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Structural modification and characterisation of a sugary maize soluble starch particle after double enzyme treatment

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ABSTRACT

Sugary maize soluble starch particles were modified by using a combined β-amylase and transglucosidase reaction, and their molecular fine structure and susceptibility to digestive enzymes were investigated. After the dual enzyme treatment, the molecular weight of starch particle decreased from 2.08×10^7 to 0.96×10^7 g/mol accompanied by the appearance of DP 2–5 chains and the degradation of DP > 12 chains, and the percentage of α-1,6 linkages increased from 8.1 to 21.7%. The digestion behaviour of enzyme-treated starch was correlated with the quantity of shorter chains and increased α-1,6 linkages. The data revealed that maize soluble starches subjected to a combined β-amylase and transglucosidase treatment for 60 min or greater, produced novel, highly branched nano-particles with slow digestion and resistance characteristics, which could be used as a potential delivery carrier for functional food components.

1. Introduction

Soluble starch is similar to glycogen, and it is also a primary amylopectin analogue with a more highly branched structure in the sugary-1 (su-1) mutants of maize, rice, sorghum or barley (Ball & Morell, 2003; BeMiller & Whistler, 2009; Boyer & Liu, 1983; Miao, Li, Jiang, Cui, Lu, et al., 2014; Tetlow, Morell, & Emes, 2004). Starch is generally formed in the amyloplasts of cereal grains, in which the reactions are catalysed by starch synthase, starch branching enzyme, and starch debranching enzyme in the starch biosynthetic pathway (BeMiller & Whistler, 2009; Powell et al., 2014; Tetlow et al., 2004). The su-1 mutant endosperm cell is deficient in debranching enzyme, which is used to trim abnormal branches that inhibit the formation of starch crystals and granules, and the more highly branched α-D-glucan then accumulates (Ball & Morell, 2003; Boyer & Liu, 1983). According to several earlier studies, soluble starch is a typical nano-scale particle ranging from 30 to 100 nm and exhibits a spherical shape (Miao, Li, Jiang, Cui, Lu, et al., 2014; Miao, Li, Jiang, Cui, Zhang, et al., 2014; Miao, Xiong, et al., 2014; Putaux, Buléon, Borsali, & Chanzy, 1999). In comparison with amylopectin, soluble starch has an average branch chain length of DP 10–12 with a 7–10% branch density (Inouchi, Glover, & Fuwa, 1987; Miao, Li, Jiang, Cui, Lu, et al., 2014; Putaux et al., 1999; Wong et al., 2003; Yun & Matheson, 1993). Moreover, there is an obvious density increment towards the external region of soluble starch, whereas the molecular density of amylopectin remains uniform during amyloglucosidase hydrolysis (Huang & Yao, 2011).

Native starch is known to be structurally too weak and functionally too restricted for most industrial applications and is often tailored through physical, chemical or physical modifications to develop desirable functional properties (BeMiller & Whistler, 2009; Miao, Jiang, Cui, Zhang, & Jin, 2013; Miao, Jiang, & Zhang, 2009; Miao, Jiang, Zhang, Jin, & Mu, 2011; Miao, Li, Jiang, Cui, Zhang, et al., 2014; Tharanathan, 2005). Among these means, enzymatic treatments have attracted greater attention because of their safe, environmentally friendly, and highly controllable nature with few by-products under mild conditions (Ao et al., 2007; BeMiller & Whistler, 2009; Le et al., 2009; Miao, Xiong, et al., 2014).

β-Amylase (4-α-D-glucan maltohydrolase, EC 3.2.1.2) is known for hydrolysing (1 → 4)-α-D-glucosidic linkages in polysaccharides to remove successive maltose units from the non-reducing ends of the chains. The hydrolase exo-reaction was stopped when the amount of glucose was between 2 and 3 unit from the branch point (α-1,6 glycosidic linkages), thereby trimming the outer branches of starch molecules for β-limit dextrin (Miao, Li, Jiang, Cui, Zhang, et al., 2014; Xia & Thompson, 2006). Transglucosidase ((1 → 4)-α-D-glucan: (1 → 4)-α-D-glucan (D-glucose) 6-α-D-glucosyltransferase, EC 2.4.1.24) transfers an α-D-glucosyl residue in a (1 → 4)-α-D-glucan to the primary hydroxy group of glucose, which is free or combined in a (1 → 4)-α-D-glucan. Transglucosidase was shown

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to produce novel glucans with an increasing branch density (α-(1→6) linkages) by hydrolytic and transfer reactions (Ao et al., 2007). A combined reaction of β-amylase and transglucosidase was recently used to produce tailor-made starch with shorter and more highly branched chains, which led to the modulation of the digestion property of granular starch (Ao et al., 2007; Miao, Xiong, et al., 2014). However, little work has been reported on structural modifications when using double enzymes to understand the structure-property relations of soluble starch. The purpose of this study was to determine the effect of dual enzyme modification on the structure and in vitro enzyme digestibility changes of soluble starch. The endosperm soluble starch from a su-1 maize mutant was used as a model system to elucidate the relationship between the branch structure and enzymatic release of glucose through a combination of chemical and instrumental analysis, which could lead to insights into the fundamental basis for reducing the overall starch digestion rate in regular processed foods.

2. Materials and methods

2.1. Materials

Sugary-1 maize kernels were purchased from the Chinese Academy of Agricultural Sciences (Beijing, China). Transglucosidase from *Aspergillus* (L-500) and β-amylase from barley (OPTIMALT BBA) were donated by DuPont Genencor International Inc. (Wuxi, China). α-Amylase from porcine pancreas (Type VI-B, ≥10 unit/mg solid) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Amyloglucosidase (3300 U/ml) and glucose oxidase–peroxidase assay kits (Cat. No. K-GLUC) were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). All chemicals were reagent grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Isolating sugary maize soluble starch particles

The soluble starch particles were isolated from sugary maize in the laboratory following the methods of as described in a previous study (Miao, Li, Jiang, Cui, Lu, et al., 2014). The sugary-1 maize fresh kernels were dehulled, and the endosperm was separated from the germ and soaked in five times its weight of deionised water at 20 °C overnight. The softened grains were ground in a laboratory blender. The mixture was filtered through 100-mesh sieves and then centrifuged at 10,000 × *g* for 10 min. The supernatant was collected, and the sediment was extracted twice with deionised water. The resulting decantate was heated in a boiling water bath for 30 min to denature the protein. After centrifugation, one volume of liquid was measured and three volumes of ethanol were added to precipitate the soluble starch. The precipitate was then collected and placed in a fume hood to remove the residual ethanol. The dried solid was ground to form a powder, which was then used for further studies within the next few months.

2.3. Dual enzyme modification of soluble starch particles

The soluble starch particles (3 g) were dissolved in 30 ml of pH 5.2, 0.02 M sodium acetate buffer solution. The temperature of the starch sample was adjusted to 55 °C and both 300 U/g β-amylase (dry weight of starch) and 50 U/g transglucosidase (dry weight of starch) was added to the solution. The enzymatic reaction was incubated for 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, or 12 h. Immediately after, the solutions were autoclaved at 121 °C for 30 min to stop the reaction; the solution was then cooled to room temperature and 3 vol. of 90% ethanol (v/v) was added to facilitate reactant precipitation. The precipitated starch was centrifuged at 5000 × *g* for 10 min, suspended in ethanol and filtered twice before collecting and drying. The collected material was ground to form a powder (100 mesh) and stored in a desiccator for further analysis. These enzymatically hydrolysed samples were named DSS1, DSS2, DSS3, DSS4, DSS5, DSS6 and DSS7 on the basis of the enzymatic reaction times listed above. The supernatant of the hydrolysed starch product was collected by stopping the enzymatic reaction at different times, and the degree of hydrolysis was determined by using the phenol–sulphuric acid method.

2.4. Amylose content and iodine binding analysis

The amylose content of the starch sample was determined by using the iodine colorimetric method (Jiang, Miao, Ye, Jiang, & Zhang, 2014). 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 reagent was stored in a non-actinic bottle at room temperature. The dried starch sample (50 mg) was dispersed in deionised water in a 10 ml screw-cap vial. The diluted solution (0.5 ml) was mixed with the iodine solution (1.5 ml) and then adjusted to a final volume of 2 ml with water. The absorbance spectra and the wavelength of maximum absorption (λ_{max}) were analysed over a wavelength scan of 500 to 800 nm.

2.5. High-performance size-exclusion chromatography (HPSEC) analysis

The molecular weight distribution of starch was analysed by using 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, USA). 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 (λ = 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 d*n*/d*c* 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, Santa Barbara, CA, USA) as described by Miao, Li, Jiang, Cui, Lu, et al. (2014). The weight-average molecular weight (M_w, g/mol) and *z*-root mean square radius of gyration (R_z, nm) were obtained using the second-order Berry method. The molecular density (ρ, g/mol nm^3) was calculated as M_w/R_z^3.

2.6. High-performance anion-exchange chromatography (HPAEC) analysis

The chain length distribution of starch was determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex Corporation, Sunnyvale, CA, USA). 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. The standard triple potential waveform was utilised, with the following period and pulse potentials: T_1 = 0.40 s, with 0.20 s sampling time,