, there-

Abbreviations: DPPH, 11-Diphenyl-2-picrylhydrazyl; FT-IR, Fourier transform infrared spectroscopy; HP-GPC, high performance gel permeation chromatography; JFP-Ps, Polysaccharides isolated from jackfruit pulp; •OH, Hydroxyl radical.

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fore, this study aims to investigate the physicochemical properties and *in vitro* antioxidant activities of a water-soluble polysaccharide from *Artocarpus heterophyllus* Lam. (jackfruit) pulp (JFP-Ps).

2. Materials and methods

2.1. Materials and reagents

The fruits of *Artocarpus Heterophyllus* Lam. were collected from Xinglong Tropical Botanical Garden, Spice and Beverage Research Institute (Wanning, Hainan, China).

The standard monosaccharides including glucose (Glc), xylose (Xyl), arabinose (Ara), rhamnose (Rha), galactose (Gal), fructose (Fru), ribose (Rib), mannose (Man), fucose (Fuc), galacturonic acid (GalA) and glucuronic acid (GlcA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). An amino acid standard solution (AA-S-18) was purchased from Fluka Ltd. (Buchs, Switzerland). Dextrans of different molecular weights (T10, T40, T70 and T2000) were from Pharmacia Co. (Uppsala, Sweden). KBr used for FT-IR analysis was of high purity purchased from Merck Co. (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, ferrouschloride, ferrozine, 1,3-diaminopropane and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, USA). Other reagents used in this study were of analytic grade.

2.2. Extraction and purification of JFP-Ps

The extraction procedure of JFP-Ps was performed with the method by Chen, Xie, Nie, Li, Wang (2008) with some modifications. Briefly, jackfruit pulps were cut into small pieces and homogenized in a mechanical grinder, and extracted with 80% ethanol for 24 h at room temperature to remove the interfering components, including monosaccharide, disaccharide, oligosaccharide, volatile compounds and polyphenols. The residues were filtrated and dried with a vacuum freeze dryer, and then extracted with ultra-pure water (30:1, water to material ratio, mL/g) at 90 °C for 2.5 h. The aqueous extracts were concentrated in a rotary evaporator under reduced pressure at 55 °C and filtered. Then the aqueous solution of polysaccharides was precipitated with four volumes of 95% (v/v) ethanol at 4 °C for 24 h, and centrifuged at 3000 × *g* for 10 min.

Then, the precipitate was dissolved in water, then deproteined by Sevag method. Briefly, the polysaccharides solution and the Sevag reagent (chloroform:n-butanol = 4:1, v/v) were mixed (3:1, v/v) and shaken vigorously for 30 min at room temperature and centrifuged at 3000 × *g* for 10 min. Then, the water layers were collected and dialyzed against 100 vols of water for 72 h. The crude polysaccharide was then separated and sequentially purified through a Sephacryl™ S-400 h column (1.6 × 60 cm) eluted with 0.15 M NaCl at a flow rate of 0.64 mL/min. This fraction was combined and freeze-dried using a vacuum freeze dryer.

Table 1
Amino acid composition of JFP.

Peak	Amino acid	Retention time (min)	Area	Content (%)
1	Asparagic acid (Asp)	9.309	17935	0.47
2	Threonine (Thr)	11.384	9647	0.23
3	Serine (Ser)	12.200	13307	0.25
4	Glutamic acid (Glu)	13.979	19593	0.54
5	Proline (Pro)	15.757	4302	0.11
6	Alanine (Ala)	20.453	16417	0.30
7	Cysteine (Cys)	23.037	666	0.03
8	Valine (Val)	24.133	13831	0.30
9	Methionine (Met)	26.173	387	0.01
10	Isoleucine (Ile)	28.333	9369	0.24
11	Leucine (Leu)	29.424	16374	0.40
12	Tyrosine (Tyr)	31.755	4540	0.16
13	Phenylalanine (Phe)	32.920	7256	0.23
14	Histidine (His)	35.672	4414	0.13
15	Lysine (Lys)	39.013	13076	0.34
16	Arginine (Arg)	42.413	6987	0.24
Total concentration of the 16 general amino acids				3.98
Proportion of the essential amino acids				44.96

2.3. Measurements of total sugar, protein, uronic acid and total phenolic contents of JFP-Ps

The total sugar content of JFP-Ps was determined by the phenol-sulfuric acid method described according to Dubois, Gilles, Hamilton, Rebers, Smith (1956) with D-glucose as a standard. Protein content was quantified according to the method by Bradford (1976), and the linear concentration range was 20–200 μg/mL of protein by using BSA as a standard. Uronic acid content was measured using the m-hydroxybiphenyl colourimetric procedure, with D-glucuronic acid as a standard (Blumenkrantz & Asboe-Hansen, 1973). The polyphenol concentration was determined by Folin-Ciocalteu colorimetry.

2.4. Ultraviolet–visible (UV) absorption spectra and amino acid compositions analysis

JFP-Ps was dissolved in distilled water at a concentration of 1 mg/mL, then UV–vis spectra was obtained on an Analytik Jena SPECORD 250 (Analytik Jena, Jena, Germany) at 25 °C in the range of 200–900 nm.

The amino acid compositions of JFP-Ps were analyzed using a Sycom S-433D automatic amino acid analyzer (Sykam, Eresing, Germany), after acid digestion (6 mol/L HCl) under vacuum in sealed glass tubes at 110 °C for 22 h. Amino acids were quantified by comparing retention times and peak areas with those of the standard curves.

2.5. Determination of homogeneity and molecular weight

High performance gel permeation chromatography (HP-GPC) is an analytical method used for measuring molecular weight of samples, and dextrans as a standard is commonly used for determining homogeneity and molecular weight of polysaccharides (Xie et al., 2014). The homogeneity and molecular weight of JFP-Ps were determined on a Waters HP-GPC system equipped with a Waters 2410 Refractive Index Detector (RI), and an Ultrahydrogel™ Linear column (7.8 × 300 mm). The column was then eluted with 0.1 mol/L NaCl at a flow rate of 0.6 mL/min, and maintained at a temperature of 35 °C. The acquisition time was 40 min. The preliminary calibration was pre-calibrated using T-series Dextran standards (Dextran T10, T40, T70, T2000 and Glc). Empower software was used to calculate average molecular weight.

2.6. Analysis of monosaccharide composition

The monosaccharide compositions of JFP-Ps were determined by high-performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) according to the method by Xie et al. (2013). Briefly, the JFP-Ps was hydrolyzed with 2.0 mol/L H_2SO_4 at 110 °C for 8 h. After neutralizing to pH 6.0 with $BaCO_3$, the supernatants were collected by filtration and centrifugation at 4000 x *g* for 10 min. The supernatants (25 μL) were analyzed on a Dionex ICS-2500 system, coupled with PAD, and equipped with a Carbo PAC™ PA10 column (2.0 mm × 250 mm). Various concentrations of NaOH and CH_3COONa were added, and the retention times of monosaccharide standards (Glc, Xyl, Ara, Rha, Gal, Fru, Rib, Man, Fuc, GalA and GlcA) were used to determine the monosaccharide compositions of JFP-Ps.