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Effect of debranching and heat-moisture treatments on structural characteristics and digestibility of sweet potato starch

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ABSTRACT

The effects of debranching treatment (DT) and debranching-heat-moisture treatment (D-HMT) on the structural characteristics and *in vitro* digestibility of sweet potato starch were investigated. The results indicated that DT and D-HMT decreased the percentage of starch fraction with degree of polymerization (DP) $\leqslant 13$, increased the percentages of the other fractions, and decreased the molecular weight of starch sample. The D-HMT starch showed a considerable SDS content of 31.60%. Compared with the DT starch sample, the T_o, T_p, T_c, $T_c - T_o$ and ΔH of D-HMT starch samples for the second endothermal were increased significantly, crystalline pattern was altered from C_a to A type, the surface became more smooth. The pasting temperatures of DT and D-HMT starch samples were higher while the peak viscosities, breakdown and setback values were lower than that of native starch. These results suggested that structural changes of sweet potato starch by D-HMT significantly affected the digestibility.

1. Introduction

Sweet potato is one of the most common crops and staple food in China, and it is highly easy to manage and cultivate. Starch is one of the main components of sweet potato root. As a major source of carbohydrate in the human diet, starch plays a very important role in supplying metabolic energy and nutrition for humans. According to the rate and extent of starch digestion, starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RDS is the starch fraction that has been rapidly digested and absorbed in the gastrointestinal tract, which causes a sudden increase in blood glucose level after ingestion, whereas SDS is the starch fraction that is digested slowly but completely and sustains glucose release over time. RS has been defined as the starch portion that cannot be digested in the upper gastrointestine, but it can be fermented by microorganisms in the large intestine, which produces short-chain fatty acids that is beneficial to colonic health (Zhang & Hamaker, 2009). Food containing high content of SDS tends to sustain plasma glucose levels that may help to control and prevent diabetes. Such foods may also be beneficial to satiety, physical performance, improved glucose tolerance, and reduced blood lipid levels in both healthy individuals and those with hyperlipidaemia (Jenkins et al., 2002). Therefore, SDS has attracted much attention as a new functional food component in novel food development in recent years.

Recently, a number of methods have been applied to prepare SDS products from various sources of starch. These methods include enzymatic modification by debranching (Guraya, James, & Champagne, 2001a, 2001b; Miao, Jiang, & Zhang, 2009; Shin et al., 2004; Zeng et al., 2014), chemical modification by citric acid treatment (Shin et al., 2007), cross-linking (Woo & Seib, 2002), and esterification (Han & BeMiller, 2007), and physical modification by hydrothermal treatment (Ahn et al., 2013; Chung, Liu, & Hoover, 2009; Lee, Kim, Choi, & Moon, 2012; Lee, Shin, Kim, Choi, & Moon, 2011; Shin, Kim, Ha, Lee, & Moon, 2005; Song et al., 2014) and retrogradation treatment (Hu, Xie, Jin, Xu, & Chen, 2014; Hu et al., 2015; Park, Baik, & Lim, 2009; Tian et al., 2012, 2013; Xie, Hu, Jin, Xu, & Chen, 2014a, 2014b; Zhang, Hu, Xu, Jin, & Tian, 2011; Zhou, Baik, Wang, & Lim, 2010; Zhou & Lim, 2012). Among these methods, pullulanase debranching and hydrothermal treatment are safe and cost-effective techniques that have significant effect on the formation of SDS.

Enzymatic modification by debranching can cleave the branching point of α-1, 6-linkages and generate short linear α-1, 4-linked glucans, accompanying reforming of double helix structure at the temperature below the melting temperature (T_m). Some studies have indicated that high debranching enzyme concentration and short debranching time are suitable to prepare SDS from rice starch

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or waxy maize starch, whereas long debranching time is beneficial to RS formation (Guraya et al., 2001b; Miao et al., 2009). Guraya et al. (2001a) demonstrated that both waxy rice starch and non-waxy rice starch were suitable to prepare SDS, but the SDS content of waxy rice starch was higher than that of non-waxy rice starch after debranching and cooling treatments. Moreover, Zeng et al. (2014) reported that pullulanase debranching and subsequent temperature-cycled crystallization treatment produced a higher yield and more thermo-stable SDS product from rice starch compared with debranching combining isothermal crystallization treatment. However, heat-moisture treatment (HMT) can cause some gelatinization or other damage of the starch granules (Stute, 1992), which leads to the structural changes within amorphous and crystalline regions in the starch granules. Pullulanase debranching followed by HMT can cause the relatively short linear chains that located in amorphous and/or semi-crystalline regions to realign the new double-helical crystalline structures, while these short linear chains can also reassociate with the crystalline structure formed with long linear chains, which leads to more perfect the crystalline regions and the increment of SDS and RS contents (Trinh, Choi, & Moon, 2013). Trinh et al. (2013) also confirmed that debranching and hydrothermal treatments could significantly increase the boiling-stable SDS and RS contents of water yam starch.

Although some studies have reported the effect of hydrothermal treatment on formation and structural characteristics of SDS from sweet potato starch (Ahn et al., 2013; Shin et al., 2005; Song et al., 2014), however, few studies regarding the effect of pullulanase debranching combined HMT on formation and structural characteristics of SDS from sweet potato starch have been reported. Therefore, the objective of the present study was to investigate the effect of pullulanase debranching combined HMT on structural characteristics and *in vitro* digestibility of sweet potato starch.

2. Materials and methods

2.1. Materials

The fresh sweet potatoes were purchased from a local market of Hefei city, China. α-amylase type VI-B from porcine pancreas (EC 3.2.1.1, A3176) was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Amyloglucosidase (EC 3.2.1.3) and pullulanase (EC 3.2.1.41) OPTIMAX L-1000 from *Bacillus licheniformis* were purchased from Shanghai Yuanye Bio-Technology Co. Ltd. (Shanghai, China) and Genencor Bio-products Co. Ltd. (Wuxi, China), respectively. Isoamylase (Cat. No. I5284-1MU, ammonium sulfate suspension, ⩾3,000,000 units/mg protein) from *Pseudomonas* sp. was obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemical reagents were of analytical grade (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China).

2.2. Isolation of starch from sweet potato

Sweet potatoes were washed, peeled and cut into cubes. The small pieces of sweet potato were soaked in 0.1% (w/v) sodium bisulfite solution for 10 min, and then smashed with the high speed blender containing sodium bisulfite solution. The resulting slurry was passed through 100-mesh sieve to remove the debris. Then, the suspension was filtered for three times using 180-mesh sieve, and allowed to settle at 8–10 °C for 24 h. The precipitated starch was suspended in distilled water and precipitated again. This procedure was repeated until the color of the precipitated starch was pure white. The starch layer was dried at 45 °C for 24 h in a drying oven. Then, it was milled and passed through a 100-mesh sieve.

2.3. Debranching treatment and heat-moisture treatment (HMT) of sweet potato starch

Starch (5.0 g) was dispersed with 45 ml phosphate buffer (pH 4.4) and cooked at 100 °C for 30 min in a water bath. The resultant gels were cooled to 58 °C and debranched by pullulanase at the concentration of 25 ASPU/g. After the reaction for 24 h, two volumes of 95% ethanol were added immediately to terminate the enzyme reaction. The mixture was centrifuged at 3500 rpm for 10 min and the precipitate was washed twice with distilled water. The collected precipitate pallet was dried in a drying oven at 45 °C for 24 h to reach final moisture content of around 10%, then milled and passed through a 100-mesh sieve.

The debranched starch sample was packed into the stainless steel can, and moisture content was adjusted to be 30% by adding an appropriate amount of distilled water, then the can was sealed and allowed to stand at room temperature overnight to reach an equilibrium state. The HMT was conducted by storing the can in an air-drying oven at 100 °C for 2 h. After the HMT, the starch sample in the can was dried in a drying oven at 45 °C for 24 h to reach final moisture content of around 10%, then milled and passed through a 100-mesh sieve.

2.4. High-performance anion-exchange chromatography analysis

The high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used to determine the chain length distribution of starch samples. The sample (10 mg) was added into sodium acetate buffer (2 ml, pH 3.5, 50 mM) and heated in a boiling water bath for 30 min. After cooled and debranched by isoamylase (0.5 U) for 24 h, the solution was heated in a boiling water bath for 20 min to deactivate the enzyme. The debranched starch sample solution was filtered through a 0.45-μm membrane filter and then injected into the HPAEC-PAD system (50 μl sample loop). The HPAEC-PAD system is a Dionex ICS-500 (Dionex Corporation, Sunnyvale, CA, USA). The standard triple potential waveform was utilized, with the following period and pulse potentials: T1 = 0.40 s, with 0.20 s sampling time, E1 = 0.05 V; T2 = 0.20 s, E2 = 0.75 V; T3 = 0.40 s, E3 = −0.15 V. The eluents were prepared in distilled and deionised water with helium sparging; eluent A was 100 mM NaOH, and eluent B consisted of 60 mM sodium acetate dissolved in 100 mM NaOH. The linear components were separated on a Dionex CarboPacPAC PA200 (250 × 4 mm I.D.) column with a gradient elution (20% of eluent B at 0 min, and 100% at 60 min) at 30 °C and a flow rate of 1 ml/min.

2.5. High-performance gel filtration chromatography analysis

The starch sample (10 mg) was added into 5 ml dimethyl sulfoxide (DMSO) containing 50 mM $NaNO_3$, stirred and boiled for 60 min and incubated at 50 °C for 24 h to completely dissolve the sample. The dissolved sample was filtered through a 0.45-μm cellulose acetate filter and then injected into a high-performance gel filtration chromatography system (HPGFC). The HPGFC instrument (Waters 600) consists of a connected column (Ultrahydrogel™ Linear 300 mm × 7.8 mm id × 2) and a 2410 differential refraction detector. The flow rate was 0.9 ml/min using 0.1 M $NaNO_3$ as the mobile phase. All data provided by HPGFC system were collected and analyzed using an Empower workstation.

2.6. Determination of in vitro digestibility of starch

The *in vitro* digestibility of the starch samples were determined according to the previously described method of Englyst et al. (1992) with a slight modification. Starch (200 mg) was dissolved in phosphate buffer (15 ml, 0.2 mol/l, pH 5.2) in centrifuge tube