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Molecular characterization and in vitro digestibility of normal maize starch hydrolyzed by maltotriohydrolase

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ABSTRACT

Normal maize starch was hydrolyzed by the glucan 1,4-alpha-maltotriohydrolase (AMTS), and the changes in molecular characteristics and digestibility of starch were evaluated. Upon hydrolysis, maltotriose purity could be modified via controlled AMTS action. The transglycosylation of AMTS possibly happened during the extensive hydrolysis of starch. No single linear association between the *z*-average radius of gyration (R_z), conformation exponent (ν), apparent molecular density (ρ) and weight average molar mass (M_w) of the starch molecules could be established in the entire process of AMTS hydrolysis. Under mild hydrolysis ($\leq$240 min), R_z and ρ displayed linear relationships with M_w. However, transitions of ν, R_z and ρ appeared after extensive hydrolysis (>240 min), due to the increase in the amount of short chains [degree of polymerization (DP) $\leq$ 5]. The spherical starch molecule tends toward less compact and a structure between sphere and random coil after extensive hydrolysis. And the increase in the amount of DP $\leq$ 12 chains and reduction of molecular dimension after AMTS hydrolysis restrict the digestibility of starch. The results of this study suggest that normal maize starch can be modulated by AMTS to produce the desired maltotriose syrup, starch molecular characteristics, and starch digestibility.

1. Introduction

Amylases have been widely used in the production of sugars and enzyme-modified starches [1]. In the presence of amylase, starch undergoes significant hydrolysis. Therefore, amylase addition may result in tailored starch molecules with discrepant molecular characterization. It is important to understand the molecular characteristics of starch to develop specific functional foods [2]. Based on the scientific literature, starch molecules of different weight average molar mass (M_w), number average molar mass (M_n), molar mass distribution, molecular dimension, and structural conformation [2] are of commercial interest. Molecular characterization of enzyme-modified starch has been partially performed [3–6]. However, the molecular degradation mechanisms of starch under some specific amylases remain unclear. Among such amylases, glucan 1,4-alpha-maltotriohydrolase (AMTS, EC 3.2.1.116) is a potential enzyme for the production of maltotriose from starch [7,8]. It has been reported that AMTS uniquely transfers maltotriosyl units during maltooligosaccharide hydrolysis [7]. Based on these, the molecular characteristics of AMTS-hydrolyzed starches were evaluated in this study.

Enzyme-modified starches of different digestibility have gained considerable interest in the past years [4,6,9–11]. Based on its rate and extent of digestibility, starch can be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [12]. The molecular structure of starch hydrolyzed by amylase has been shown to affect cooked starch digestibility [3,4,10]. However, to the best of our knowledge, no studies have focused on the relationships between the molecular characteristics and digestibility of AMTS-hydrolyzed starch.

The objective of this work is to understand the influence of AMTS hydrolysis on the soluble oligosaccharides released, starch molecular characteristics and digestibility. To achieve this aim, normal maize starch was hydrolyzed by AMTS to different extents. Data on the molecular characteristics of AMTS-hydrolyzed starches were derived from high performance size exclusion chromatography equipped with a multi-angle laser light scattering detector and a refractive index detector (HPSEC-MALLS-RI; Wyatt Technology, Santa Barbara, CA, USA). Additionally, the correlations between molecular characteristics and starch digestibility were studied. By

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doing this, it is hoped that useful information is shed about the molecular structure-property relationships of starch after AMTS hydrolyzed.

2. Materials and methods

2.1. Materials

Normal maize starch was kindly donated by Puluoxing Starch Co., Ltd. (Hangzhou, China). AMT 1.2 L was purchased from Amano Enzyme Inc. (Nagoya, Japan); α-amylase from porcine pancreas and glucose oxidase–peroxidase assay kit were purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA). Amyloglucosidase and isoamylase were acquired from Megazyme International Ireland Co. (Wicklow, Ireland).

2.2. AMTS preparation

AMTS was purified from the commercial enzyme AMT 1.2 L according to a previously described laboratory procedure [7]. Enzyme activity was assessed by quantifying the amount of reducing sugars released from soluble starch (1%, pH 6.5, containing 5 mM $CaCl_2$) at 50 °C. One enzymatic unit (U) was defined as the amount of enzyme required to release 1.0 mmol of maltotriose equivalents per min.

2.3. Normal maize starch hydrolyzed by AMTS

Normal maize starch (5%, w/v) suspended in 100 mM phosphate buffer (pH 6.5, 5 mM $CaCl_2$) was heated in a boiling water bath for 60 min. The starch suspension was autoclaved at 121 °C for 30 min and subsequently allowed to cool to 50 °C. AMTS (40 U/g starch) was added to the suspension, which was incubated at 50 °C for 0, 5, 10, 30, 60, 120, 240, 480, and 960 min. Twofold ethanol was added to terminate the reaction and centrifuged at 5000 × *g* for 10 min. The precipitated starch was washed three times with distilled water by centrifugation. The pellet was subsequently freeze-dried, pulverized, and passed through a 100-mesh sieve. The resulting supernatant was analyzed by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD; Dionex DX 500, Sunnyvale, CA, USA) [7]. To monitor the progress of hydrolysis, aliquots were collected as a function of time and the reducing values [13] and total sugar contents [14] were measured. The degree of hydrolysis (DH) was calculated according to Eq. (1).

$$\text{Degree of hydrolysis}\ (\%) = \frac{\text{Reducing value}\ \ (\text{expressed as maltotriose equivalents})}{\text{Total sugar content}\ \ (\text{expressed as glucose equivalents})} \times 100 \quad (1)$$

2.4. Molecular characterization

The AMTS-hydrolyzed starch (10 mg) was completely dissolved in dimethyl sulfoxide (DMSO, 50 mM $NaNO_3$), passed through a 0.45 μm membrane filter, and injected into HPSEC-MALLS-RI (Wyatt Technology, Santa Barbara, CA, USA). The system consisted of a Dawn EOS laser photometer coupled to a He–Ne laser (λ = 658.0 nm), a K-5 flow cell, and an RI detector (model 2414, Waters, USA). Two series tandem columns (7.8 mm × 300 mm, Styragel HMW 6E DMF 250 and 1000, Waters, USA) were used. The flow rate was set at 0.6 mL/min and the mobile phase was pure DMSO containing 50 mM $NaNO_3$. This experiment was performed as previously reported by Wyatt [15]. The M_w and *z*-average radius of gyration (R_z) were calculated using the following equations [15,16],

$$\frac{Kc}{R_\theta} = \frac{1}{M_w P(\theta)} - 2A_2 c \quad (2)$$

$$K = \frac{4\pi^2 n_0^2 (dn/dc)^2}{N_A \lambda_0^4} \quad (3)$$

$$\frac{1}{P(\theta)} = 1 + \frac{16\pi^2 R_Z^2}{3\lambda^2} \sin^2(\theta/2) + f_4 \sin^4(\theta/2) + \cdots \quad (4)$$

where *c* is the mass concentration of the sample, R_θ is the Rayleigh ratio of scattered intensity at angle θ, A_2 is a second viral coefficient, *K* is an optical parameter calculated from Eq. (3), *n* is the refractive index of the solvent, *dn*/*dc* is the refractive index increase, λ_0 is the wavelength of the scattered light in vacuum, and P(θ) in Eq. (4) describes the angular dependence of scattered light. Curve fitting was performed from a Debye plot using the second order Berry method (Kc/R_θ vs. $\sin^2(\theta/2)$) and A_2 set at zero. M_w and R_z were calculated from the intercept and slope, respectively, of Eq. (2). A *dn*/*dc* value of 0.066 was used in this calculation.

Data from the chromatograms were converted into molecular weight and radius of gyration using ASTRA software 5.3.4.20 (Wyatt Technology, Santa Barbara, CA, USA). Molecular characteristics of AMTS-hydrolyzed starches were calculated and compared.

2.5. Chain length distribution analysis

Modified maize starch dispersions (0.5%, w/v) were prepared according to our laboratory protocol [7]. Starch was dispersed in DMSO (90%, w/v) under constant stirring for 60 min in a boiling water bath. The dispersion was further stirred for 12 h at 30 °C. Four volumes of ethanol were added to precipitate the starch. Following centrifugation, the precipitate was suspended in acetone, vacuum-dried, dispersed in boiling sodium acetate solution (pH 4.0, 0.1 M), and heated in a water bath for 10 min. The starch dispersion was equilibrated at 40 °C; 0.1 mL of isoamylase (0.5 U) was added and the mixture was incubated under constant shaking at 40 °C for 24 h. The de-branched starch solutions were passed through a 0.45 μm membrane filter and analyzed by HPAEC-PAD [7].

2.6. In vitro digestibility

The digestibility of AMTS-hydrolyzed starch was assessed according to the method of Englyst with some modifications [12]. Briefly, dissolved starch samples (200 mg) were incubated in a simulated intestinal solution (30 U/mL α-amylase, 20 U/mL amyloglucosidase, and 0.2 M phosphate buffer at pH 5.2 and 37 °C) under constant shaking. Aliquots of hydrolyzed solution (4 mL) were removed at different time intervals, mixed with ethanol, and centrifuged. The glucose content in the supernatant was determined using a glucose oxidase–peroxidase assay kit (GAGO20, Sigma). RDS was defined as the starch that was digested within 20 min, SDS represented the starch that was digested from 20 min to 120 min, and RS was the starch that was not digested within 120 min.

2.7. Statistical analyses

Data were analyzed using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) and expressed as mean ± SD from triplicate measurements. Correlation coefficients were calculated using Pearson product moment correlation. Statistical significance was set at $p < 0.05$.