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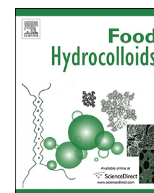

Dual-enzymatic modification of maize starch for increasing slow digestion property

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ABSTRACT

Maize starch was modified using β-amylase and transglucosidase and their molecular fine structure and *in vitro* digestibility were investigated. By dual-enzymes treatment, the molecule weight decreased, the amount of short chains and α-1, 6 linkages increased. This indicated that α-1,4 linkage of starch was cleaved and non-reducing D-glucosyl residues of maltose was transferred to forming α-1,6 branch linkage. A maximum SDS content (33.5%) was obtained using double enzymes hydrolysis for 6 h compared to native starch. Both the increase in amount of shortened chain length and α-1,6 linkage were likely attributed to slow digestion property of starch. The results suggested that starches using combined β-amylase and transglucosidase treatment produced new branched structures with slowly digestible character.

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1. Introduction

Obesity, diabetes and coronary heart disease have become major public health concerns worldwide, with the number of cases increasing exponentially in recent years. The multi-factorial etiology of this worldwide epidemic, and the idea that dietary factor may contribute to it, is now well recognized (Abete, Astrup, Martinez, Thorsdottir, & Zulet, 2010; Aston, 2006; Barclay et al., 2008; FAO/WHO, 2002; Hauner et al., 2012; Manuel-y-Keenoy & Perez-Gallardo, 2012; Mozaffarian, Hao, Rimm, Willett, & Hu, 2011). Compelling evidence from epidemiologic studies indicates that the blood glucose from ingested carbohydrate sources plays an important role in energy metabolism and glucose homeostasis (Barclay et al., 2008; Hauner et al., 2012; Ludwig, 2002; Miao, Jiang, Cui, Zhang, & Jin, in press; O'Keefe, Abuannadi, Lavie, & Bell, 2011; Semjonous et al., 2009). New developments in food and nutritional science have led to the conclusion that slowing down the rate of carbohydrate digestion helps to maintain proper blood glucose levels and to provide extended energy absorption related to human health (Aston, 2006; Ludwig, 2002; Miao, Jiang, et al., in press; Miao, Xiong, et al., in press; Semjonous et al., 2009; Zhang & Hamaker, 2009).

Starch is one of the major the glycemic carbohydrate materials in cereal- and tuber-based food products. On the basis of the rate and extent of digestion, starch has been classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). Starch can be quantified into different fractions using the *in vitro* Englyst assay: the starch fraction digested within 20 min of incubation is classified as RDS, the starch fraction digested between 20 and 120 min corresponds to SDS, and the remaining fraction that is not further digested is RS. RDS usually leads to a rapid increase of plasma glucose and insulin levels, whereas SDS is digested slowly with a moderate glycemic and insulinemic response (Englyst et al., 1992; Ludwig, 2002; Miao, Jiang, et al., in press; Miao, Xiong, et al., in press). Long-term intake of foods with high contents of RDS has been shown to be associated with a series of health complications such as diabetes and pre-diabetes, cardiovascular diseases, obesity and overweight through generating a high stress on regulatory systems related to glucose homeostasis (Ludwig, 2002; Manuel-y-Keenoy & Perez-Gallardo, 2012). Therefore, improving food quality with higher amounts of SDS is becoming an area of interest for food industry and health professionals, but the amount of SDS in regular food products is very low and there is no commercially available SDS in the current food market. In the past decades, considerable research effort has been devoted to novel ways for SDS preparation using different techniques (Zhang & Hamacker, 2009; Miao et al., 2013). For example, Shin, Kim, Ha, Lee, and Moon (2005) reported that the granular sweet potato starch with 50% moisture

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content heated at 55 °C increased by 200% in terms of heat-stable SDS compared to raw starch. Miao, Jiang, and Zhang (2009) attempted the production of SDS based on partial hydrolysis of gelatinized waxy maize starch with pullulanase following controlled retrogradation. They found high enzyme concentration and less debranching time increased the amount of SDS, whereas longer times accelerated the production of RS. Esterification with octenyl succinic anhydride (OSA) was shown to be the more effective of waxy starch modifications used for making SDS, followed by crosslinking-hydroxypropylated or crosslinking-acetylated starch than cross-linking starch (Han & BeMiller, 2007). Dry heating (130 °C) of OSA-starch increased the SDS content and decreased RS content. He, Liu, and Zhang (2008) showed SDS (42.8%) in heat-moisture treatment of OSA-starch (10% moisture, at 120 °C for 4 h) was higher than that of OSA-starch (28.3%). However, the earlier research on producing SDS was done using physical, chemical or enzymatic methods, and little work has been reported on the structure modification using double enzymes for understanding of the slow digestion property of starch. In this study, the effect of dual-enzymes modifications using β-amylase and transglucosidase on molecular structure and its slow digestion property of starch were investigated. The relationship between slow digestion property and fine structure was also studied to gain insight into the fundamental basis for increasing the SDS content in regular processed food products.

2. Materials and methods

2.1. Materials

The normal native maize starch with 28.1% amylose was a generous gift from Changchun Dacheng Industrial Group Co. Ltd. (Jilin, China). β-Amylase from barley (OPTIMALT BBA) and transglucosidase from *Aspergillus* (L-500) were donated by DuPont Genencor International Inc. (Wuxi, China). α-Amylase from porcine pancreas (type VI-B) was purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA). Amyloglucosidase, isoamylase and the glucose oxidase-peroxidase assay kits were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). All chemicals were of reagent grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of starch samples

The maize starch slurry (10% w/v in diluted pH 5.0, 0.02 M sodium acetate buffer solution) was heated in a thermostatic water bath at 95 °C for 60 min. When the temperature of the starch sample was adjusted to 55 °C, both 0.5% β-amylase (dry weight of starch) and 8 TGU/g transglucosidase (dry weight of starch) were added to the solution. The enzymatic reaction was incubated for 10 min, 20 min, 30 min, 1 h, 2 h, 3 h, 6 h or 12 h. Immediately after the reaction, the solutions were autoclaved at 121 °C for 30 min to stop the reaction; the solution was then cooled to room temperature and 1 volume of 90% ethanol (v/v) was added to facilitate the precipitation of reactant. The precipitated starch was centrifuged at 5000 g for 10 min, washed with deionised water and then re-centrifuged twice before collecting and freeze-drying ad suggested in a previously study (Miao, Xiong, et al., in press). The collected material was ground to form a powder (120 mesh) and stored in a desiccator for further analysis. These enzymatically hydrolysed samples were named DS1, DS2, DS3, DS4, DS5, DS6, DS7 and DS8, respectively. The supernatant of the hydrolysed starch product was collected by stopping the enzymatic reaction at different times and the degree of hydrolysis was determined using the phenol-sulphuric method.

2.3. Iodine binding analysis

The iodine binding analysis was measured using a UV/visible Spectrophotometer (UV-2102PC, Unico Instrument Co., Ltd., Shanghai, China). An iodine reagent was prepared by adding 2 mg of I_2 and 20 mg of KI to 1 ml of deionised water; the solution was stored in a non-actinic bottle at room temperature. The dried starch sample (50 mg) was dissolved in deionised water in 10 ml screw-cap vials. The diluted solution (0.5 ml) was mixed with the iodine solution (1.5 ml) and was then adjusted to a final volume 2 ml of with water. The absorbance spectra and the wavelength of maximum absorption (λ_{max}) were analysed over a wavelength scan of 500–800 nm.

2.4. High-performance size-exclusion chromatography analysis

Starch samples (10 mg) were added to 5 ml deionised water, stirred and boiled for 15 min to completely dissolve the samples. The dissolved samples were filtered through 5 μm cellulose acetate filters (Whatman, Maidstone, UK) and were injected into a high-performance size-exclusion chromatography system with a multi-angle laser light scattering detector and a refractive index detector (HPSEC-MALLS-RI) (Wyatt Technology, Santa Barbara, CA). Two series tandem columns (300 × 8 mm, Shodex OH-pak SB-806 and 804, Showa Denko K.K., Tokyo, Japan) with an OH-pak SB-G guard column, a DAWN HELEOS II laser photometer fitted with a He–Ne laser ($\lambda = 632.8$ nm) with a K-5 flow cell, and an OPTILAB® T-rEX Interferometric Refractometer were used. The flow rate was set at 0.5 ml/min with a mobile phase of distilled–deionised water (pH 6.8, 18.2 MΩ cm) containing 0.02% NaN_3. A dn/dc value of 0.138 was used in molecular weight calculations, and data processing was performed using ASTRA software (Version 5.3.4.14, Wyatt Technology).

2.5. High-performance anion-exchange chromatography analysis

The chain length distribution of starch was determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The samples (10 mg) were dissolved with 2 ml $NaNO_3$ solution (pH 4.0, 0.1 M) and heated in a boiling water bath for 10 min. Isoamylase (0.5 U) was added to each dispersion, and the mixtures were incubated at 40 °C with shaking for 24 h. Then, the solution was heated in a boiling water bath for 10 min to deactivate the enzyme. The debranched sample solutions were filtered through a 0.45-μm membrane filter and then injected into the HPAEC-PAD system (50 μl sample loop). The HPAEC-PAD system consisted of a Dionex DX 600 equipped with an ED 50 electrochemical detector with a gold working electrode, GP 50 gradient pump, LC 30 chromatography oven, and an AS 40 automated sampler (Dionex Corporation, Sunnyvale, CA, USA). The standard triple potential waveform was employed, 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. Data were collected using Chromeleon software, version 6.50 (Dionex Corporation, Sunnyvale, CA, USA). The eluents were prepared in distilled–deionised water with helium sparging; eluent A was 150 mM NaOH, and eluent B was 50 mM sodium acetate in 150 mM NaOH. Linear components were separated on a Dionex CarboPac™ PA1 column with a gradient elution (40% of eluent B at 0 min, 50% at 2 min, 60% at 10 min, and 80% at 40 min) at 30 °C and a flow rate of 1 ml/min.

2.6. Proton nuclear magnetic resonance spectroscopy (^1H NMR)

^1H NMR analysis of starch samples were performed using an AVANCE III 400 MHz Digital NMR Spectrometer (Bruker Biospin

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