

## Structural and Rheological Properties of Sweet Potato Starch Modified with 4- $\alpha$ -Glucanotransferase from *Thermus aquaticus*

Seung Hee Lee, Seung Jun Choi, Sang Ick Shin<sup>1</sup>, Kwan Hwa Park, and Tae Wha Moon\*

Department of Agricultural Biotechnology, Center for Agricultural Biomaterials and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

<sup>1</sup>Korea Yakult R&D Center, Korea Yakult, Yongin, Gyeonggi 446-901, Korea

**Abstract** Sweet potato starch was modified using *Thermus aquaticus*  $\alpha$ -1,4-glucanotransferase (T $\alpha$ GT), and its structural and rheological properties were investigated. T $\alpha$ GT-modified starch had a lower amylose level and molecular weight than raw starch. The chain length distribution showed an increased number of short and long branched chains and the formation of cycloamyloses. Compared with raw starch, T $\alpha$ GT-modified starch displayed a lower gelatinization enthalpy and a wider melting temperature range. The X-ray diffraction of T $\alpha$ GT-modified starch was a weak V-type pattern with distinct sharp peaks at 13 and 20°. Scanning electron micrographs of modified starch exhibited big holes on the surface and the loss of granular structure. The frequency sweep measurement revealed that the gel of T $\alpha$ GT-modified starch was more rigid than raw starch gel. However, the structure of modified starch gel was destroyed by heating at 75°C, and a firm gel was re-formed by subsequent storage at 5°C, indicating thermoreversible property.

**Keywords:** 4- $\alpha$ -glucanotransferase, rheological property, structural property, sweet potato starch, *Thermus aquaticus*

### Introduction

Starch is a major storage polysaccharide of many economically important crops such as wheat, rice, maize, tapioca, and potato (1,2). Sweet potato is an important economic crop that can successfully adapt to a wide range of habitats including marginal regions (3). However, raw starch has several physical and chemical disadvantages such as low solubility, irreversible retrogradation, and low fluidity at room temperature of the starch gel. These factors are not appropriate for food application (4,5). Therefore, researches have been conducted to improve the solubility and retrograding properties of starch by reducing its molecular weight using enzymatic, chemical, or physical treatment (6-8). Recently, enzymatic approaches for the starch modification have been taken (9-11), because the starch modification with enzyme is an environmentally friendly alternative to chemical methods using harsh conditions (12,13). The functional properties of modified starches produced by various carbohydrate enzymes are of great interest in food industry. Many enzymes, including  $\alpha$ -amylase, amylosucrase, and 4- $\alpha$ -glucanotransferase, have been used to modify the structural properties of starch (14-16).

*Thermococcus aquaticus*  $\alpha$ -1,4-glucanotransferase (T $\alpha$ GT), a member of the  $\alpha$ -amylase super-family (17,18), was used to change the physical and structural properties of starch in this study. This enzyme catalyzes the hydrolysis of amylose molecules and long branched chains of amylopectin molecules and the transfer of  $\alpha$ -glucan chains from non-reducing end of one  $\alpha$ -glucan molecule to the

non-reducing end of another (19). It can also catalyze an intra-molecular glucan transfer reaction within a single linear glucan molecule to form cyclic  $\alpha$ -1,4-glucan (20).

In this work, the effects of the modification with T $\alpha$ GT on the structural characteristics of sweet potato starch were investigated using gel permeation chromatography (GPC), high performance anion exchange chromatography (HPAEC), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM), and the changes in rheological properties of sweet potato starch by T $\alpha$ GT treatment were studied with a rheometer.

### Materials and Methods

**Materials** Sweet potato starch was obtained from Samyang Genex (Incheon, Korea). The proximate composition of raw sweet potato starch was as follows: moisture 10.8 $\pm$ 0.1%, crude lipid 0.1 $\pm$ 0.0%, crude ash 0.4 $\pm$ 0.1%, crude protein 0.0 $\pm$ 0.0%, and carbohydrate 88.7 $\pm$ 0.1%. Pancreatin (P-7545, activity 8 $\times$ USP/g) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and pullulanase (Promozyme<sup>®</sup> D2) and amyloglucosidase (AMG 300L, activity 300 AGU/mL) from Novozymes (Bagsvaerd, Denmark), where USP and AGU stand for United States Pharmacopoeia and amyloglucosidase activity, respectively. All other chemicals were of analytical reagent grade.

**Preparation of T $\alpha$ GT-modified starch** The *Escherichia coli* transformant carrying recombinant plasmid (p6 $\times$ HisT $\alpha$ GT) was obtained from the Food Enzymology Laboratory of Seoul National University. After the cell extract was centrifuged, the supernatant was passed through a nickel-nitrilotriacetic acid (Ni-NTA) column (Qiagen, Hilden, Germany). The column was washed with 50 mM Tris-HCl buffer (pH 7.5) containing 300 mM NaCl and 20 mM imidazole and then with 50 mM Tris-HCl

\*Corresponding author: Tel: +82-2-880-4854; Fax: +82-2-873-5095

E-mail: twmoon@snu.ac.kr

Received August 31, 2007; Revised December 28, 2007;

Accepted December 29, 2007

buffer (pH 7.5) containing 300 mM NaCl and 250 mM imidazole. The purified protein was dialyzed against 50 mM Tris-HCl buffer (pH 7.5). The purity was electrophoretically assured. Raw sweet potato starch (2.0 g) was suspended in 20 mL of water for 10 min by heating and stirring on a stirrer, and then in 20 mL of 50 mM Tris-HCl buffer (pH 7.5) in a 250-mL flask. After temperature equilibration at 70°C for 1 hr, Ta $\alpha$ GT solution (20 mL, 20 units) was added to the suspension. One unit was defined as the amount of enzyme required to degrade 1 mg of amylose per min at pH 7.5 and 70°C. The mixture was shaken (165 rpm) at 70°C for 16 hr. Protease solution was added to the mixture to inactivate the Ta $\alpha$ GT at 60°C for 30 min. The residue was precipitated with 400 mL of absolute ethanol for 3 days. The mixture was centrifuged at 3,000 $\times$ g for 20 min, and the supernatant was removed. The residue was dried at room temperature. For a control, sweet potato starch without Ta $\alpha$ GT was used with the same thermal history.

**Determination of apparent amylose content and iodine absorption spectra** The apparent amylose content of the starch was determined using a modified procedure described by Chrastil (21). Briefly, the starch was dissolved in 10 mL of 1 M NaOH at 95°C for 30 min, and 0.1 mL of the mixture was added to 1.5 mL of 0.5% trichloroacetic acid solution. Afterwards, 0.05 mL of I<sub>2</sub>-KI solution (2.5 mM I<sub>2</sub> and 6.5 mM KI) was added to the solution, reacted for 15 min at room temperature, and the absorbance was measured at 620 nm.

The mixture for determination of apparent amylose was used to investigate wave-scanning pattern. The absorption was measured at the wavelength from 400 to 800 nm.

**Gel permeation chromatography (GPC)** The molecular weight distribution of Ta $\alpha$ GT-modified starch was analyzed by GPC using a Sepharose CL-2B column following the procedure of Jane and Chen (22). A sample solution (1 mL) containing starch (about 0.3 %, w/v) was injected into a 850 $\times$ 18 mm column packed with Sepharose CL-2B gel (Pharmacia, Piscataway, NJ, USA). The column was eluted with a solution of 0.25 N KOH (0.2 mL/min) and fractions of 1 mL were collected in each test tube. Total carbohydrate of each fraction was determined by the phenol-sulfuric acid method (23).

The branch chain distribution of enzymatically modified starch was analyzed by GPC using a Bio-gel P-6 (Bio-Rad, Hercules, CA, USA) column following the procedure of Jane and Chen (22). Raw, control, and enzymatically modified starches (1%) were treated with Promozyme, a debranching enzyme, at 55°C for 12 hr. Reaction mixtures were boiled for 5 min, centrifuged at 10,000 $\times$ g for 10 min, injected into a column (100 $\times$ 1 cm) packed with Bio-gel P-6 gel, and eluted with deionized water. The flow rate was 0.27 mL/min. Fractions of 2.3 mL were collected. All eluted carbohydrates were detected by the phenol-sulfuric acid method (23).

**High performance anion exchange chromatography (HPAEC)** For the analysis of branch chain length distribution of Ta $\alpha$ GT-modified sweet potato starch, the sample was debranched by Promozyme at 55°C for 16 hr.

The reaction mixtures were boiled for 5 min to stop the debranching reaction. The branch chain length distribution was analyzed by using a HPAEC system (Dionex-300; Dionex, Sunnyvale, CA, USA) with an electrochemical detector (ED40; Dionex). A CarboPac™ PA-1 column (250 $\times$ 4 mm, Dionex) and a guard column were used for the separation of debranched samples. After the column was equilibrated with 150 mM NaOH, the sample was eluted with varied gradients of 600 mM sodium acetate in 150 mM NaOH at a flow rate of 1 mL/min. The applied gradients of sodium acetate were as follows: a linear gradient from 10 to 30% for 0-10 min, from 30 to 40% for 10-16 min, from 40 to 50% for 16-27 min, from 50 to 60% for 27-44 min, and from 60 to 64% for 44-60 min. As for the cycloamylose analysis, a linear gradient of sodium acetate from 0 to 100% was used over 70 min.

**Differential scanning calorimetry (DSC)** Thermal properties of starch samples were investigated using a DSC 120 (Seiko, Chiba, Japan). Indium was used as standard. Starch sample (5 mg) was weighed in a Seiko high pressure stainless steel pan and 15  $\mu$ L of distilled water was added. The sample pan was sealed and kept at room temperature for 24 hr. Water was used as reference. The sample pan was heated from 3 to 150°C at a rate of 5°C/min.

**X-ray diffraction** X-ray diffraction analysis was performed with an X-ray diffractometer (D5005; Bruker, Karlsruhe, Germany) operating at 30 kV and 40 mA with Cu-K $\alpha$  radiation of 0.154 nm (nickel filter). The scanning region of the diffraction angle 2 $\theta$  was 4-30°.

**Scanning electron microscopy (SEM)** Raw starch and starch modified with Ta $\alpha$ GT were analyzed using a scanning electron microscope (JSM 5410LV; Jeol, Tokyo, Japan). Dried and finely ground sample was placed on double-stick Scotch tape mounted on aluminum specimen holder. The samples were coated with a thin film of gold (30 nm) and examined at 20 kV.

**Starch digestion** Enzyme solution was prepared according to the method of Englyst *et al.* (24) with a slight modification. Pancreatin (1 g) was added to 12 mL of water in a beaker and stirred by a magnetic stirrer for 10 min. This suspension was centrifuged at 1,500 $\times$ g for 10 min. A 10 mL of the cloudy supernatant was transferred to a conical flask containing 0.2 mL amyloglucosidase solution and made up to 12 mL with distilled water.

Starch sample (30 mg) was weighed into a 2.0 mL microtube for determination of starch fraction. A 0.75 mL of sodium acetate buffer (pH 5.2) and a glass ball were added to each microtube. The microtubes were incubated in a shaking incubator at 37°C to equilibrate for 10 min. Afterward, 0.75 mL of the prepared enzyme solution was added to each microtube. The shaking incubator was adjusted to a stroke speed of 250 rpm. After 10 min and a further 230 min, each tube was boiled to stop digestion. The glucose in the supernatant obtained from centrifugation (5,000 $\times$ g, 5 min) was measured with the GOD-POD kit (BCS Co., Anyang, Korea).

**Rheological properties** Starch suspensions (8%, w/w)

were heated to make starch gel at 95°C for 30 min. The heated samples were transferred to conical tubes, cooled for 1 hr at room temperature, and stored at 5°C for 12 hr.

The dynamic viscoelasticity of starch gel was measured using an oscillatory rheometer (Rheostress RS1; HAAKE, Karlsruhe, Germany) with a cone-plate system (35 mm in diameter, cone angle 1°). Expelled materials were trimmed off. The sample at the edge of the system was covered by silicone oil to prevent drying. The linear viscoelastic region was determined by means of a strain sweep measurement. The frequency sweep measurement was carried out in a frequency range of 0.1-10 Hz at 25°C. The rheological properties during temperature ascending were measured in the range of 25-80°C at constant frequency (1 Hz) and strain (1%) with the temperature sweep rate of 5.5°C/min. For analysis of the gel properties during heating and cooling cycle, the storage modulus ( $G'$ ) and loss modulus ( $G''$ ) were measured at constant frequency (1 Hz) and strain (1%) after heating at 70°C for 1 hr, cooling at 5°C for 12 hr, re-heating at 70°C for 1 hr, and cooling at 5°C for 12 hr. All the measurements were performed in the linear viscoelastic region.

**Statistical analysis** Experiments were performed in triplicate, and data were expressed as mean $\pm$ standard deviation using the SAS system for windows (version 9.1; SAS, Cary, NC, USA) or SigmaPlot (version 8.02; SPSS, Chicago, IL, USA). The Duncan's multiple range test was applied to compare means at the 5% significance level.

## Results and Discussion

**Apparent amylose contents and iodine absorption spectra** Raw starch showed apparent amylose content of 20.8%, which was in agreement with the report of Subramony (25). Ta $\alpha$ GT-modified starch exhibited lower amylose content (5.9%) than raw starch, because the amylose in starch was hydrolyzed by Ta $\alpha$ GT. The  $\lambda_{\max}$  values of raw and control starches were presumably originated from amylose, as the  $\lambda_{\max}$  of amylose is in general between 640 and 660 nm, but that of Ta $\alpha$ GT-modified starch appeared to originate from amylopectin, which has the  $\lambda_{\max}$  of 530-550 nm (26). As compared with raw and control starches, the  $\lambda_{\max}$  of Ta $\alpha$ GT-modified starch was slightly shifted to the left, and its degree decreased (Fig. 1). These results indicate the decreases in the amylose content and the branched chain length in modified starch.

**Molecular weight distribution** The molecular weight distribution of Ta $\alpha$ GT-modified sweet potato starch determined by GPC is presented in Fig. 2. The GPC chromatogram of raw starch was similar to that of control. In control, with reference to the retention time, the GPC chromatogram could be divided into 2 parts: the early fraction, eluted from 10 to 70 min (amylopectin) and the later fraction, eluted from 70 to 200 min (amylose). However, in Ta $\alpha$ GT-modified starch, the early fraction, which corresponds to the amylopectin macromolecules, decreased in height and a new fraction (eluted from 120 to 240 min) developed. This new fraction was likely originated from the degraded amylopectin, rearranged

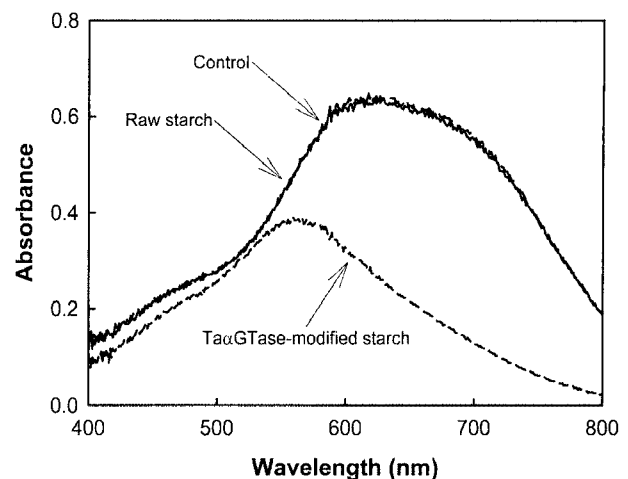


Fig. 1. The wave-scanning patterns of starches.

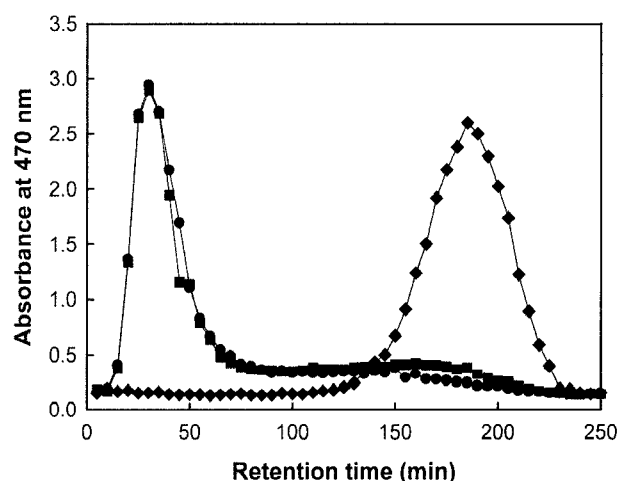
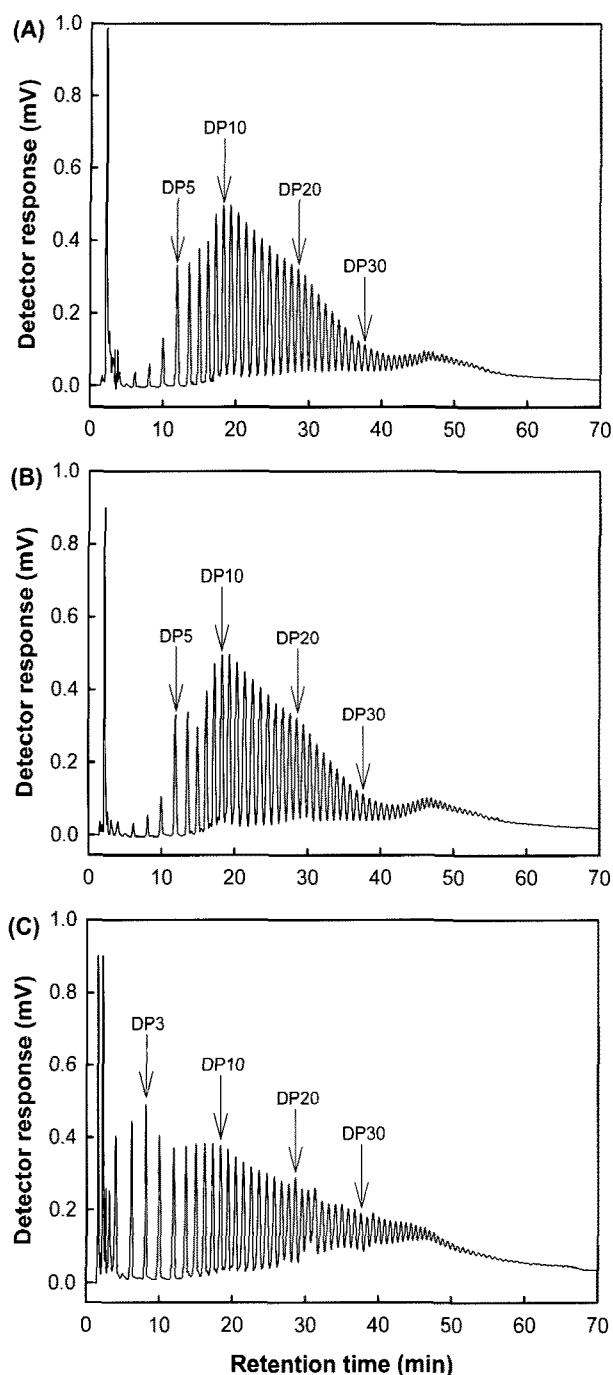


Fig. 2. Sepharose CL-2B gel permeation profiles of starches. (●) Raw sweet potato starch; (■) control; (◆) Ta $\alpha$ GT-modified sweet potato starch.

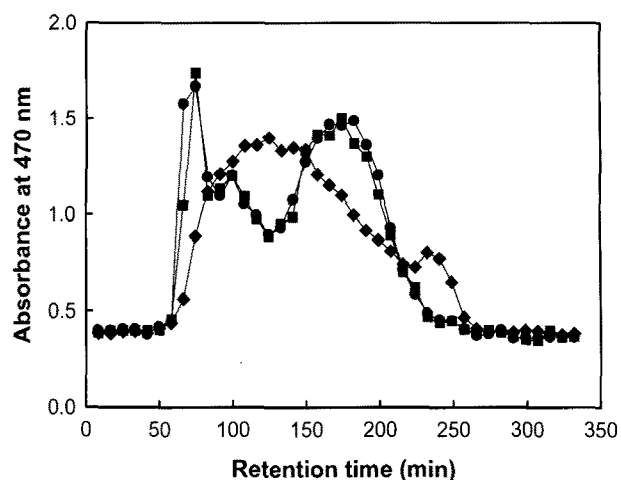
amylopectin which had short branched-chain length, and degraded amylose, because apparent amylose content of Ta $\alpha$ GT-treated starch was lower than that of raw starch as determined by the colorimetric method. Therefore, the present results revealed that the contents of amylose and high molecular weight fraction of amylopectin were decreased and long branched-chains of amylopectin were degraded and rearranged by Ta $\alpha$ GT treatment.

**Branch chain length distribution** The branch chain length distribution of raw starch (Fig. 3A) was very similar to that of control starch (Fig. 3B). The branch chain length distribution of control starch showed the greatest amount of branches with chain length of a degree of polymerization of 11 (DP11, Fig. 3A). The branch chain length distributions of control and Ta $\alpha$ GT-modified starches differed, as observed after debranching. Analysis of the branch chain length distribution of the Ta $\alpha$ GT-treated starch revealed that the number of short (<DP10) and long (>DP30) side chains increased as compared with raw and control starches, whereas the number of medium chains (10<DP<30) decreased (Fig. 3C). Kaper *et al.* (27)

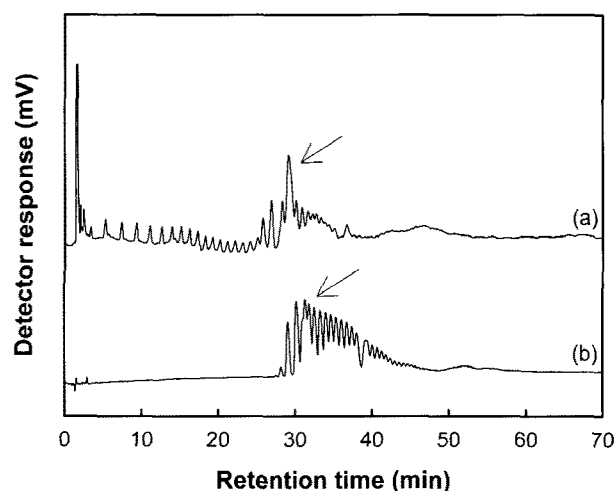


**Fig. 3.** HPAEC analysis for branched chain length distribution of starches. (A) Raw sweet potato starch; (B) control; (C) Ta $\alpha$ GT-modified sweet potato starch.

reported a broad branch chain length distribution in potato starch after treatment with 4- $\alpha$ -glucanotransferase from the hyperthermophilic archaeon *Pyrobaculum aerophilum* IM2, displaying an increase in very small and longer branch chains compared with raw potato starch. Gel permeation chromatogram was in accordance with the result of HPAEC (Fig. 4). The very long branch chains, eluted around 75 min, of raw starch disappeared in the modified starch. The amounts of long chain, eluted from 75 to 150 min, and short chain, eluted around 220 min, of the modified starch increased compared with raw starch, whereas the amount of medium branch chain, eluted from



**Fig. 4.** Bio-gel P-6 gel permeation profiles of starches debranched by pullulanase. (●) Raw sweet potato starch; (■) control; (◆) Ta $\alpha$ GT-modified sweet potato starch.



**Fig. 5.** HPAEC analysis of cycloamyloses produced by Ta $\alpha$ GT. (a) Ta $\alpha$ GT-modified sweet potato starch; (b) cycloamylose standard (DP21-33). An arrow indicates a group of cycloamylose peaks.

150 to 220 min, decreased. The increase in the number of longer side chains could be due to the intermolecular transfer from amylose to amylopectin that partially elongated the amylopectin side chains and the partial hydrolysis of inner long chains (probably B or C chains). The increase in the number of shorter side chains and the decrease in the number of medium side chains were possibly due to the partial hydrolysis and intramolecular rearrangement of branch chains of amylopectin.

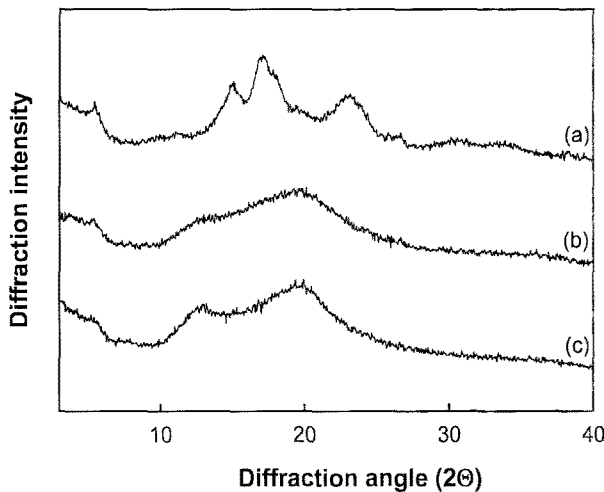
Ta $\alpha$ GT can catalyze an intramolecular glucan transfer reaction within a single linear glucan molecule to form cyclic  $\alpha$ -1, 4-glucan (28). The ability of Ta $\alpha$ GT to produce cycloamylose from sweet potato starch was examined using HPAEC (Fig. 5). HPAEC analysis showed that cycloamyloses during Ta $\alpha$ GT treatment were eluted at the similar elution time to cycloamylose standard (DP21-33). Cycloamylose may be converted from amylose because amylose content decreased, previously determined by colorimetric method, although some amylose was used for the intermolecular transfer from amylose to amylopectin.

**Table 1. Thermodynamic data of raw, control, and Ta $\alpha$ GT-modified sweet potato starches<sup>1)</sup>**

	$T_o$	$T_p$	$T_c$	$T_c-T_o$	$\Delta H$ (J/g)
Raw starch	50.2 $\pm$ 0.0 <sup>(c2)</sup>	71.5 $\pm$ 0.5 <sup>B</sup>	82.4 $\pm$ 0.2 <sup>B</sup>	30.4 $\pm$ 0.2 <sup>A</sup>	12.2 $\pm$ 0.3 <sup>C</sup>
Control	33.9 $\pm$ 0.1 <sup>B</sup>	56.5 $\pm$ 0.2 <sup>A</sup>	75.5 $\pm$ 0.1 <sup>A</sup>	41.5 $\pm$ 0.2 <sup>B</sup>	1.6 $\pm$ 0.2 <sup>A</sup>
Modified starch	47.4 $\pm$ 0.6 <sup>A</sup>	81.5 $\pm$ 0.7 <sup>C</sup>	95.0 $\pm$ 0.5 <sup>C</sup>	47.6 $\pm$ 0.4 <sup>C</sup>	5.3 $\pm$ 0.6 <sup>B</sup>

<sup>1)</sup> $T_o$ , onset temperature;  $T_p$ , peak temperature;  $T_c$ , conclusion temperature;  $T_c-T_o$ , temperature range of gelatinization;  $\Delta H$ , gelatinization enthalpy.

<sup>2)</sup>The values with different superscripts within a column are significantly different ( $p < 0.05$ ) by Duncan's multiple range test



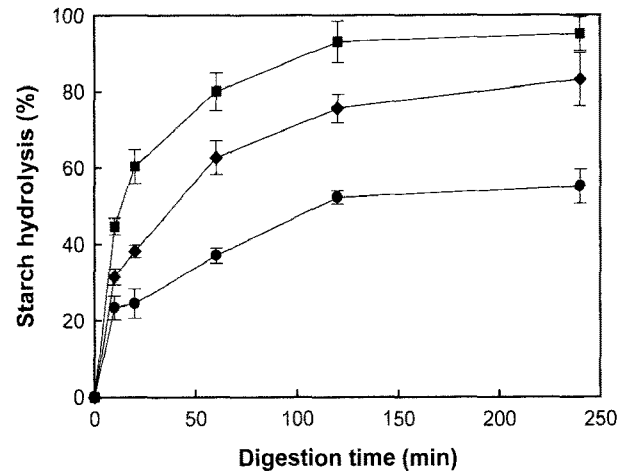
**Fig. 6. X-ray diffractograms of starches.** (a) Raw sweet potato starch; (b) control; (c) Ta $\alpha$ GT-modified sweet potato starch.

**Thermal properties** The thermal properties of raw, control, and Ta $\alpha$ GT-modified sweet potato starches are presented in Table 1. Control showed thermal properties similar to those of typical gelatinized starch. The peak temperature ( $T_p$ ) and the gelatinization enthalpy ( $\Delta H$ ) of modified starch increased compared with control. One possible cause of this is the easy retrogradation of small amylose molecules. Jane and Chen (22) reported that the gel prepared with a mixture of long branch chain amylopectin and small molecular amylose had a high gel strength. Another possible reason for our results is the cycloamylose formed by Ta $\alpha$ GT because cycloamylose is thermally stable (28).

Ta $\alpha$ GT-modified sweet potato starch showed the greatest gelatinization temperature range,  $T_c-T_o$ . A broader melting range implies a more heterogeneous structure (29). Because the Ta $\alpha$ GT-treated starch had lower levels of apparent amylose and amylopectin having low molecular weight, it appeared to have a more heterogeneous structure than raw starch.

#### Crystalline properties determined by X-ray diffractometry

X-ray diffractograms of sweet potato starch are exhibited in Fig. 6. The X-ray diffraction patterns of raw starch had peaks at 5.5, 15.0, 17.1, 23.1, and 26.7° (Fig. 6a). It is known that X-ray diffraction peaks at 5.5, 15, 17, and 22 or 23° are characteristics of C-type starch that is common to most sweet potato starches, and the C-type is a mixture of A- and B-types by various proportions (30). The X-ray diffraction pattern for Ta $\alpha$ GT-modified starch was similar to that of control, but the peaks at around 13 and 20° were

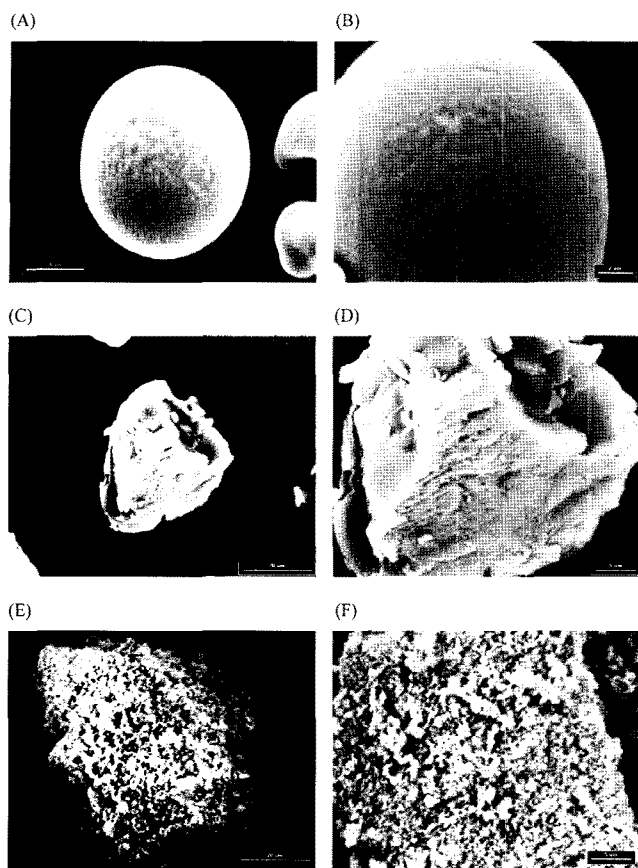


**Fig. 7. The hydrolysis patterns of raw (●), control (■), and Ta $\alpha$ GT-modified sweet potato starches (◆).**

pronounced compared with control, corresponding to the V-polymorph (31). Control starch also displayed a weak V-type pattern, but its peak intensity was lower than that of Ta $\alpha$ GT-treated starch. The weak V-type pattern, that is formed from collapsed amylose helices in which chemical adjuncts are trapped inside (32), of control and modified starches could be due to cooking effects. The difference in peak intensity of control and modified starches was probably due to an increased number of shorter and longer branch chains of amylopectin and the rearrangement of amylopectin in Ta $\alpha$ GT-modified starch. Those changes influenced the crystalline structure resulting in the different relative crystallinity of Ta $\alpha$ GT-modified starch (33).

#### Enzymatic hydrolysis pattern

The *in vitro* enzyme digestibility of sweet potato starch was determined by hydrolyzing starch granules with pancreatin and amyloglucosidase. Figure 7 shows the hydrolysis rate of starches. The digestibility of control was higher than that of treated starch. Holm *et al.* (34) reported that digestibility of wheat starch was increased by heat treatment in comparison with that of raw starch. The hydrolysis rate of Ta $\alpha$ GT-treated starch was lower than that of control starch, presumably due to an increase in crystallinity and the cycloamylose formation. Cycloamyloses are less susceptible to amylase action than are linear oligosaccharides (35). They are completely resistant to  $\beta$ -amylases as cycloamyloses do not contain end groups susceptible to the attack of this enzyme. However,  $\alpha$ -amylases do not require end groups and are therefore capable of hydrolyzing cycloamyloses, although at a slow rate (5). This result is in agreement with

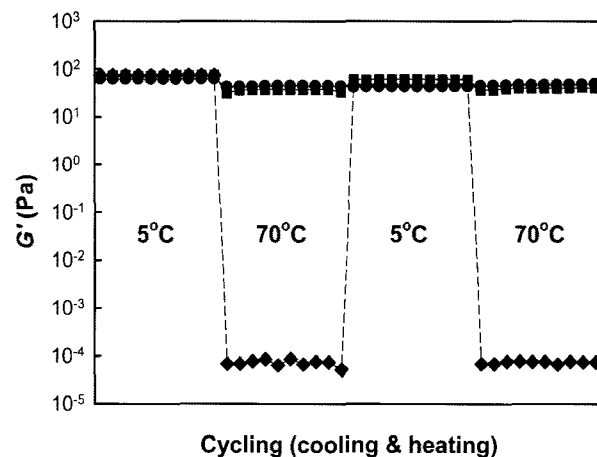


**Fig. 8.** Scanning electron micrographs of starches. (A) Raw sweet potato starch (5,000 $\times$ ); (B) raw sweet potato starch (7,500 $\times$ ); (C) control (1,500 $\times$ ); (D) control (3,500 $\times$ ); (E) Ta $\alpha$ GT-modified sweet potato starch (1,500 $\times$ ); (F) Ta $\alpha$ GT-modified sweet potato starch (3,500 $\times$ ).

the X-ray diffraction intensity, gelatinization temperature, and gelatinization enthalpy change of the Ta $\alpha$ GT-treated starch.

**Microscopic observation** Scanning electron micrographs of raw and treated starches are presented in Fig. 8. The raw starch (Fig. 8A and 8B) displayed spherical or angular-shaped granules with smooth surface. However, control and modified starches showed disrupted and reassociated structure, and bigger, irregularly-shaped particles as compared with raw starch (Fig. 8C, 8D, 8E, and 8F). These morphological changes could be due to the enzyme treatment conditions under which the granular structure of raw sweet potato starch was completely destroyed and reassociated. The surface of modified sweet potato starch was rougher than that of control. This could be explained by considering that the lower amylose level and increased number of long and short branch chains of amylopectin in the Ta $\alpha$ GT-modified starch probably caused the dissimilarity in reassociation pattern leading to different surface characteristics.

**Thermoreversibility of Ta $\alpha$ GT-modified sweet potato starch gel** The raw and Ta $\alpha$ GT-modified sweet potato starch gels exhibited a lack of frequency dependence and large differences between  $G'$  and  $G''$  over the frequency



**Fig. 9.** The changes in storage modulus ( $G'$ ) with various thermoreversible cycles. ( $\bullet$ ) Raw sweet potato starch; ( $\blacksquare$ ) control; ( $\blacklozenge$ ) Ta $\alpha$ GT-modified sweet potato starch.

range tested (0.1–10 Hz), confirming that a solid-gel structure was formed (data not shown). The  $G'$  of Ta $\alpha$ GT-modified starch gel was higher than that of raw starch gel, implying that the modified starch gel had stronger gel structure than raw starch gel. Hosoney (36) described that when starch resulted from acid treatment was cooled, its chains tended to interact more easily and thus form a more rigid gel than raw starch. Leached-out amylose during cooking plays a role in forming the gel network (37). In the present study, however, the apparent amylose content of Ta $\alpha$ GT-modified starch was lower than that of raw starch. Although the content of amylose and the molecular weight of amylose and amylopectin decreased to a certain level with Ta $\alpha$ GT treatment, the modified starch could be large enough to form a rigid gel through reassociation. This result agrees well with the report by Lee *et al.* (14).

Many researchers reported the formation of thermo-reversible starch gel after the treatment of starch with 4- $\alpha$ -glucanotransferase (14,38,39). In this study, the strength of Ta $\alpha$ GT-modified sweet potato starch gel structure was dramatically weakened as temperature increased. The heating and cooling cycle was repeated and  $G'$  measured at the end of each cycle are presented in Fig. 9. The dynamic viscoelasticity of raw starch gel was slightly changed during the heating and cooling cycle. On the other hand, the  $G'$  of Ta $\alpha$ GT-modified sweet potato starch gel showed reversible changes during heating and cooling cycle. This could be explained as follows. First, the content of amylose, which has large chains for strong double-helical bond, was decreased, whereas the content of amylopectin having short chains for weak double-helical bond, was increased. The longer chains have been reported to require a higher temperature to dissociate completely than that required for shorter double helices (40). Therefore, the structure of Ta $\alpha$ GT-modified starch gel is easily collapsed with an increase in temperature. Second, as stated above, the gel having high strength could be prepared from a mixture of long branched chain amylopectin and small amylose molecules (22). The increase in long branched chains of amylopectin and the decrease in length of amylose after enzyme treatment allowed gel to be harder, but their

association were dissolved by heating because of the increase in short branch chains of amylopectin in modified starch. Third, the opportunities for the formation of double-helical bond without steric hindrance is increased due to its small size of chains and large mobility of amylopectin in the Ta $\alpha$ GT-modified starch gel, which gives the less kinetic energy for dissociation. Consequently, the  $G'$  and  $G''$  of Ta $\alpha$ GT-modified starch gel were higher than those of raw starch gel at relatively low temperature where the dissociation of double-helical bond could not occur. However, removal of all the long linear chains from starch could induce the formation of weak gel. The increase in the number of short chains and the decrease in average chain length of amylopectin are presumably required for the gel thermoreversibility.

In conclusion, sweet potato starch could be modified with a glucanotransferase originated from *Thermus aquaticus*. The molecular weight of enzyme-modified starch molecules decreased. The amounts of short and long chains in amylopectin increased by Ta $\alpha$ GT treatment, but the amount of medium chains decreased. The gel prepared with this modified starch had thermoreversible property. The Ta $\alpha$ GT treatment, therefore, could be useful considering a demand for novel starchy ingredients having thermoreversibility in food industry.

## Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-005-J04703).

## References

- van der Maarel MJEC, van der Veen B, Uitdehaag JCM, Leemhuis H, Dijkhuizen L. Properties and applications of starch-converting enzymes of the  $\alpha$ -amylase family. *J. Biotechnol.* 94: 137-155 (2002)
- Srichuwong S, Jane J-L. Physicochemical properties of starch affected by molecular composition and structure: A review. *Food Sci. Biotechnol.* 16: 663-674 (2007)
- Zhang T, Oates CG. Relationship between  $\alpha$ -amylase degradation and physico-chemical properties of sweet potato starches. *Food Chem.* 65: 157-163 (1999)
- Smith PS. Starch derivatives and their use in foods. pp. 237-269. In: *Food Carbohydrates*. Lineback DR, Inglett GE (eds). AVI, Westport, CT, USA (1982)
- Xie SX, Liu Q, Cui SW. Starch modification and application. pp. 357-405. In: *Food Carbohydrates: Chemistry, Physical Properties, and Application*. Cui SW (ed). CRC Press, Boca Raton, FL, USA (2005)
- Bechtel WG. Staling studies of bread made with flour fractions. V. Effect of a heat-stable amylase and a cross-linked starch. *Cereal Chem.* 36: 368-372 (1959)
- Han X-Z, Ao Z, Janaswamy S, Jane J-L, Chandrasekaran R, Hamaker BR. Development of a low glycemic maize starch: Preparation and characterization. *Biomacromolecules* 7: 1162-1168 (2006)
- Hebeda RE, Bowles LK, Teague WM. Development in enzymes for retarding staling of baked goods. *Cereal Food World* 35: 453-457 (1990)
- Park JH, Park KH, Jane J-L. Physicochemical properties of enzymatically modified maize starch using 4-glucanotransferase. *Food Sci. Biotechnol.* 16: 902-909 (2007)
- Alexander RJ. New starches for food application. *Cereal Food World* 41: 796-798 (1996)
- Davis JP, Supatcharee N, Khandelwal RI., Chibbar RN. Synthesis of novel starches in planta: Opportunities and challenges. *Starch/Stärke* 55: 107-120 (2003)
- Brandam C, Mayer XM, Proth J, Strehaiano P, Piaguad H. An original kinetic model for the enzymatic hydrolysis of starch during mashing. *Biochem. Eng. J.* 13: 43-52 (2003)
- Hamdi G, Ponchel G. Enzymatic degradation of epichlorohydrin crosslinked starch microspheres by alpha-amylase. *Pharm. Res.* 16: 867-875 (1999)
- Lee KY, Kim YR, Park KH, Lee HG. Effect of  $\alpha$ -glucanotransferase treatment on the thermo-reversibility and freeze-thaw stability of a rice starch gel. *Carbohydr. Polym.* 63: 347-354 (2006)
- Rolland-Sabaté A, Colonna P, Potocki-Véronèse G, Monsan P, Planchot V. Elongation and insolubilisation of  $\alpha$ -glucan by the action of *Neisseria polysaccharea* amylosucrase. *J. Cereal Sci.* 40: 17-30 (2004)
- Kamasaka H, Sugimoto K, Takara H, Nishimura T, Kuriki T. *Bacillus stearothermophilus* neopullulanase selective hydrolysis of amylose to maltose in the presence of amylopectin. *Appl. Environ. Microb.* 68: 1658-1664 (2002)
- Kuriki T, Imanaka T. The concept of the  $\alpha$ -amylase family: Structural similarity and common catalytic mechanism. *J. Biosci. Bioeng.* 87: 557-565 (1999)
- Svensson B. Protein engineering in the  $\alpha$ -amylase family: Catalytic mechanism, substrate specificity, and stability. *Plant Mol. Biol.* 25: 141-157 (1994)
- Takaha T, Yanase M, Okada S, Smith SM. Disproportionating enzyme (4- $\alpha$ -glucanotransferase; E.C 2.4.1.25) of potato: Purification, molecular cloning, and potential role in starch metabolism. *J. Biol. Chem.* 268: 1391-1396 (1993)
- Takaha TM, Yanase H, Takata S, Smit SM. Potato D-enzyme catalyzes the cyclization of amylose to produce cycloamylose, a novel cyclic glucan. *J. Biol. Chem.* 271: 2902-2908 (1996)
- Chrastil J. Improved colorimetric determination of amylose in starches or flours. *Carbohydr. Res.* 159: 154-158 (1987)
- Jane J, Chen J. Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.* 69: 60-65 (1992)
- Dubois M, Gilles KA, Hamilton K, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350-356 (1956)
- Englyst HN, Kingman SM, Cummings JH. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* 46: S33-S50 (1992)
- Subramony NM. Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke* 54: 559-592 (2002)
- Hizukuri S, Abe J, Hanashiro I. Starch: Analytical aspects. pp. 305-390. In: *Carbohydrates in Food*. 2<sup>nd</sup> ed. Eliasson A-C (ed). CRC Press, Boca Raton, FL, USA (2006)
- Kaper T, Talik B, Ettema TJ, Bos H, van der Maarel MJEC, Dijkhuizen L. Amylomaltase of *Pyrobaculum aerophilum* IM2 produces thermoreversible starch gels. *Appl. Environ. Microb.* 71: 5098-5106 (2005)
- Tedra Y, Fujii K, Takaha T, Okada S. *Thermus aquaticus* ATCC 33923 amyloamylase gene cloning and expression and enzyme characterization: Production of cycloamylose. *Appl. Environ. Microb.* 65: 910-915 (1999)
- Marchant JL, Blanshard MV. Studies of the dynamics of the gelatinization of starch granules employing a small angle light scattering system. *Starch/Stärke* 30: 257-264 (1978)
- Imberty A, Buleon A, Tran V, Perez S. Recent advances in knowledge of starch structure. *Starch/Stärke* 43: 375-384 (1991)
- Hizukuri S, Takeda Y, Usami S, Takese Y. Effect of aliphatic hydrocarbon groups on the crystallization of amylopectin: Model experiments for starch crystallization. *Carbohydr. Res.* 83: 193-199 (1980)
- Zobel HF. Molecules to granules: A comprehensive starch review. *Starch/Stärke* 40: 44-50 (1988)
- Perera C, Lu Z, Sell J, Jane J. Comparison of physicochemical properties and structures of sugary-2 cornstarch with normal and waxy cultivars. *Cereal Chem.* 78: 249-256 (2001)
- Holm J, Lundquist I, Björck I, Eliasson A-C, Asp NG. Degree of starch gelatinization, digestion rate of starch *in vitro*, and metabolic

- response in rats. *Am. J. Clin. Nutr.* 47: 1010-1016 (1988)
35. Kainuma K. Starch oligosaccharides: Linear, branched, and cyclic. pp. 125-152. In: *Starch: Chemistry and Technology*. 2<sup>nd</sup> ed. Whistler RL, BeMiller JN, Paschall EF (eds). Academic Press, Inc., Orlando, FL, USA (1984)
  36. Hosney RC. Starch. pp. 29-64. In: *Principles of Cereal Science and Technology*. American Association of Cereal Chemists, St. Paul, MN, USA (1994)
  37. Miles MJ, Morris VJ, Ring SG. Gelation of amylose. *Carbohydr. Res.* 135: 257-269 (1996)
  38. Euverink GJW, Binnema DJ. Use of modified starch as an agent for forming a thermoreversible gel. U.S. patent 6,864,063 (2005)
  39. Kaper T, van der Maarel MJEC, Euverink GJW, Dijkhuizen L. Exploring and exploiting starch-modifying amylomaltases from thermophiles. *Biochem. Soc. T.* 32: 279-282 (2004)
  40. Yuan RC, Thompson DB, Boyer CD. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three *wx*-containing genotypes in two inbred lines. *Cereal Chem.* 70: 81-89 (1993)