



Physicochemical, functional, and biological properties of water-soluble polysaccharides from *Rosa roxburghii* Tratt fruit

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ABSTRACT

Water-soluble polysaccharides (RTPF) were extracted from *Rosa roxburghii* Tratt fruit by hot water method. The physicochemical, functional, and hypoglycemic properties of RTPF were investigated. The results revealed that RTPF mainly contained carbohydrates ($63.79 \pm 0.73\%$, g/g), uronic acids ($14.8 \pm 0.06\%$, g/g), and proteins ($4.10 \pm 0.58\%$, g/g). RTPF was composed of arabinose, galactose, glucose, mannose, xylose, and fucose with molar percentages of 33.8, 37.3, 20.7, 1.74, 3.43, and 2.95%, respectively. Functional analyses indicated that RTPF had good oil-holding capacity, foaming properties, and emulsifying capacity. The rheological results showed that RTPF exhibited typical shear-thinning behavior and viscoelastic properties influenced by sample concentration, temperature and inorganic salt. Additionally, RTPF exhibited favorable inhibitory activities against α -glucosidase in a mixed inhibition type, and against α -amylase in an uncompetitive inhibition type. These results suggest that RTPF can be exploited as a multi-functional additive or hypoglycemic agent in foods, pharmaceuticals and cosmetics.

1. Introduction

Natural polysaccharides have attracted great attention world-wide due to their multi-functional bioactivities including antioxidant, immunomodulatory, hypoglycemic and prebiotic activities in recent decades (Srichamroen, Thomson, Field, & Basu, 2009; Wang et al., 2018). Besides, some plant polysaccharides are drawing increasing interest due to its ideal functional properties, such as emulsifying, thickening, foaming, and gelling properties (Gao et al., 2015; Xu, Shi et al., 2016). The strong hydrophilic character and favorable rheological property of these polysaccharides are the main reasons for their increasing application as texture modifiers, thickeners, gelling agents, and emulsifiers in food and biomedical industries (Bayar, Kriaa, & Kammoun, 2016; Tabarsa, Anvari, Joyner, Behnam, & Tabarsa, 2017; McClements, Bai, & Chung, 2017). However, the functional properties of polysaccharides are closely related to their monosaccharide compositions, molecular weight, chain-chain interaction and conformation of the glycosidic bonds (Funami et al., 2011). Therefore, investigation on the physicochemical characteristics and functional properties of polysaccharides could help direct potential applications in foods and pharmaceuticals.

Rosa roxburghii Tratt (*R. roxburghii*), belonging to the *Rosaceae* family, has been widely used as an edible and medicinal resource in Asian

countries (He et al., 2016). Its juice is consumed as a delicious beverage and herbal tea in folk and claimed to have functions of tonifying spleen, curing diarrhea, and clearing summerheat. *R. roxburghii* fruit have been found to exert antioxidant, antimutagenic, antiatherogenic, antitumor, and radioprotective activities (He et al., 2016; Xu, Cai et al., 2016). There are various functional ingredients in *R. roxburghii* fruit, such as flavonoids, organic acids, triterpenes, polysaccharides and etc. To date, plenty of studies have been focused on the flavonoids and organic acids of *R. roxburghii* fruit (Xu, Cai et al., 2016; Xu et al., 2017). To the best of our knowledge, only a few literatures have reported that RTPF exhibited anti-tumor and anti-oxidation activities (Chen & Kan, 2018; Chen et al., 2014). RTPF is a good source of water-soluble gel, but little information was available on the functional and hypoglycemic properties of RTPF.

Therefore, the aim of the present study was to extract and investigate the physicochemical characteristics and functional properties of RTPF. Moreover, the hypoglycemic activities *in vitro* of RTPF were evaluated by the assay of α -amylase and α -glucosidase inhibitory activities. These results would help guide potential application of this natural polysaccharides as a functional additive or pharmaceutical supplement.

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2. Materials and methods

2.1. Materials and reagents

R. roxburghii fruit were provided by Guizhou Lvyuan Food Co. Ltd (Guizhou Province, China). The fruit were washed, de-seeded, and dried in a drying oven at 55 °C for 48 h. Dried fruits were then ground using a FW135 grinder (Taisite, Tianjin, China) and sieved through a 60-mesh sieve to obtain the powder. Dextran standards, bovine serum albumin (BSA), p-nitrophenyl- α -D-glucopyranoside (pPNG), α -amylase and α -glucosidase were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Standards of monosaccharides including arabinose, glucose, galactose, xylose, mannose, rhamnose, galacturonic acid, and glucuronic acid were purchased from Aladdin Chemistry Co. (Shanghai, China).

2.2. Preparation of RTFP

The polysaccharides were extracted from *R. roxburghii* fruit according to the previous method (Chen et al., 2016). The dried powder of *R. roxburghii* fruit was refluxed with ethanol (95% v/v) twice at 70 °C for 3 h to remove liposoluble compounds and impurities. The residue was separated from the ethanol by vacuum filtration and dried in an air drying oven at 50 °C for 24 h. The dried residue was extracted in a designed extraction temperature (55–95 °C), extraction time (1.5–3.5 h) and water/material ratio (15–35 mL/g) by single-factor experimental design. The optimal extraction conditions were as follows: extraction temperature 95 °C, extraction time 3 h, and water/material ratio 30:1 (mL/g). The mixture was centrifuged using at 5000g for 10 min to obtain the supernatant. After extraction twice, the supernatant was concentrated to 1/3 its original volume using a rotary evaporator under vacuum at 55 °C (Yarong RE-3000A, Shanghai, China). The concentrated solution was deproteinized with Sevag method (chloroform:butyl alcohol = 4:1) and then decolorized using AB-8 macroporous resin. The solution was adjusted to a final concentration of 80% (v/v) by adding dehydrated ethanol and kept overnight at 4 °C. The precipitate was separated by centrifugation at 5000g for 10 min and lyophilized to yield the polysaccharides (RTFP). Three replicate samples were carried out in parallel. The extraction yield of polysaccharides was calculated by the following formula:

$$\text{Extraction yield (\%)} = (W_1/W_0) \times 100 \quad (1)$$

where W_1 and W_0 are the weights of crude polysaccharides and dried powder of RTFP, respectively.

2.3. Chemical composition and monosaccharide composition analysis

The content of total carbohydrates was measured by the method of phenol-sulfuric acid. Protein content was measured by the Comassie Brilliant Blue G-250 method using BSA as the standard. Moisture content was determined by the AOAC method.

The monosaccharide composition and uronic acids of RTFP were measured according to our previous method (Chen et al., 2016). Briefly, RTFP was hydrolyzed with trifluoroacetic acid (2 M) at 105 °C for 6 h in a sealed tube. The residue was re-dissolved in deionized water and filtered through 0.22 μ m microporous filtering film for measurement, which was performed on an ion chromatography instrument (ICS 3000, Dionex Corp. Sunnyvale, CA, USA). The monosaccharide content was calculated according to the calibration curve (peak area-concentration) of each monosaccharide standard.

2.4. Determination of molecular weight

The molecular weight distribution of RTFP was measured by high-performance gel permeation chromatography (HPGPC) instrument (Agilent Co., USA) according to our previous method (Chen et al. 2016).

2.5. Infrared (IR) spectrometry

The dried RTFP was evenly mixed with KBr powder, ground and then pressed into a 1 mm pellet. Then the pellets were determined using a Vector 33 FT-IR spectrophotometer (Bruker, Ettlingen, Germany). The IR spectrum was recorded in the wavelength range of 400–4000 cm^{-1} .

2.6. Thermal properties

The thermal properties of RTFP were evaluated using a TGA/DSC simultaneous thermal analyzer (Netzsch, Sta449 F3, Germany) in the temperature range of 25–600 °C, at a heating rate of 10 °C/min, under N_2 atmosphere. The sample (5–10 mg) was weighed and placed in the equipment alumina crucible, with an empty aluminum pan used as the reference.

2.7. Scanning electron microscopy (SEM)

The powder samples were placed on a specimen holder and then sputtered with gold under reduced pressure. The SEM images were captured by a SEM system (Helios Nanolab G3, FEI, USA) at a 15 kV acceleration voltage.

2.8. Functional properties

2.8.1. Water and oil holding capacity

Water-holding capacity (WHC) and oil holding capacity (OHC) were measured according to a partially modified method of Jeddou et al. (2016). For WHC, 30 mL of RTFP solution (1%, w/v) was placed in centrifuge tubes and weighed. The solution was stirred using a magnetic stirrer every 5 min, and held for 30 min followed by centrifugation at 3000g for 25 min. Then, the supernatant was removed and excess water was drained at 50 °C for 25 min. The experiment was conducted in triplicate. The WHC was calculated as follows:

$$\text{WHC (g/g)} = \frac{\text{Water absorbed weight (g)}}{\text{Sample weight (g)}} \quad (2)$$

For OHC, 0.5 g of RTFP was mixed with 10 mL soybean oil (Yihai Kerry Group, Singapore). The mixture was stirred using a magnetic stirrer for 1 min. After a holding period of 30 min, the tube was centrifuged at 3000g for 25 min. The upper phase was removed and the tube was drained for 30 min on a filter paper prior to reweighing. The experiment was conducted in triplicate. The OHC was calculated as follows:

$$\text{OHC (g/g)} = \frac{\text{Oil absorbed weight (g)}}{\text{Sample weight (g)}} \quad (3)$$

2.8.2. Foaming property

Foaming capacity (FC) and foaming stability (FS) were measured according to the method of Rezaei, Nasirpour, and Tavanai (2016). A series of RTFP solutions at different concentrations (0.5, 1, 2, 4, and 6%; w/v) were homogenized at 10,000 rpm for 1 min using a high-speed shear homogenizer (T25, IKA Co., Germany) at room temperature. The foaming volume was measured at $t = 0$ s to determine the FC and after 30 min to evaluate the FS. FC was expressed as the percentage of volume increase after homogenization at 0 min, and FS was expressed as the percentage of foam volume after 30 min. The FC and FS were calculated as follows:

$$\text{FC (\%)} = \frac{\text{Initial foam volume}}{\text{Total suspension volume}} \times 100 \quad (4)$$

$$\text{FS (\%)} = \frac{\text{Final foam volume}}{\text{Total suspension volume}} \times 100 \quad (5)$$

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