Food Chemistry 251 (2018) 33–40

Contents lists available at ScienceDirect

Food Chemistry

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

Structural properties and prebiotic activities of fractionated lotus seed resistant starches

Hongliang Zeng[a,b,c], Peilin Chen[a], Chuanjie Chen[a], Cancan Huang[a], Shan Lin[a], Baodong Zheng[a,b,c,*], Yi Zhang[a,b,c,*]

[a] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China
[b] Fujian Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[c] China-Ireland International Cooperation Centre for Food Material Science and Structure Design, Fujian Agriculture and Forestry University, Fuzhou 350002, China

ARTICLE INFO

Keywords:
Fractionation
Lotus seed resistant starch
Structural properties
Prebiotic activities
Bifidobacterium adolescentis
Lactobacillus acidophilus

ABSTRACT

The objective of this study was to fractionate lotus seed resistant starch (LRS3) and investigate their structural properties and prebiotic activities. Two main fractions of resistant starch precipitated gradually by ethanol at concentrations of 20% and 30% were named as LRS3-20% and LRS3-30%, respectively. The swelling power and solubility of LRS3-20% were smaller compared to LRS3-30%, and their moisture and resistant starch contents were not significantly different. LRS3-20% and LRS3-30% had molecular weights mainly of 2.0×10^4–4.0×10^4 and 1.0×10^4–2.0×10^4 g/mol. Layered strips and gully shapes were evident on the rough surfaces of LRS3-20%, while LRS3-30% displayed a relatively smooth surface. Both LRS3-20% and LRS3-30% had a B-type crystalline structure with LRS3-20% containing more ordered structures and double-helices. Furthermore, LRS3-20% displayed higher prebiotic activities against *Bifidobacterium adolescentis* and *Lactobacillus acidophilus* compared to LRS3-30% and high amylose maize starch. This effect was related to its rough surface and double helix structure.

1. Introduction

Resistant starch cannot be digested and absorbed in the human small intestine but can be fermented or partially fermented by the gut microbiota in the large intestine (Sonnenburg & Backhed, 2016). Resistant starch type 3 (RS3) has been known as retrograded or crystalline starch formed during the cooling of gelatinized starch. Resistant starch often has molecular weights distributed over a wide range (Perera, Meda, & Tyler, 2010). Its broad molecular-weight range restricts its application; resistant starch with different molecular weights often has different physicochemical, structural, and functional properties (Hu, Liu, Jin, & Tian, 2015; Zeng et al., 2015). At present, size exclusion chromatography, ultrafiltration, and antisolvent precipitation are the primary methods for the fractionation of carbohydrate polymers. Chromatography and ultrafiltration are costly, and the former is not suitable for producing fractions on a large scale (Chen, Liu, Xiao, Huang, & Liu, 2016). Antisolvent precipitation has an advantage in terms of economy, and it can be used for preparing a large amount of polymers with a narrow molecular-weight distribution. In this method, the starch can be dissolved in dimethyl sulfoxide (DMSO) solution or partially dissolved in NaOH solution, and then the amylose can be separated from amylopectin by precipitation using organic solvents such as butanol and ethanol (Gelders, Bijnens, Loosveld, Vidts, & Delcour, 2003; Krishnaswamy & Sreenivasan, 1948). Thus, the following hypothesis can be raised that resistant-starch fractions of different sizes may be separated using an alkali–ethanol system.

Lotus seeds are the mature seeds from the genus *Nelumbo*, which is an important commercial crop in China (Zheng, Zhang, & Zeng, 2016). A high content of amylose (about 40%, w/w) in lotus seed is conducive to the formation of lotus seed resistant starch type 3 (LRS3) (Zheng et al., 2016). This resistant starch is composed of several fractions with a molecular weight range of 7.0×10^3–3.0×10^6 g/mol (Wu, 2015). LRS3 has a B-type crystal structure, as well as a rougher surface, a greater degree of molecular order, and a higher content of double-helix structure compared with native starch and high amylose maize starch (HAMS) (Zhang, Zeng, Wang, Zeng, & Zheng, 2014). Furthermore, LRS3 can promote the proliferation of *Bifidobacterium adolescentis*, which is affected by its rough surface and its short-chain fatty acids produced during fermentation (Zhang, Wang, Zheng, Lu, & Zhuang, 2013). Different molecular-weight prebiotics usually displayed different effects on gut microbiota or intestine health, such as inulin. Long-chain inulin but not short-chain inulin delayed the development of type 1 diabetes via

* Corresponding authors at: College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China.
E-mail addresses: zbdfst@fafu.edu.cn (B. Zheng), zyifst@fafu.edu.cn (Y. Zhang).

https://doi.org/10.1016/j.foodchem.2018.01.057
Received 17 August 2017; Received in revised form 3 January 2018; Accepted 6 January 2018
Available online 06 January 2018

modulation of gut-pancreatic immunity, barrier function, and microbiota homeostasis (Chen et al., 2017). However, little work has been on the prebiotic activities of different molecular-weight fractions of resistant starch, as well as the relationship between their structural properties and prebiotic activities.

The aim of this study, therefore, was to establish a method for separating differently sized fractions of lotus seed resistant starch using an alkali–ethanol system and investigate the proliferation of different molecular-weight resistant starch on *Bifidobacterium adolescentis* (*B. adolescentis*) and *Lactobacillus acidophilus* (*L. acidophilus*). The structural properties of the fractionated resistant starch were characterized and compared using high-performance size-exclusion chromatography connected with multi-angle laser light-scattering and refractive index (HPSEC-MALLS-RI) system, environmental scanning electron microscopy (ESEM), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, and ^{13}C cross-polarization and magic angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR) spectroscopy. Additionally, their prebiotic activities on *B. adolescentis* and *L. acidophilus in vitro* were investigated.

2. Materials and methods

2.1. Materials

Fresh lotus seed was provided by Green Field Food Co., Ltd. (Sanming, China). Lotus seed starch was prepared according to a published method (Zhang et al., 2014). Briefly, fresh lotus seeds with twice mass of distilled water were homogenized in a fruitpulper (MJ-60BM01A, Midea Group Co., Ltd., Guangzhou, China). After filtering, static settlement, washing twice with distilled water, settling twice and drying at 60 °C for 12 h, the lotus seed starch was obtained and stored in a vacuum desiccator (9.57 ± 0.42% of moisture content). DMSO was purchased from Shanghai Aladdin Bio-chem Technology (Shanghai, China). Other reagents used were of analytical grade; they were obtained from Sinopharm Group Chemical Reagent Co. (Shanghai, China). *B. adolescentis* was purchased from Livzon Pharmaceutical Group Inc. (Guangzhou, China). *L. acidophilus* was provided by the China Center of Industrial Culture Collection (Beijing, China).

2.2. Fractionation and purification of lotus seed resistant starch

Lotus seed starch (30 g dry weight) was suspended in 1 L of distilled water in a 2 L conical flask. The starch suspensions were heated at 121 °C for 15 min in an autoclave (SYQ-DSX-280B; Shenan Medical Devices, Shanghai, China), cooled to room temperature using cooling fan of the autoclave, and subsequently stored at 4 °C for 24 h in a fridge (BCD-236WM, Midea Group Co., Ltd., Guangzhou, China). The gelatinous suspension was evenly dispersed in 0.2 mol/L of NaOH solution (pH 9.0) by shaking at a constant temperature of 30 °C for 3 min and then passed through a 100-mesh sieve. The obtained suspension was sequentially fractionated at 30 °C for 8 h by graded precipitation at ethanol concentrations of 20%, 30%, 40%, 50%, and 60% (v/v), respectively. The precipitates were purified using α-amylase and glucoamylase (Zhang et al., 2014). Lotus seed resistant starch fractions of different sizes were thus obtained by drying at 60 °C for 12 h in a drying oven (DJG-9053A, Yiheng Instrument, Shanghai, China), grinding, and passing through a 100-mesh sieve. The yields of the different precipitates and LRS3 were calculated from equations (1) and (2), respectively.

$$\text{Yield of precipitate (\%, W/W)} = \frac{\text{Weight of dried precipitate}}{\text{Weight of lotus seed starch}} \times 100 \quad (1)$$

$$\text{Yield of LRS3 (\%, W/W)} = \frac{\text{Weight of purified LRS3}}{\text{Weight of dried precipitate}} \times 100 \quad (2)$$

2.3. Physicochemical properties

The moisture content of lotus seed resistant starch fractions was determined according to the Association of Official Analytical Chemists (AOAC) 920.151 methodologies. The content of resistant starch in the samples was determined using a Megazyme resistant starch assay kit (Megazyme International, Wicklow, Ireland), which was based on AOAC methods (Perera et al., 2010). Resistant starch was calculated as the amount of glucose × 0.9. Each sample was analyzed in triplicate.

The swelling power and solubility of the samples were analyzed using the method described by Zeng et al. (2015) with a slight modification. Briefly, 0.2 g of sample was dispersed in 20 mL of distilled water to form a suspension. The starch suspension was heated to 85 °C for 30 min in a water bath with vigorous shaking every 5 min. After centrifuging at 1500*g* for 30 min, the supernatant was dried and weighed to measure the amount of dissolved starch and the sediment was used to calculate the swelling power. The swelling power and solubility were calculated as follows:

$$\text{Swelling power (g/g)} = \frac{\text{Weight of sediment}}{\text{Weight of dry starch} - \text{Weight of dissolved starch}} \quad (3)$$

$$\text{Solubility (\%)} = \frac{\text{Weight of dissolved starch}}{\text{Weight of dry starch}} \quad (4)$$

2.4. HPSEC-MALLS-RI analysis

The molecular and structural characteristics as well as molar mass distributions of the fractions were analyzed using an HPSEC-MALLS-RI system through a reported method with slight modifications (Pérez-Quirce, Lazaridou, Biliaderis, & Ronda, 2017). A Waters HPSEC system was equipped with a Waters model 12-6 pump (515 HPLC; Waters, Milford, MA, USA) and an injector with a 1 mL loop (Waters). The loop was adjacent to an 18-angle MALLS detector (Dawn-Heleos II; Wyatt Technology, Santa Barbara, CA, USA), which had a laser wavelength of 664.1 nm, and a refractive index detector (Shodex RI-101; Shodex, Tokyo, Japan). DMSO solution with 50 mmol/L LiBr was the mobile phase, and the flow rate was 0.3 mL/min. The dried sample was dissolved in the mobile phase and heated at 90 °C in a water bath for 2 h, and then stirred at 25 °C for 24 h. The solutions were centrifuged at 13,500*g* and 20 °C for 20 min and then filtered through a 0.45 μm PTFE filter film before injection. OHpak SB-G, SB-805M HQ, and SB-803M HQ columns (Shodex) connected in series were maintained at 50 °C, and the RI detector was maintained at 35 °C. The data obtained by SEC-MALLS-RI were analyzed by ASTRA 6.1 software (Wyatt Technology, Santa Barbara, CA, USA).

2.5. ESEM examination

The microstructural morphology of the fractions was examined by ESEM (Philips-XL30 ESEM; Philips-FEI, Amsterdam, The Netherlands). The dried samples were deposited on a copper stub using a double-sided adhesive tape and then coated with gold using a sputter coater (Cressington Scientific Instruments, Watford, UK) to produce a conductive sample. Images were taken at an accelerating voltage of 15 keV. Micrographs were recorded at 200× and 5000× magnifications.

2.6. XRD measurement

XRD measurements of the fractions were performed using an X'Pert Pro MPD diffractometer (Philips, Amsterdam, The Netherlands) equipped with a θ-θ goniometer. The diffractometer uses Cu Kα radiation (λ = 0.25 nm) produced in a sealed tube at 40 kV and 30 mA. The diffractograms were collected over a 2θ range of 5°–45°, a step interval of 0.0128916°, and a scanning rate of 0.02°/min for continuous