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Structural characteristics and crystalline properties of lotus seed resistant starch and its prebiotic effects

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ABSTRACT

Lotus seed resistant starch (LRS) is a type of retrograded starch that is commonly known as resistant starch type 3 (RS3). The structural and crystalline properties of unpurified LRS (NP-LRS3), enzyme purified LRS after drying (GP-LRS3), and enzyme purified LRS (ZP-LRS3) were characterized. The result showed that the molecular weights of NP-LRS3, GP-LRS3, and ZP-LRS3 were 0.102×10^6, 0.014×10^6, and 0.025×10^6 Da, respectively. Compared with native starch and high amylose maize starch (HAMS), LRS lacked the polarization cross and the irregularly shaped LRS granules had a rougher surface, B-type crystal structure, and greater level of molecular order. The FT-IR measurements indicated no differences in the chemical groups. Analysis by ^{13}C NMR indicated an increased propensity for double helix formation and higher crystallinity in LRS than in the two other types of starch. Moreover, LRS was more effective than either glucose or HAMS in promoting the proliferation of bifidobacteria.

1. Introduction

Lotus seeds are obtained from members of the genus *Nelumbo*, especially *Nelumbo nucifera*, which is an important specialty crop that has been grown in China for thousands of years, and is currently cultivated mainly in Fujian, Hebei, Hubei, Jiangsu, Hunan, Zhejiang, Jiangxi, and Taiwan (Wu et al., 2007). The content of starch in lotus seeds is approximately 500 g/kg (dry basis) with a high amount of amylose (~40%, w/w)(Yuan-yuan, Bao-dong, Shao-xiao, Fan, & Shu-zheng, 2011), which may contribute to the formation of resistant starch type 3 (RS3).

Resistant starch (RS) consists of starch and products of starch digestion that are poorly absorbed by the small intestine (Escarpa, Gonzalez, Morales, & Saura-Calixto, 1997) and are completely or partially fermented in the colon (Wang, Jin, & Yuan, 2007). The RS3 variant is primarily formed by hydrogen bonding between retrograde amylose molecules. The destruction of the crystal structure and dissolution of amylose during the gelatinization and retro-gradation process causes migration of the starch molecular chain and binding of its terminal region to form a double helical structure. Together with these changes, extension, curling, and folding of the original disordered free starch enables hydroxyl groups on the molecular chains to form hydrogen bonds easily. A crystalline structure is formed by ordering a number of different double helices over a particular region or by folding starch chains (Eerlingen & Delcour, 1995). Some potential physiological effects of resistant starch are the prevention of gastrointestinal diseases; reduction of blood glucose levels, the insulin response, and levels of serum cholesterol; prevention of cardiovascular disease; modification of colonic micro-flora; promotion of bacterial growth; and promotion of mineral absorption (Brouns, Kettlitz, & Arrigoni, 2002). The prebiotic properties of RS3 have been studied recently. The results have shown that RS3 increases the growth of bifidobacteria in the gastrointestinal tracts of rats (Rodríguez-Cabezas et al., 2010). In addition, in vitro studies have reported that high amylose maize starch (HAMS) enhances the survival of bifidobacteria in acidic buffer or bile acid solutions as a result of a bacterial adhesive effect (Barczynska, Slizewska, JoChym, Kapusniak, & Libudzisz, 2012). No studies have been conducted on the effect of LRS3 on bifidobacteria proliferation.

The present study characterized the structural and crystalline properties of lotus seed RS3 by using size exclusion chromatography (SEC), refractive index (RI) analysis, multi-angle laser light scattering (MALLS), polarized optical microscopy (POM), scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, and solid state ^{13}C nuclear

Abbreviations: POM, polarized optical microscopy; SEM, scanning electron microscopy; XRD, X-ray diffraction; FT-IR, Fourier transform infrared spectroscopy; ^{13}C NMR, solid state ^{13}C nuclear magnetic resonance spectroscopy; HAMS, high amylose maize starch; RS, resistant starch; RS3, resistant starch type 3.

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magnetic resonance (NMR) spectroscopy. The particle characteristics of lotus seed RS3 were compared with native starch and high amylose maize starch (HAMS), which is a typical resistant type-2 starch. In addition, the effects of LRS on the proliferation of bifidobacteria were assessed and compared with those of glucose (GLU) and HAMS by assessing the changes in optical density (OD). This investigation has provided a theoretical foundation for understanding the structural changes that occur during the preparation of LRS, and provides preliminarily insight into the relationship between the structure and function of LRS.

2. Materials and methods

2.1. Preparation of lotus seed native starch

Approximately 2.5 kg of fresh lotus seeds (Green acres (Fujian) food Co., Ltd, Fujian, China) were homogenized in a fruitpulper (MJ-60BM01A, Guangdong Midea Electric Manufacturing Co., Ltd, Guangdong, China) with twice mass as much water. The filtrate was passed through 149-μm mesh screen and left to settle for 6 h, and the supernatant was discarded. The precipitate was washed with distilled water, and then put aside for 4 h. The supernatant was then discarded and the washed precipitate was placed in an oven (DJG-9053A, Shanghai Yiheng Technology Co., Ltd, Shanghai, China) for 13 h at 45 °C to obtain the lotus seed native starch (Zeng, 2007). The moisture of obtained lotus seed native starch was 11.8% determined by using a halogen rapid moisture tester (SFY-6, Shenzhen Electronic Technology Co., Ltd, Shenzhen, China). The dried lotus seed native starch was then extracted using a grinder (FW135, Tianjing Taisite Instrument Co., Ltd, Tianjing, China), and passed through a 149-μm mesh screen.

2.2. Preparation of lotus seed resistant starch

For NP-LRS3, 250 g (dry weight) of native lotus seed starch was suspended in 1 L of distilled water in a 2-L conical flask. The starch suspensions were heated at 121 °C for 10 min in an autoclave (SYQ-DSX-280B, SHENAN Medical Devices, Shanghai, China), cooled to room temperature, and stored at 4 °C for 24 h. The gelatinous suspensions were subsequently dried at 45 °C in a drying oven (DJG-9053A, YIHENG Instrument, Shanghai, China), ground, and passed through a 185-μm mesh screen. The resulting product was NP-LRS3 (Onyango, Bley, Jacob, Henle, & Rohm, 2006; Zhang & Jin, 2011).

For GP-LRS3, 250 g (dry basis) of LRS3 was suspended in 500 ml of citric acid buffer (pH 6.0) in a 1-L conical flask. The suspensions were hydrolysed by the addition of thermostable α-amylase (10,000 U/ml, obtained from ANKOM, New York, USA) at 95 °C for 1 h in an orbital incubator-shaker (SHA-C, Guo Hua Electric Applicance, Changzhou, China) at 128 rpm. After adjusting the pH to 4.5 with a citric acid solution (4 mol/L), glucoamylase (300 U/ml, obtained from Sigma, St. Louis, USA) was added (5000 U/g of starch) and incubated at 60 °C for 1 h in an orbital incubator-shaker at 128 rpm. The suspensions were centrifuged (L-530, Xiang Yi Laboratory Instrument Development Co., Ltd, Hunan, China) at 2850g for 10 min. The resulting precipitates or residues were washed 3 times with distilled water and ethanol solutions of different concentrations (75%, 85%, and 95%; all determined on a v/v basis). The residues were dried at 45 °C, ground, and passed through a 185-μm mesh screen. The resulting product was GP-LRS3. The GP-LRS3 content in LRS3 was determined as the ratio of the weight of GP-LRS3 (dry basis) relative to the weight of LRS3 (dry basis).

For ZP-LRS3, 250 g (dry weight) of native lotus seed starch was suspended in 1 L of distilled water in a 2-L conical flask. The starch suspensions were heated at 121 °C for 10 min in an autoclave, cooled to room temperature, and stored at 4 °C for 24 h. The gelatinous suspensions were subsequently stirred well and hydrolysed by incubation in the presence of thermostable α-amylase at 95 °C for 2 h in an orbital incubator-shaker. The processing method was the same as that used to prepare GP-LRS3.

2.3. Determination of M_w and M_w/M_n

A 20-mg sample of starch was dispersed in 5 ml of 50 mM LiBr in 90% DMSO (HPLC grade, Sigma Chemical Co., St. Louis, MO) at 90 °C for 2 h on a stirrer-heater module, followed by stirring for 24 h using a magnetic stirrer at room temperature. Dispersed samples were centrifuged for 15 min at 13,500g. The supernatants were filtered through 0.45-μm nylon syringe filters, and then 1-ml samples were injected into the SEC-MALLS-RI system that comprised a pump (P2000, Spectra System, San Hose, CA), injector with a 1-ml loop, SEC column, MALLS (632.8 nm, DAWN DSP, Wyatt Technology, Santa Barbara, CA), and an RI detector (Optilab DSP, Wyatt Technology, Santa Barbara, CA) connected in series. The flow rate of the eluent (50 mM LiBr in DMSO) was 0.6 ml/min

2.4. Polarized optical microscopy

A small amount of starch sample was placed on an object slide with a drop of mixed liquor (1:1 glycerol/water (v/v)). The morphologies of dispersed starch granules and changes in cross polarization were observed using a polarized optical microscope (BA300POL, Mike Audi Industrial Group Co., Ltd, Xiamen, China) equipped with a camera set (Deheng Image DH-M3100UC, Shanghai, China) (Li, Ward, & Gao, 2011).

2.5. Scanning electron microscopy

The dried starch materials were deposited on copper stubs using double-adhesive tape and coated with gold. The particle morphology of starch samples were visualized using SEM (PHILIPS-XL30 ESEM, Philips-FEI, Netherlands) at an acceleration of 20 keV.

2.6. X-ray diffraction

The starch powder was scanned through the 2θ of 5°–45° using X-ray diffractograms (X'Pert Pro MPD, Philips, Netherlands). Traces were obtained using a Cu-Kα radiation detector with a nickel filter and scintillation counter operating under the following conditions: 40 kV, 30 mA, scattering slit 0.25 nm, K-Alpha1 wavelength 1.78901 Å, K-Alpha1 wavelength 1.7929 Å, Ratio K-Alpha2/K-Alpha1 0.5, and scanning rate of 0.02°/min. The degree of crystallinity of samples was quantitatively estimated and analysed with Peakfit v4.12 (SeaSolve Software Inc., Framingham, USA) (Ciolacu, Kovac, & Kokol, 2010; Tester, Karkalas, & Qi, 2004):

$$C_{CL} = \frac{S_C}{S_T} \times 100\,(\%) \quad (1)$$

$$SC_{CL} = \frac{S_{SC}}{S_T} \times 100\,(\%) \quad (2)$$

$$C_L = C_{CL} + SC_{CL} \quad (3)$$

where C_{CL} is the proportion of crystalline region, S_C is the crystallization area, S_T is the total area, SC_{CL} is the proportion of sub-crystalline region, S_{SC} is the sub-crystallization area, and C_L is the degree of crystallinity.