Pasting and thermal properties of waxy corn starch modified by 1,4-α-glucan branching enzyme

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ABSTRACT

Waxy corn starch was modified with the 1,4-α-glucan branching enzyme (GBE) from *Geobacillus thermoglucosidans* STB02. Incubating waxy corn starch with GBE increased the number of α-1,6 branch points and reduced the average chain length. Enzymatic modification also decreased the breakdown and setback values of Brabender viscosity curves, indicating that the modified starch had higher paste stability. Preheating the starch at 65 °C for 30 min before incubation with GBE could promote enzymatic modification of starch. Linear regression was used to describe the relationships between starch structure and its pasting and thermal properties. The setback value showed a negative linear correlation with the α-1,6 branch point content ($R^2 = 0.9824$) and a positive linear correlation with the average chain length ($R^2 = 0.8954$). Meanwhile, the gelatinization enthalpy was also linearly correlated to the α-1,6 branch point content ($R^2 = 0.9326$) and the average chain length ($R^2 = 0.8567$). These insights provide a useful reference for food processors.

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1. Introduction

The food industry produces a wide variety of valuable products using starch as a raw material. Starch usually undergoes some form of processing, such as heating, shearing, or cooling, before being consumed by humans. Starch gelatinization, a swelling of starch granules and melting of starch crystallites that leads to the destruction of molecular order, occurs when starchy food is heated in the presence of water [1]. In contrast, starch retrogradation and disorder-order phase transitions, which lead to the reassociation of gelled structures and the restoration of an ordered crystalline structure, occur during cold storage [2]. The changes in the properties of starch that occur during these processing operations are seen as desirable by the food industry. Previous studies have suggested that these phenomena occur during pasting, and that thermal treatment substantially affects the digestibility and enzymatic hydrolysis of starch [3,4], as well as the hardness and taste of starchy food [2,3,5]. Thus, pasting and thermal behaviors are thought to have an effect

on the edibility of starchy foods, which is a key consumer criterion. The pasting and thermal properties of starch are determined by the amylose/amylopectin ratio, crystalline structure, and amylopectin chain length distribution of the starch [6,7], as well as its degree of branching [2]. Although a series of physical [5,8,9], chemical [10–12], and enzymatic methods [13–15] have been used to modify starch to improve its pasting and thermal properties, there is growing interest in the use of 1,4-α-glucan branching enzyme for the enzymatic modification of starch due to its specificity [2].

A member of the α-amylase family [16], 1,4-α-glucan branching enzyme (GBE, EC 2.4.1.18) cleaves α-1,4 glucosidic bonds, and then reconnects the released chains by forming new α-1,6 glucosidic bonds. This process leads to an increase in the degree of branching. For example, treating rice starch with the GBE from *Streptococcus mutans* resulted in a decrease in retrogradation enthalpy, which suggested a trend toward retarded retrogradation [17]. Similarly, a branching enzyme isolated from developing waxy rice endosperm was used to modify high-amylose maize starch [18], leading to highly branched side chains. The results suggested branching enzyme might inhibit starch retrogradation and control blood glucose levels. The thermostable branching enzyme from *Rhodothermus obamensis* was used to modify starch, exploring its potential to extensively increase the number of branch points in

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starch. The results suggested that branching enzyme modifies pure amylose differently than it modifies amylopectin and the molecular configuration of the modified glucans restricted dietary degradation [19]. Suppression of starch branching enzyme activity with RNAi technology was used to modify starch directly in the potato tuber [20], leading to an elevated amylose content, longer amylopectin chains and elevated gelatinization temperature. What's more, Jensen reported the effect of GBE modification at very high concentrations of starch. After GBE treatment, products from potato starch and corn starch were characterized by increased branching and decreased molecular size [21,22].

However, there is little information about GBE-modified waxy starch. Waxy starch has a high amylopectin content, leading to a high degree of branching compared with normal starch. Meanwhile, GBE treatment can lead to an increase in the degree of branching in starch. Thus, treating waxy starch with GBE can lead to a product with an extremely high branch point content (significantly higher than that found in normal starch), which we believe will be valuable to study.

In this study, waxy corn starch (WCS) was modified by GBE. The structure, pasting, and thermal properties of the modified WCS were investigated. The relationships among these properties were also studied. The results may provide a useful reference for the further development of starchy food.

2. Materials and methods

2.1. Materials

WCS was obtained from Cargill (Beijing, China). The GBE was produced using *Escherichia coli* BL21(DE3) harboring the plasmid pET-20b(+)/*gbe*, which was constructed by insertion of the *gbe* gene from *G. thermoglucosidans* STB02 into the T7-driven expression vector pET-20b(+) [2]. Isoamylase was purchased from Sigma Chemical Co. [21].

2.2. Preparation of GBE-modified WCS

GBE-modified WCS (M-WCS) was prepared using a previously reported method [2], with slight modification. WCS (30% dry starch basis, w/v) was suspended in 100 mL Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 7.5). This mixture was incubated with GBE (200 U/g starch) in a shaker bath at 55 °C for 4 h or 8 h. Then, five volumes of deionized water were added to wash the mixture. The collected solids were dried in a vacuum dryer at 45 °C for 12 h, and then ground and sieved through a 100-mesh sieve. The materials modified by GBE for 4 h and 8 h will be referred to as M-WCS-4 and M-WCS-8, respectively.

2.3. Preparation of GBE-modified WCS after preheat treatment

WCS (30% dry starch basis, w/v) was suspended in 100 mL Na_2HPO_4-NaH_2PO_4 buffer (50 mM, pH 7.5) and heated in a shaker bath at 65 °C for 30 min. Then, GBE (200 U/g starch) was added and the suspension was incubated at 55 °C for 4 h or 8 h. The final mixtures were washed and dried as described above for M-WCS. The dried products will be referred to as P-M-WCS-4 and P-M-WCS-8, respectively.

2.4. Glycosidic linkage ratio analysis

The α-1,6 linkages/total linkages ratios was analyzed using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy [23]. Samples were dissolved in deuterium oxide (80 mg/mL) and then gelatinized in boiling water for 30 min. After freeze-drying and re-dissolution in deuterium oxide (80 mg/mL), the samples were analyzed using ^{1}H NMR spectroscopy. The α-1,6 linkages/total linkages ratio was quantified by dividing the area of the peak corresponding to the anomeric protons from the α-1,6 glycosidic linkages by the total area of the peaks corresponding to the anomeric protons of the α-1,6 glycosidic linkages and the α-1,4 glycosidic linkages.

2.5. Chain length distribution analysis

The chain length distribution of the debranched starch was determined using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) [24,25]. Samples were suspended in acetate buffer (pH 3.5, 0.5% starch) and then gelatinized in boiling water for 30 min. After cooling to room temperature, samples were stirred at 37 °C for 10 min and incubated with isoamylase (100 U/g starch) at 37 °C for 24 h. The solutions were heated in a boiling water bath for 20 min and cooled. After centrifugation at 13,000g for 10 min, the debranched starch solutions were filtered through a 0.45 μm syringe filter. The filtrate (20 μL) was analyzed using an HPAEC system equipped with a CarboPac PA200 chromatographic column. The eluents were prepared in ultrapure water and sparged with nitrogen. Eluent A consisted of 250 mM NaOH, eluent B consisted of 1 M sodium acetate, and eluent C consisted of ultrapure water. The linear components were separated with gradient elution (4% eluent B at 0 min, 40% eluent B at 40 min and 4% eluent B at 41 min, 38% of eluent A continuously, and the remainder eluent C) at 30 °C and a flow rate of 0.5 mL/min [24]. The chain length distribution was characterized as a percentage of the total peak area [2,18].

2.6. Pasting properties of starch

The pasting properties of starch samples were analyzed using a Brabender amylograph [26]. Samples were prepared by adding deionized water to WCS, M-WCS and P-M-WCS (6% dry starch basis). The heating schedule was as follows: samples were heated from 30 °C to 95 °C at 1.5 °C/min, held at 95 °C for 30 min, cooled to 50 °C at 1.5 °C/min, and then held at 50 °C for 30 min. The control was WCS treated at 55 °C for 4 h in the absence of GBE. The pretreatment control (P-control) was WCS treated at 65 °C for 30 min and then at 55 °C for 4 h in the absence of GBE.

2.7. Thermal properties of starch

The thermal properties of WCS, M-WCS and P-M-WCS were analyzed using differential scanning calorimetry [24,27]. Samples (2.0 mg, dry basis) were weighed into aluminum pans and deionized water (4.0 mg) was added with a microsyringe [2,28,29]. The aluminum pans were sealed, stored at 4 °C for 24 h, and then heated from 25 to 95 °C at a rate of 10 °C/min. The onset temperature (T_o), peak temperature (T_p), conclusion temperature and gelatinization enthalpy ($\triangle H_g$) were quantified during the heating process. Gelatinized samples were stored in the refrigerator at 4 °C for 1, 3, 5, 7, 14, or 21 days, and then the thermal analysis was repeated to measure retrogradation [30].

2.8. Statistical analysis

Data shown are the means of measurements performed in triplicate. Statistical analyses were conducted with SPSS 17.0 software (SPSS Inc., Chicago, Illinois, USA). The statistical significance of the differences were assessed using the Student–Newman–Keuls (SNK) test. Differences with values of $p < 0.05$ were considered statistically significant.