*Food Sci. Biotechnol. Vol. 16, No. 6, pp. 902 ~ 909 (2007)*

Food Science and Biotechnology



# Physicochemical Properties of Enzymatically Modified Maize Starch Using 4-α-Glucanotransferase

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**Abstract** Granular maize starch was treated with *Thermus scotoductus* 4-α-glucanotransferase (α-GTase), and its physicochemical properties were determined. The gelatinization and pasting temperatures of α-GTase-modified starch were decreased by higher enzyme concentrations. α-GTase treatment lowered the peak, setback, and final viscosity of the starch. At a higher level of enzyme treatment, the melting peak of the amylose-lipid complex was undetectable on the DSC thermogram. Also, α-GTase-modified starch showed a slower retrogradation rate. The enzyme treatment changed the dynamic rheological properties of the starch, leading to decreases in its elastic (G') and viscous (G") moduli. α-GTase-modified starch showed more liquid-like characteristics, whereas normal maize starch was more elastic and solid-like. Gel permeation chromatography of modified starch showed that amylose was degraded, and a low molecular-weight fraction with $M_w$ of $1.1\times10^5$ was produced. Branch chain-length (BCL) distribution of modified starch showed increases in BCL (DP>20), which could result from the glucans degraded from amylose molecules transferred to the branch chains of amylopectin by inter-/intra-molecular transglycosylation of α-GTase. These new physicochemical functionalities of the modified starch produced by α-GTase treatment are applicable to starch-based products in various industries.

**Keywords:** modified starch, 4-α-glucanotransferase, physicochemical property, dynamic rheology, retrogradation, transglycosylation

## Introduction

Starch is one of the most abundant biomasses; both native and modified starches are used extensively in various industries, including paper, textile, and food. Enzymatic modifications of starch have been conducted by treating starch with amylolytic enzymes that cause molecular structure changes in the starch and reduce the rate of staling of bread (1-3). Amylases, as effective antistaling enzymes, hydrolyze amylose and amylopectin into smaller chains, which lead to limitation of retrogradation (4). Wursch and Gumy (5) report that β-amylase inhibits the retrogradation of amylopectin. This effect is attributed to the shortening of the external amylopectin chains to the lengths that do not crystallize. Uses of combinations of amylolytic enzymes such as glucoamylase, pullulanase, and α-amylase isolated from thermophiles develop an ideal starch saccharification process (6). Recently, it has been reported that a promising approach of modifying the structure of starch is the selective removal of the long chains in amylose. The substrate specificity of neopullulanase was analyzed toward amylose and amylopectin. Although it completely hydrolyzed amylose to maltose as the main product, it scarcely hydrolyzed amylopectin. The neopullulanase selectively hydrolyzed amylose when starch was used as a substrate (7). The enzymatically modified starches have significantly different properties from the native starch, leading to use in various starch-based food products and other industrial applications.

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Received February 15, 2007; accepted May 29, 2007

The enzyme 4-α-glucanotransferase (α-GTase, EC 2.4.1.25) is a glycosyl transferase belonging to family 13 of amylolytic enzymes, which has $(\beta/\alpha)_8$-barrel fold in the catalytic domains (8). The enzyme can catalyze the transfer of α-glucan chains from the non-reducing end of one α-glucan molecule to the non-reducing end of another α-glucan chain as an intermolecular transglycosylation (9-12). It also catalyzes an intramolecular transglycosylation, creating a cyclic glucan (cycloamylose) (13-15). The α-GTase used in this study is originated from *Thermus scotoductus* (GenBank accession number: AY459351). The maximum activity of α-GTase shows at pH 7.0 and 75°C. α-GTase exhibits a high thermal stability, retaining 90% activity after 120 min incubation at 80°C. The half-life of α-GTase is 210 and 29 min at 85 and 90°C, respectively (16). Many benefits, such as higher substrate concentrations, limited risk of bacterial contamination, increased reaction rate, decreased reaction time, and lower costs in enzyme purification, are produced by using thermal stable enzyme at high temperature (17).

In this study, we investigated the effect of α-GTase treatment on the structure and properties of normal maize starch. Granular starch was treated with various concentrations of α-GTase, and the enzymatically modified starches were characterized for their physicochemical properties.

## Materials and Methods

**Materials** Normal maize starch used in this study was a gift of Cerestar USA (Hammond, IN, USA). Purified α-GTase originating from *Thermus scotoductus* was provided by the laboratory of food enzymology of Seoul National

University (Seoul, Korea). Isoamylase from *Pseudomonas amyloderomosa* was purchased from Hayashibara Co. (Okayama, Japan). Pullulan standards (Shodex Standard P-82) were purchased from Showa Denko K.K. (Tokyo, Japan). All other chemicals were of analytical grade.

**Assay of α-GTase activity** The activity of α-GTase was determined by measuring the absorbance change in iodine-staining blue color during the conversion of amylose by the enzyme (18). The enzyme reaction mixture contains 250 μL of 0.2% amylose, 50 μL of 1% maltose, 600 μL of 50 mM Tris-HCl buffer (pH 7.0), and 100 μL of enzyme solution. The mixture was incubated at 75°C for 10 min. The reaction was stopped by boiling for 10 min. Aliquots (0.1 mL) were mixed with 1 mL of Iodine solution (0.02% iodine and 0.2% potassium iodide solution), and the absorbance at 620 nm was measured immediately with a spectrophotometer (Ultraspec DU® 520; Beckman, Fullerton, CA, USA). One unit (U) of α-GTase activity was defined as the amount of enzyme which degrades 0.5 mg/mL of amylose per min under the conditions described above. Protein concentrations were measured by using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA), using bovine serum albumin standard (Sigma-Aldrich Co., St. Louis, MO, USA).

**Preparation of α-GTase-modified starch** Normal maize starch (10 g, d.b.) was suspended in distilled water (100 mL). Various amounts of α-GTase per g starch (10, 25, 50, and 75 U α-GTase/g starch) were added to the starch slurry, and the mixture was incubated in a water bath at 65°C with shaking at 120 rpm for 12 hr. After the α-GTase reaction, 3 volumes of ethanol were added to the enzyme-treated starch to precipitate the starch. The ethanol-precipitated starch was separated by centrifugation at 8,300×g for 20 min. The starch was washed twice with ethanol and recovered by filtration using a Whatman No. 4 filter paper. The modified starch was dried in a convection oven at 35°C for 24 hr. Normal maize starch incubated at the same condition without α-GTase was used as a control.

**Starch molecular weight distribution** The molecular weight distributions of the control and the modified starches were determined by using a gel-permeation chromatography (GPC) following to the method reported by Jane and Chen (19). Starch (0.5 g) was wetted with water (5 mL) and dispersed in dimethyl sulfoxide (DMSO, 45 mL). The starch suspension was heated in a boiling water bath for 1 hr and then stirred overnight at room temperature. Ethanol (6 mL) was added to a starch dispersion (2 mL) to precipitate the starch, and followed by centrifugation. The precipitated starch was redissolved in 10 mL boiling water and stirred for 30 min, and the solution was filtered through a 5.0 nylon filter. The starch solution (5 mL) containing 25 mg starch was injected onto a Sepharose CL-2B gel (Pharmacia, Piscataway, NJ, USA) column (2.6×90 cm). The eluent was an aqueous solution with 25 mM NaCl and 1 mM NaOH at a flow rate of 0.5 mL/min in an ascending direction. The elution profiles were analyzed for total carbohydrates (phenol-sulfuric acid method) and amylose-iodine blue value at 490 and 630 nm, respectively, using an Ultra Microplate reader (Bio-Tek Instrument, Winooski, VT, USA) (20).

**Branch chain-length (BCL) distribution** The BCL distributions of the control and the modified starches were determined following the procedure of Jane *et al.* (21). Starch was debranched using *P. amyloderamosa* isoamylase (60 U) and incubated at 40°C for 6 hr. Branched chain distributions were analyzed by using a high-performance anion-exchange chromatography (HPAEC, Dionex-300; Dionex, Sunnyvale, CA, USA) equipped with an on-line amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) following the method reported by Wong and Jane (22). A CarboPac™ PA100 anion-exchange column (4×250 mm, Dionex) and a guard column (3×25 mm) were used for the separation of debranched samples. The sample was eluted with 100 mM NaOH with a gradient of sodium nitrate (300 mM) at a flow rate of 0.5 mL/min. The separation gradient was 1% at 0 min, 5% at 39 min, 8% at 50 min, 30% at 170 min, and 45% at 220 min (23). The results were obtained from at least 2 replicates.

**Thermal properties of starch** Thermal properties of the native, control, and modified starches were analyzed using a differential scanning calorimeter (DSC) equipped with an Intracooling II system (DSC-7; Perkin-Elmer, Norwalk, CT, USA) following the method of Chen and Jane (24). Starch (3 mg) was precisely weighed in an aluminum pan, mixed with 9 mg of deionized water and sealed. The sealed sample was heated at a rate of 10°C/min over a temperature range of 20-110°C. An empty pan was used as the reference. The gelatinization onset temperature ($T_o$), peak temperature ($T_p$), completion temperature ($T_c$), and enthalpy change ($\Delta H$) were computed using a Pyris Manager (Perkin-Elmer). The properties of retrograded starch were determined using the same method (24). Gelatinized starch samples were stored at 4°C for 7 days.

**Pasting properties of starch** The pasting properties of the native, control, and modified starches were obtained using a Rapid Visco™ Analyzer (RVA) (RVA-4; Newport Scientific, Sydney, Australia). A starch suspension (8%, w/w) was prepared by weighing starch (2.24 g, d.b.) into an aluminum canister and making up the total weight to 28 g with distilled water. The sample suspension was heated at a rate of 6°C/min from 50 to 95°C, held at 95°C for 5 min, and then cooled down to 50°C at a rate of 6°C/min. The sample suspension was stirred at 960 rpm paddle speed for the first 10 sec, and followed by a 160 rpm paddle speed for the remainder of the analysis. The pasting temperature and viscosity factors of paste including peak, breakdown, final, and setback viscosity were recorded.

**Gel strength of starch** The starch gels of the native, control, and modified starches were prepared following the procedure of Takahashi and Seib (25). The hot starch pastes (8%) prepared by using an RVA were poured immediately into petri dishes (3 cm i.d × 3 cm height). The depth of each dish increased approximately 5 mm by taping aluminum foil around its rim. The petri dishes were covered, and the pastes were stored at 4°C for 24 hr to develop gel. After the aluminum foil was removed, a smooth, freshly cut surface was obtained by removing the

excess gel above the rim with a wire cheese cutter. The gel strength was measured using a TA-XT2*i* texture analyzer (Texture Technologies Corp., Scarsdale, NY, USA) equipped with a Texture Expert software program. The gels were compressed for a distance of 25% at a penetration speed of 1 mm/sec using a 6 mm cylinder probe with a flat end. The force applied to break the gel was recorded. The results were reported as the mean value of 10 measurements.

**Rheological properties of starch** The dynamic rheological properties of the native, control, and modified starches were measured using small deformation oscillation techniques with a controlled stress rheometer (Rheostress 1; Thermo Haake, Ettlingen, Germany). Starch paste (6%, w/v) was prepared by heating the starch slurry in a boiling water bath and stirring for 30 min. The starch paste sample was placed immediately between the cone and plate (60 mm diameter, 2° angle), and equilibrated at 30°C. The gap was 0.105 mm. Vaseline oil was used to prevent the evaporation of water. All the measurements were conducted within the linear viscoelastic region. A frequency sweep from 0.1 to 10 Hz was performed at a constant stress of 5 Pa at 30°C. The rheological properties of the starch paste were evaluated by comparing the log plots of G' and G" with frequency. All the measurements were in duplications.

## Results and Discussion

**Molecular weight distribution of α-GTase-modified starch** The size distributions of amylopectin and amylose of the control or α-GTase-modified starches were studied using GPC. Results are shown in Fig. 1. Amylopectin was eluted in fractions 26-34 from the control starch, and its

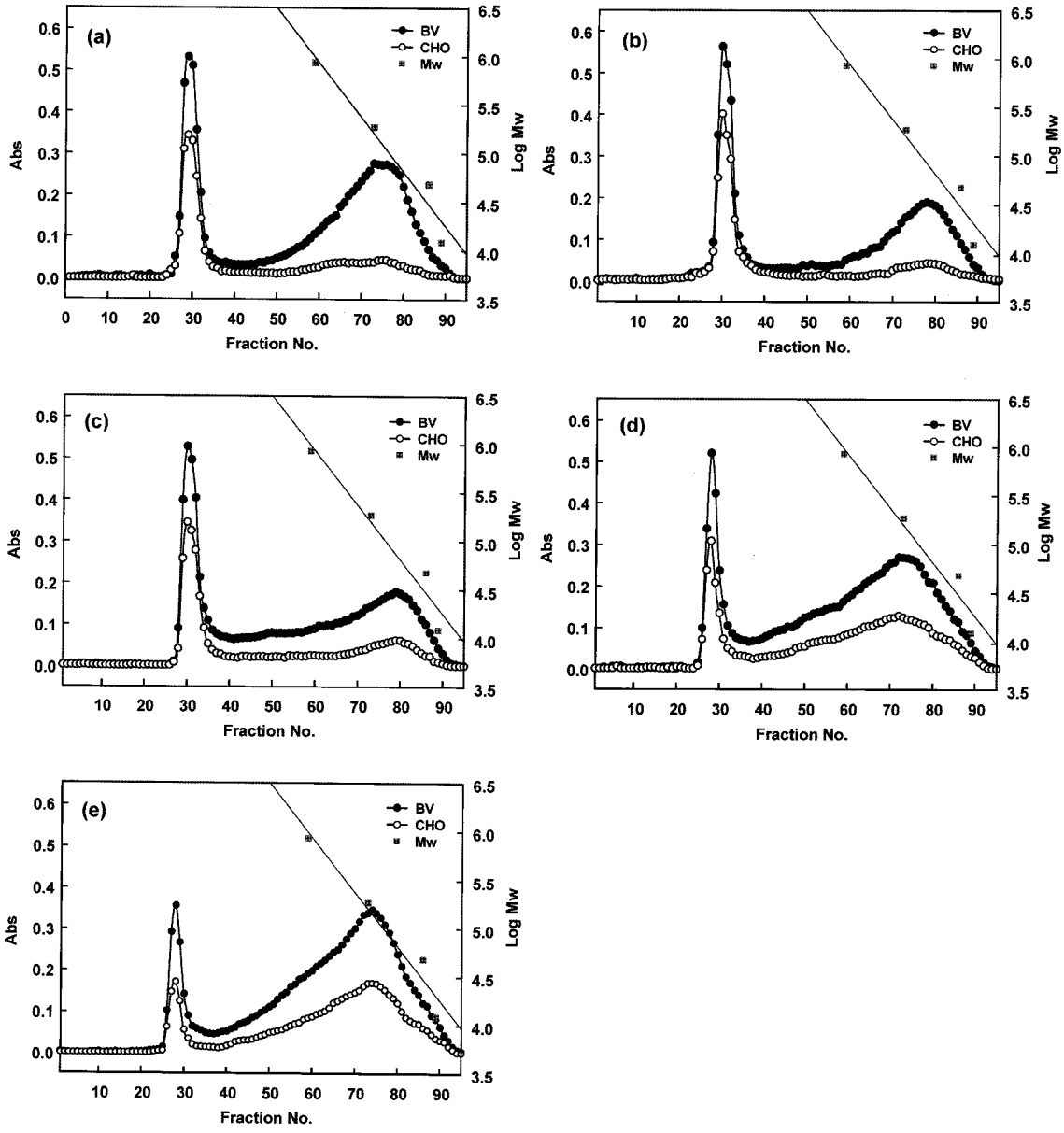


**Fig. 1. Sepharose CL-2B GPC profiles of the control and modified starches.** The control starch (a) and α-GTase treated starch including 10 U/g starch (b), 25 (c) 50 (d), and 75 U/g (e). BV, blue value; CHO, total carbohydrates; $M_w$, molecular weights.

weight-average molecular weight ($M_w$) was $3.0\times10^8$ by the HPSEC-MALLS-RI system. Amylose was eluted in fractions 50-95 from the control starch, and its $M_w$ was $1.6\times10^5$ determined using pullulan as a standard. Amylose and amylopectin fractions showed blue and purple colors by iodine staining, respectively. The ratio of blue values to total carbohydrates (BV/CHO) of the amylopectin peak was ca.1.5, whereas that of the amylose peak was ca.6.0 (Fig. 1a). For the α-GTase-treated starch (10 U/g starch), the amylose peak shifted to fractions 60-95 and the blue value of the amylose peak decreased, suggesting that α-GTase degraded the amylose and the structure of amylose changed (Fig. 1b). For the 25 U/g α-GTase-treated starch, both the total carbohydrate and blue value of the fractions 40-65 increased, indicating the increase in the intermediate components. And peaks appeared at fractions 65-95, which had the $M_w$ of $6.4\times10^4$ and the BV/CHO ratio of ca.2.8 instead of ca.6.0 (Fig. 1c). For the 50 U/g α-GTase-treated starch, the amylopectin peak decreased and the intermediate and small size fraction further increased which the BV/CHO ratio of ca.2.1 (Fig. 1d). For the 75 U/g α-GTase-treated starch, the amylopectin fraction decreased considerably and the small molecular weight fraction increased (Fig. 1e). The change in the ratio of BV/CHO and the decrease in the molecular weight of α-GTase-modified normal maize starch were results of the activity of α-GTase on amylose and amylopectin. Takaha *et al.* (26) have proposed a model for the transglycosylation of a whole amylopectin cluster unit from a larger cluster to another cluster in α-GTase. Larger amylopectin clusters in the earlier fraction is retained without further reduction in size due to the enzyme's certain rate of cluster transfer activity.

**BCL distribution of α-GTase-modified starch** The BCL distributions of the control and α-GTase-modified

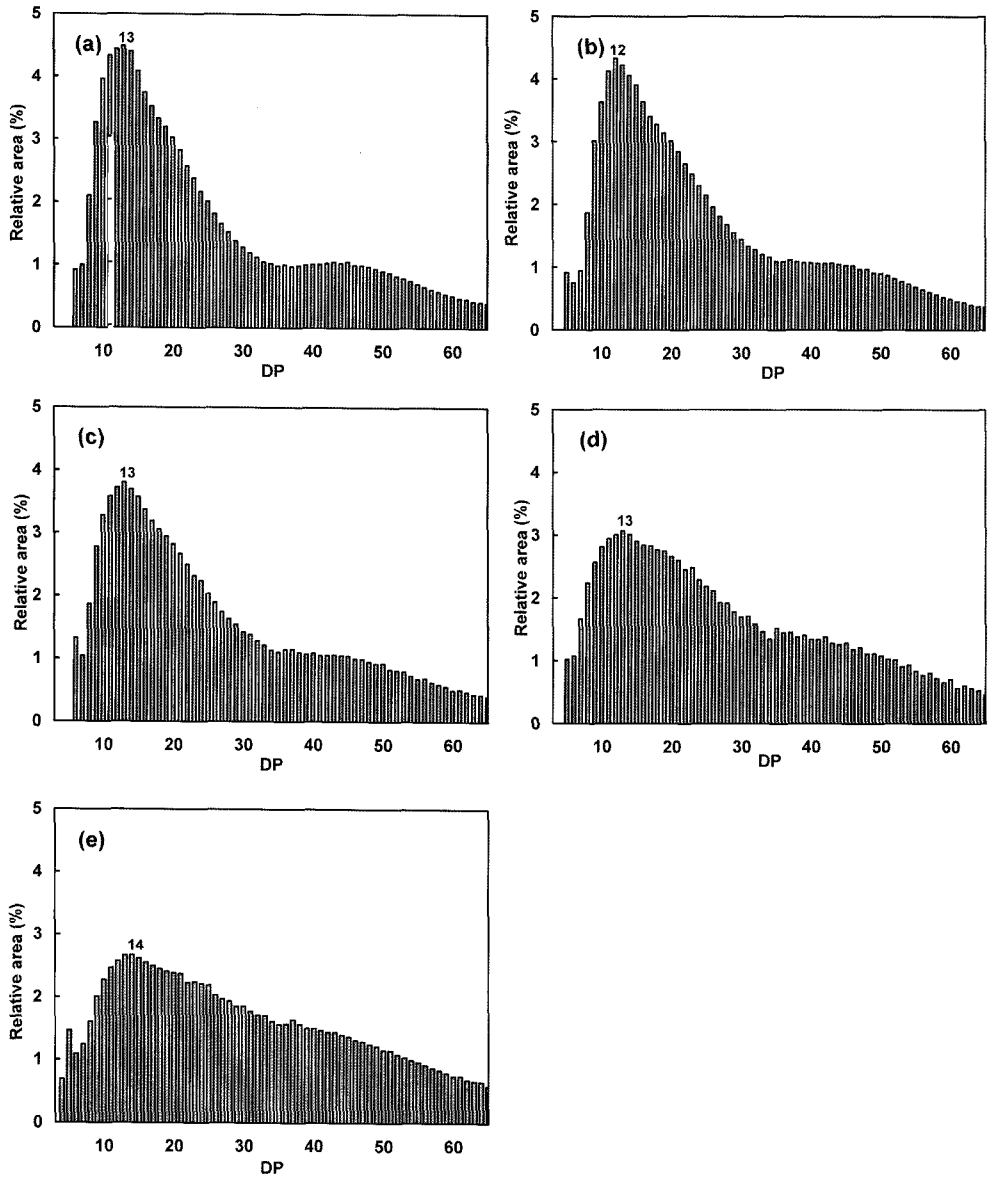


**Fig. 2. HPAEC analysis of branched chain distributions of the control and modified starches.** The control starch (a) and α-GTase treated starch including 10 U/g starch (b), 25 (c) 50 (d), and 75 U/g (e). DP, degree of polymerization.

starches were analyzed using the HPAEC-ENZ-PAD, and the results are shown in Fig. 2. The control starch showed a bimodal distribution (first peak at DP 13, second peak at DP 45). The 10 and 25 U/g α-GTase-treated starches also had bimodal distributions, whereas the 50 and 75 U/g α-GTase-treated starches had unimodal patterns of BCL distribution. The α-GTase modified starches displayed a smaller proportion of branch chains with DP 7-20 and a larger proportion of branch chains of DP>20 than the control. The results showed that the BCL of the native maize starch increased as results of α-GTase treatment. Previous studies have shown that the amylose is hydrolyzed, and the fragments are transferred to the branch chains of amylopectin through hydrolyzing and disproportion reactions of α-GTase (16). It also has been reported that α-1,4 cyclic structures (cycloamyloses) with various DP's are produced from amylose or long branch chains of amylopectin through the intramolecular transglycosylation of α-GTase (10). A previous study has shown that the minimum DP of the cycloamylose produced from amylose using α-GTase was DP 17 (16). The molecular weights of the cycloamylose ranged from DP 17 to more than 60 (16). Using a large concentration of enzyme treatment (Fig. 2d and 2e), the peaks of DP>16 shown in the HPAEC chromatograms were mixtures of α-1,4 linear and cyclic glucans. Cycloamylose is highly soluble in water, and its solution has low viscosity. Cycloamylose has been proposed to be used in food and beverage compositions, or starch substitutes for biodegradable plastics (27). It was also reported that the transfer of α-glucan from amylose to amylopectin to partly elongate amylopectin side chains after the treatment of starch with *Thermus thermophilus* α-GTase. However, the increase could also be attributed to shortened and branched amylose, even though the branched portion of amylose is much less compared to that amylopectin in starch (28). Lee *et al.* (29) claimed the formation of thermoreversible gel after the treatment of rice starch with *T. scotoductus* α-GTase. The formation of amylopectin clusters with re-organized branch chains might be responsible for the formation of thermoreversible gel, which can not be obtained from popular starch hydrolyzing enzymes, such as α-amylase.

**Thermal properties of α-GTase-modified starch** The thermal properties of the native, control, and modified starches were studied using DSC and are summarized in Table 1. Onset gelatinization temperature ($T_o$) of the native, control, 10, 25, 50, and 75 U/g α-GTase-treated starch were 68.3, 80.4, 79.6, 79.0, 78.8, and 78.4°C, respectively. The control starch showed a higher gelatinization temperature (80.4°C of the control starch versus 68.3°C of the native starch) and lower enthalpy changes ($\Delta H$) (2.3 vs. 13.9 J/g) than the native starch, indicating some starch was gelatinized in the control sample during incubation at 65°C, the same condition used for the enzyme treatment of the starch. The gelatinization temperatures and enthalpy changes of the α-GTase-modified starches decreased with the increase in the level of enzyme treatment. The DSC thermograms of the 25, 50, and 75 U/g α-GTase-treated starches showed no melting peak for the amylose-lipid complex, whereas the native, control, and 10 U/g α-GTase-treated-starches showed an amylose-lipid complex melting peak at 84-106, 89-105, and 91-102°C, respectively. The modified starches showed decreased enthalpy changes (Table 1). The loss of the amylose-lipid complex melting peak was attributed to the enzyme degradation of the amylose.

The DSC data of retrograded starches that were stored at 4°C for 7 days are shown in Table 2. The onset thermal transition temperatures of dissociating retrograded starches ranged between 40.5 and 40.6°C, showing no significant difference between the control and the modified starches.

**Table 2. Thermal properties of the retrograded native, control, and modified starches**

| Starches | Retrograded starch[1)] | | | |
|---|---|---|---|---|
| | $T_o$ (°C) | $T_p$ (°C) | $T_c$ (°C) | $\Delta H$ (J/g) |
| Native | 41.4±0.2[2)] | 52.0±0.4 | 61.2±0.3 | 4.9±0.2 |
| Control | 40.6±0.2 | 55.0±0.2 | 62.0±0.4 | 1.8±0.1 |
| α-GTase treatments | | | | |
| 10 U/g | 40.5±0.1 | 58.2±0.3 | 63.4±0.3 | 1.5±0.0 |
| 25 U/g | 40.6±0.0 | 58.0±0.2 | 60.5±0.2 | 1.5±0.1 |
| 50 U/g | 40.8±0.2 | 55.8±0.4 | 61.0±0.5 | 1.3±0.1 |
| 75 U/g | 40.6±0.2 | 54.2±0.3 | 60.4±0.3 | 0.8±0.2 |

[1)]After storage at 4°C for 7 days.
[2)]Values were obtained as mean values from 3 replicates± SD.

**Table 1. Thermal properties of the native, control, and modified starches**

| Starches | Gelatinized starch[1)] | | | | |
|---|---|---|---|---|---|
| | $T_o$ (°C) | $T_p$ (°C) | $T_c$ (°C) | $\Delta H$ (J/g) | Amylose-lipid complex, $\Delta H$ (J/g) |
| Native | 68.3±0.1[2)] | 73.5±0.4 | 78.3±0.3 | 13.9±0.4 | 3.0±0.1 |
| Control | 80.4±0.1 | 82.0±0.1 | 85.3±0.2 | 2.3±0.0 | 1.5±0.1 |
| α-GTase treatments | | | | | |
| 10 U/g | 79.6±0.1 | 81.2±0.1 | 84.0±0.3 | 2.0±0.0 | 0.9±0.2 |
| 25 U/g | 79.0±0.0 | 81.0±0.2 | 84.9±0.3 | 2.1±0.1 | ND[3)] |
| 50 U/g | 78.8±0.1 | 80.8±0.2 | 83.9±0.2 | 2.0±0.0 | ND |
| 75 U/g | 78.4±0.2 | 80.3±0.1 | 82.6±0.3 | 1.5±0.2 | ND |

[1)]$T_o$, $T_p$, $T_c$, and $\Delta H$ are onset, peak, conclusion temperature, and enthalpy change, respectively.
[2)]Values were obtained as mean values from 3 replicates±SD.
[3)]Not detectable.

The control starch showed a lower enthalpy change ($\Delta H$) than did native starch (1.8 J/g of the control starch vs. 4.9 J/g of the native starch). The enthalpy changes of the dissociation of retrograded modified starches showed decreased retrogradation of the modified starches with the increase in enzyme treatment. This could be attributed to the absence of long chains of amylose, which would have led to re-crystallization of α-GTase modified starches, and cycloamylose. It is generally accepted that the long unbranched amylose chains have a greater tendency to retrograde than the highly branched and much softer amylopectin chains. The favorable property in retrogradation of gelatinized starch is suitable to be applied in starch-based products during storage.

**Pasting properties and gel strength** The pasting behaviors of the native, control, and modified starches, analyzed using an RVA, are shown in Fig. 3 and are summarized in Table 3. The pasting temperature of the α-GTase-modified starch ranged between 77.2 and 79.2°C, which were lower than that of the control starch, 80.6°C. The pasting temperature and the viscosity of the α-GTase-modified starch decreased with the increasing level of enzyme treatment. The peak viscosity of the α-GTase-modified starch decreased to 4.0 RVU with a 75 U/g α-GTase-treatment. The final and setback viscosities of the α-GTase-modified starches were also lower than those of the native maize starch and the control starch. This indicates that the α-GTase-treated starches can not form gel-networks.

The effects of α-GTase treatments on the strength of starch gel are shown in Fig. 4. The gels of α-GTase-modified starch were turbid and weaker than the native and the control starch gel. The gel strengths decreased with increasing level of enzyme treatment. The α-GTase-modified starches did not form strong gels, which could be attributed to the fact that amylose was degraded and some converted to cyclic amylose, which could not form strong networks with other molecules to form strong gels. Amylopectin molecules were also degraded to smaller molecules with longer branch chains. The long branched chains of the amylopectin interact with one another and maintained 47% of gel strength after 75 U/g α-GTase treatment. The weak gels derived from α-GTase treated starch agreed resulted in low viscosity of the starch pastes (Fig. 3). Recently, it has been reported that *T. thermophilus* α-GTase can modify starch in a way that the modified starch gel demonstrates thermo-reversiblity (30). The favorable property in pasting of starch is suitable to be applied in

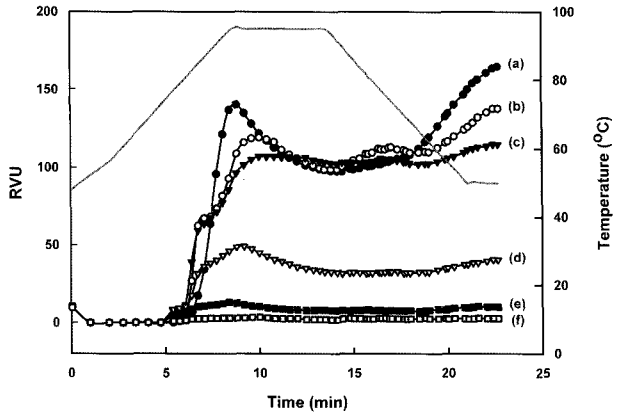


**Fig. 3. Rapid ViscoAnalyzer (RVA) pasting profiles of the native, control, and modified starches.** The native starch (a), control starch (b), and α-GTase treated starch including 10 U/g starch (c), 25 (d) 50 (e), and 75 U/g (f).

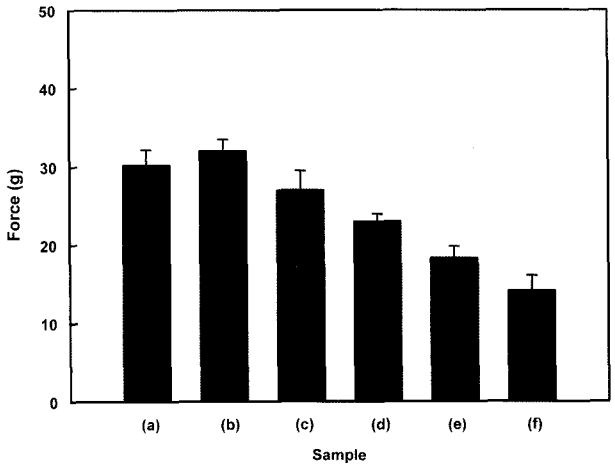


**Fig. 4. Gel strength of the native, control, and modified starches.** The native starch (a), control starch (b), and α-GTase treated starch including 10 U/g starch (c), 25 (d) 50 (e), and 75 U/g (f).

**Table 3. Pasting properties of the native, control, and modified starches**

| Starches | Pasting temperature (°C) | Viscosity (RVU)[1)] | | | |
|---|---|---|---|---|---|
| | | Peak | Breakdown | Final | Set-back |
| Native | 81.3 | 140.7 | 44.9 | 167.2 | 71.4 |
| Control | 80.6 | 120.6 | 19.8 | 137.7 | 37.0 |
| α-GTase treatments | | | | | |
| 10 U/g | 79.2 | 108.0 | 5.8 | 117.3 | 15.0 |
| 25 U/g | 78.5 | 49.4 | 17.5 | 39.9 | 8.0 |
| 50 U/g | 77.7 | 13.3 | 5.5 | 10.6 | 2.8 |
| 75 U/g | 77.2 | 4.0 | 2.1 | 2.8 | 0.9 |

[1)]Measured in Rapid ViscoAnalyzer units.

starch-based products containing the 'chilled sushi' and 'retort pouched rice and noodle'.

**Rheological properties of the α-GTase-modified starch** The dynamic rheological properties of the native, control, and modified starches were studied using a controlled stress rheometer. Figure 4 shows changes in the G' and G" as a function of log frequency for 6% starch dispersions. The G' is a measure of elasticity or energy stored in the material. The G" is a measure of viscosity or energy lost. The treatment of normal maize starch with α-GTase resulted in a decrease in both elastic and viscous properties. For both native and control starches, the storage modulus was greater than the loss modulus at all frequencies, which showed elastic, solid-like and hard gels. In the α-GTase-modified starches, the G" was greater than the G'. They showed viscous, liquid-like, soft gels, depending on frequencies. Fifty and 75 U/g α-GTase-treated starches showed a cross of the G' and G" curves, which indicating the balance between the solid and liquid-like structure. The α-GTase-modified maize starch displayed more viscous (liquid-like) behavior than the native starch. This result was in agreement with the data obtained from the analysis of gel strengths (Fig. 4). Lee *et al.* (29) investigated the effect of a thermostable α-GTase on the rheological properties of rice starch paste. The modified rice starch paste showed a yield stress. The enzymatic modified starch showed considerably low moduli with higher G" than G', indicating a significant liquefaction. It is known that acid treatment of starch causes partial hydrolysis of starch chains, resulting in much lower paste viscosity compared to native starch. This result suggests considerable potential for α-GTase modified rice starch gel in numerous industrial applications (29, 31).

In conclusion, carbohydrate engineering is one of the most important areas in food biotechnology. The use of

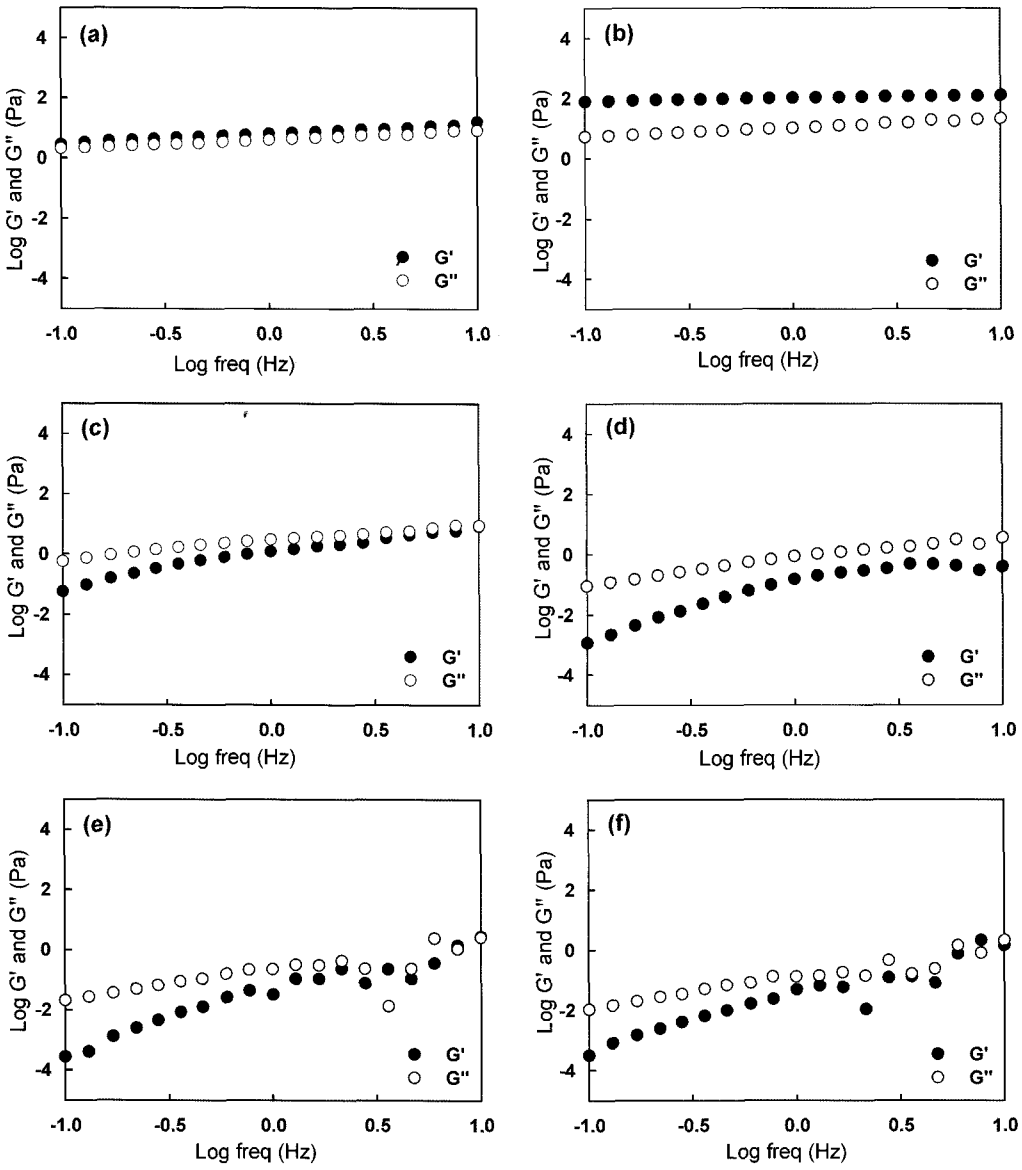


**Fig. 5. Dynamic rheological properties of the native, control, and modified starches.** The native starch (a), control starch (b), and α-GTase treated starch including 10 U/g starch (c), 25 (d) 50 (e), and 75 U/g (f).

native starches in food processing is limited by the undesirable textural changes under the conditions of temperature, shear, and pH commonly applied to processed foods (32). Recently significant progress has been conducted to improve the functional properties of starch and starch-based foodstuff (33). Moreover, recent advances in protein engineering and molecular biology for carbohydrate enzyme have made it possible to create many structured modified starch. This study attempts to modify normal maize starch using *Thermus* α-GTase. This approach results in the improvement of the properties of starch and related compounds. In this way, it is possible to promote the formation of desirable structure of starch and soon new varieties of starch-based products will be offered in food industry.

## Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (M01-2004-000-10137-0).

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