Food Sci. Biotechnol. 21(6): 1707-1714 (2012)
DOI 10.1007/s10068-012-0227-6

RESEARCH ARTICLE

Texture Properties of Rice Cakes Made of Rice Flours Treated with 4-α-Glucanotransferase and Their Relationship with Structural Characteristics

Yang Kim, Young-Lim Kim, Khanh Son Trinh, Yong-Ro Kim, and Tae Wha Moon

Received: 11 May 2012 / Revised: 12 July 2012 / Accepted: 13 July 2012 / Published Online: 31 December 2012

Abstract Rice flours were treated using a thermostable 4-α-glucanotransferase (GTase) from *Thermus aquaticus* for 1, 3, and 48 h. Molecular weight of modified rice starch rapidly decreased within 1 h of reaction, then slowed down. As the reaction proceeded, the proportions of short (<DP 11) and long (>DP 30) branched chains of modified starch increased, whereas the proportion of medium chains decreased. Rice cakes were prepared with native and GTase-treated rice flours (substitution at 5%) and kept for 3 and 21 h. Texture profile analysis indicated significantly increased hardness, adhesiveness, chewiness, and resilience in rice cakes containing treated rice flours. Sensory analysis revealed that the rice cakes containing 48 h-treated flour had significantly increased springiness, hardness, toughness, and adhesiveness during both storage periods. Meanwhile, it displayed the lowest starch-like attribute and crumbliness. These results suggested that the substitution for 48 h GTase-treated rice flour could retard the retrogradation of rice cakes.

Keywords: rice cake, 4-α-glucanotransferase, texture profile analysis, sensory descriptive analysis, multivariate analysis

Yang Kim, Yong-Ro Kim, Tae Wha Moon (✉)
Center for Food and Bioconvergence, Seoul National University, Seoul 151-921, Korea
Tel: +82-2-880-4854; Fax: +82-2-873-5095
E-mail: twmoon@snu.ac.kr

Young-Lim Kim, Yong-Ro Kim
Department of Biosystems & Biomaterial Science and Engineering, Seoul National University, Seoul 151-921, Korea

Khanh Son Trinh†, Tae Wha Moon
Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea
†Present address: Faculty of Chemical and Food Technology, University of Technical Education Ho Chi Minh City, Vo Van Ngan 1, Thu Duc District, Vietnam

Introduction

Rice cakes, which have been made and enjoyed at festivals and feasts since ancient times, are one of the most widely consumed traditional foods in many Asian countries. A wide variety of rice cakes exist in countries such as Korea, China, and Japan, and they are consumed every day as well as at holidays and important celebrations. The basic white steamed non-waxy rice cake (called *baekseolgi* in Korean) contains high moisture, is soft and chewy, and is served as a dessert. However, rice cakes require daily production due to the rapid retrogradation. Starch retrogradation occurs during cooling and aging and involves rapid gelation of amylose and slow development of recrystallization of amylopectin within the granules (1,2). The retrogradation of starch depends on the amylose/amylopectin ratio, moisture content, temperature, and additives (3). The rapid retrogradation progress of rice cakes is mainly due to high moisture content, high starch concentration, and low fat content compared to bread and baked products (4). Researches for enhancing the shelf-life of *baekseolgi* mainly focused on the addition of hydrocolloids (5), oligosaccharides, sugars, and sugar alcohols (6). Many attempts to slow down the retrogradation progress of starch products have been made via enzymatic modifications of starch (7,8). Enzymes such as cyclodextrin glucanotransferase, maltogenic amylase, and α-glucanotransferase have been investigated (8,9). Among them, 4-α-glucanotransferase (GTase) catalyzes disproportionation reaction, which transfers the malto-oligosaccharide moiety inter-molecularly (10,11). Thus, GTase-treatment causes the reduction in amylose content, the rearrangement of amylopectin, and the production of malto-oligosaccharides (9). Recently, Seo *et al.* (12) reported that the treatment of GTase effectively inhibited starch retrogradation in non-waxy rice cake, based on the results of X-ray diffraction pattern and instrumental texture profile analysis.

The retrogradation of starch in rice cake causes rapid changes in texture within 1 day. Elucidating the structural properties of modified starch and texture properties of rice cakes would be valuable; however, such information is limited. In this study, the changes in structural characteristics of GTase-treated rice flour were investigated according to the treatment time. Also, rice cakes were prepared with partial substitution of GTase-treated rice flour and the sensory and instrumental texture properties of non-waxy rice cake were evaluated on the day of the production and after 1 day storage to verify the effects of modified starch on texture properties and the retrogradation of rice cake. Additionally, the relationships between structural properties of GTase-treated rice flour and textural properties in rice cake systems were examined using multivariate statistical analysis.

Materials and Methods

Materials Wet rice flour with 35% moisture content was provided from Samlip General Foods (Siheung, Korea). 4-α-Glucanotransferase (GTase) from *Thermus aquaticus* was cloned and purified as described by Seo *et al.* (12). Rice flour (30 g, dry basis) was suspended in deionized water at concentration of 5%(w/v) and gelatinized by keeping it in a water bath (95°C) for 30 min with mechanical stirring. After cooling to 75°C, 5 units of GT/g of rice flour was added and incubated for 1, 3, and 48 h. One unit of GTase activity was defined as the amount of enzyme that degraded 0.5 mg/mL of amylase/min under the assay conditions used. The enzyme reaction was stopped by boiling for 10 min. Five volumes of ethanol were added to precipitate the enzyme-treated flour and then centrifuged at 652×*g* for 20 min. The supernatant was removed, and the pellet was washed with deionized water and centrifuged 3 times, air-dried, ground, and sieved (45 mesh).

Starch isolation To examine Mw, branched chain distribution and crystalline structure of starch in rice flour, starch was isolated by removing protein from rice flour. Starch suspension (5%, w/v) was treated with protease (50 unit; Sigma-Aldrich, St. Louis, MO, USA) at 30°C for 1 h and precipitated with 3 volumes of ethanol. It was washed with deionized water and centrifuged (10,000×*g*, 20 min) 3 times, lyophilized, ground, and sieved (100 mesh).

Preparation of rice cakes Control rice cake was prepared with 200 g wet rice flour, 30 g sugar, 2 g NaCl, and 31.5 g water. Modified starch was substituted for rice flour at 5% level (dry basis). All the ingredients were mixed, sieved (20 mesh), and steamed in a steam cooker for 30 min. Samples were tempered at room temperature (about 18°C) for 30 min. The final moisture content of rice cakes was made up to 38%. Rice cakes were cut and stored for 3 (0 day storage) and 21 h (1 day storage) before sensory evaluation and instrumental texture measurement. One batch of each rice cake was made and used for sensory analysis or texture profile analysis.

Molecular weight distribution of starch isolated from GTase-treated rice flour Molecular weight distribution of the GTase-treated rice flour was analyzed based on the size using multiangle laser light scattering (MALLS) system (Dawn DSP; Wyatt Technology, Santa Barbara, CA, USA) with a differential refractive index (RI) detector (Opti-Lab; Wyatt Technology) coupled with size exclusion chromatography (SEC). OH-Pak 804 and 806 columns (8×300 mm, Shodex, Kawasaki, Japan) were used consecutively. The flow rate of the mobile phase (100 mM $NaNO_3$ containing 0.02% NaN_3) was 0.4 mL/min. Sample (25 mg) was dispersed in 5 mL dimethyl sulfoxide (DMSO, 90%) and heated for 15 min. The solution was mixed with 25 mL of ethanol to precipitate starch and was centrifuged at 10,000×*g* for 10 min to remove DMSO. The precipitated starch was redissolved in 5 mL of 100 mM $NaNO_3$, then, autoclaved (121°C) for 20 min, and filtered through a 5-μm membrane filter (Millipore, Billerica, MA, USA). The sample was injected into the SEC-MALLS-RI system.

Branched chain distribution of starch isolated from GTase-treated rice flour To analyze the branched chain length distribution of the enzyme-treated rice starch in rice cake, isolated starch samples (15 mg each) were dissolved in 3 mL DMSO and debranched with isoamylase (10 U, Megazyme International Ireland Ltd., Bray, Ireland) in 50 mM sodium acetate buffer (pH 4.3) at 45°C for 2 h. After the reaction was stopped by boiling for 5 min, the branched chain length distribution was analyzed using a high-performance anion-exchange chromatography (HPSEC) system (Dionex-300; Dionex, Sunnyvale, CA, USA) coupled with a pulsed amperometric detection (HPAEC-PAD) detector (ED40; Dionex). A CarboPacTM PA-1 anion-exchange column (250×4 mm, Dionex) and a guard column were used to separate the 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. In order to analyze malto-oligosaccharide composition of rice cakes, 1 g of rice cake was dispersed in 100 mL of distilled water and boiled for 1 h. After filtration using a 5-μm disposable syringe filter, the solution was analyzed in a similar manner as above, except for isoamylase treatment.

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-Kα radiation of 0.154-nm (nickel filter). The scanning region of the diffraction angle 2θ was 4-30°.

Instrumental texture analysis of rice cakes Samples stored for 3 (0 day) and 21 h (1 day) were prepared as 20×20×10 mm slabs. Texture profile analysis (TPA) was performed using a texture analyzer (TA-XT2i; Stable Microsystems, Surrey, UK) in combination with a 5 kg load cell, fitted with a 50 mm diameter cylinder aluminum probe at a constant speed of 2.0 mm/s with strain rate of 75%. The parameters recorded include hardness, adhesiveness, springiness, cohesiveness, and chewiness. For resilience testing, a plunger compressed the samples to 20% of their original height at a speed of 1.0 mm/s. Each sample was analyzed in triplicate.

Sensory evaluation of rice cakes containing GTase-treated rice flour Sensory attributes of rice cakes were evaluated using descriptive analysis method with partial adoption of spectrum descriptive analysis method. Seven female and 2 male subjects (23-28 year of age), who were interested and experienced in sensory evaluation, were selected as panelists in the College of Agriculture and Life Sciences at Seoul National University (Seoul, Korea). The panelists were initially trained to communicate with each other, understand the attributes, and use the rating scale properly. They were also trained with various types of starchy products including rice products to develop and define texture attributes. Thirteen texture, flavor, and taste attributes were determined and defined. The reference standards were selected for each attribute to help panelists understand each attribute better. At each session, panelists evaluated 4 rice cakes - control, GT 1, GT 3, and GT 48 - with the same storage period to test the effects of GTase-treated flour substitution on sensory attributes. Because the difference in strength between samples within the same storage period became negligible due to the big difference of strength in sensory attribute between the storage periods, samples with different storage periods were not evaluated simultaneously. Rice cakes were cut in about 2×2×1 cm slabs and stored in an air-tight container for 3 or 22 h. Four pieces of each rice cake were placed in a Pyrex cup coded with random 3-digit numbers and presented to each panelist. The intensity of each sensory attribute was rated on a 15-point category scale, and all samples were evaluated 3 times.

Statistical analysis For each sensory attribute measured, a 3-way (type of rice cake, replicate, and panel) multivariate analysis of variance (MANOVA) with interaction of 2 factors was applied. It was conducted not only to test significant effects of substitution of GTase-treated rice flour on the attributes of rice cakes but also to monitor panel performance for reproducibility and to determine the attributes that were discriminated among the samples. As Wilks' λ value of the sample effect was significant in MANOVA, analysis of variance (ANOVA), and Duncan's multiple range tests were performed to test the significance of the effect of rice cake types on the individual sensory attribute. Flavor and taste attributes, which did not have significant discrimination between rice cake types ($p > 0.05$), were excluded in further analysis. Triplicate TPA measurements were analyzed by one-way ANOVA. The relationships between structural, TPA parameters, and sensory data were investigated by using correlation coefficients and principal component analysis (PCA). Analyses were performed using the Unscrambler (Windows version 7.6 software package; CAMO ASA, Trondheim, Norway).

Results and Discussion

Molecular weight distributions of starch isolated from GTase-treated rice flour The chromatograms from the RI detector for the GTase-treated rice flours are shown in Fig. 1. The Mw rapidly decreased within 1 h of the reaction, and slowed down afterward. The elution profile of the GTase-treated sample was divided into 3 fractions according to the elution time, although those fractions were overlapped, whereas that of control rice flour could be divided into 2, which are amylopectin and amylose. The elution time of the 1st fraction of GT 1 and GT 3 matched well with that of control rice flour, indicating that this fraction corresponded to amylopectin macromolecules. However, the 1st fraction of GT 48 showed delayed retention time, suggesting that the Mw of this fraction had been reduced. As the treatment time increased, the proportions of the 2nd fractions of modified flours decreased, whereas those of the 3rd fractions dramatically increased. The decrease in the 2nd fraction might be due to degradation of amylose, and the remainders in the 2nd fractions of modified starches were likely to be originated either from undegradaded amylose or from the degraded/rearranged amylopectin which had short branched chain length (8). The 3rd fraction seemed to consist of small amylopectin clusters and degraded amylose reorganized and disproportionated (9,13,14). The present results suggested that the long branched amylopectin with large molecular weight was degraded and rearranged by the GTase treatment. The decrease in amylopectin and amylose macromolecules and the increase in small amylopectin clusters and oligosaccharides might induce the reduction of Mw

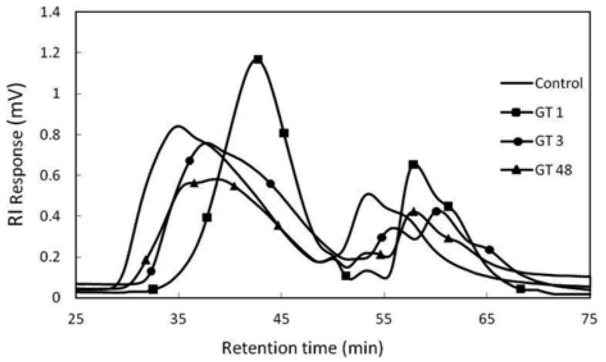

Fig. 1. Molecular weight distributions of starches in control and GTase-treated rice flours. GT 1, GT 3, and GT 48 represent rice cakes containing 5% 4-α-glucanotransferase-treated rice flour for enzyme treatment time 1, 3, and 48 h, respectively. Weight average Mw (g/mol; 2 replicates) of control, GT 1, GT 3, and GT 48 were 2.51±0.12 (×10^7), 6.14±0.64 (×10^6), 2.80±0.28 (×10^6) and 4.32±0.2 (×10^5), respectively.

through cleaving and reorganizing the starch molecules. This enzymatic modification of starch molecules would be responsible for the alteration of textural properties and staling rate of GTase-treated rice cake.

Branched chain distribution of GTase-treated rice starch After debranching with isoamylase, the branch chain length of control and GTase-treated starches differed among the samples (Fig. 2). The control starch showed the greatest amount of branches with chain length of a degree of polymerization (DP) of 10, whereas GTase-treated samples showed the greatest amount with DP 5. Analysis of branch chain length distribution revealed that the proportion of short (DP 1-10) and long (>DP 30) branched chains increased as the reaction proceeded, whereas the proportion of medium chains (DP 11-30) decreased in GTase-treated samples. A similar distribution of branch chain length was reported in sweet potato starch after treatment with GTase from *Thermus aquaticus*, displaying an increase in very small and longer branch chains compared with raw starch (14). The increase in the proportion of longer branched chains could be due to the intermolecular transfer from amylose to amylopectin that partially elongated the amylopectin branch chains and the partial hydrolysis of inner long chains. The increase in the proportion of shorter chains and the decrease in the proportion of medium branch chains were possibly due to the partial hydrolysis and intramolecular rearrangement of branch chains of amylopectin. And increased proportion of chains with over DP 30 would enhance the retrogradation rate of starch, whereas short chains with DP 6 to 10 were thought to reduce starch retrogradation (15). Consequently, the branch chain profiles of the amylopectin molecules altered by GTase would reduce the retrogradation rate of starch. Meanwhile, it is known that GTase causes the cyclization reaction of both linear and branched molecules (9,13). These cyclic molecules can be identified from the unusual peaks at around DP 20 through DP 35 in the HPAEC chromatogram (9). However, in Fig. 2, the sign of unusual peaks was scarce, indicating that no significant amount of cycloamylose or cycloamylopectin was formed in the GTase-treated rice flour.

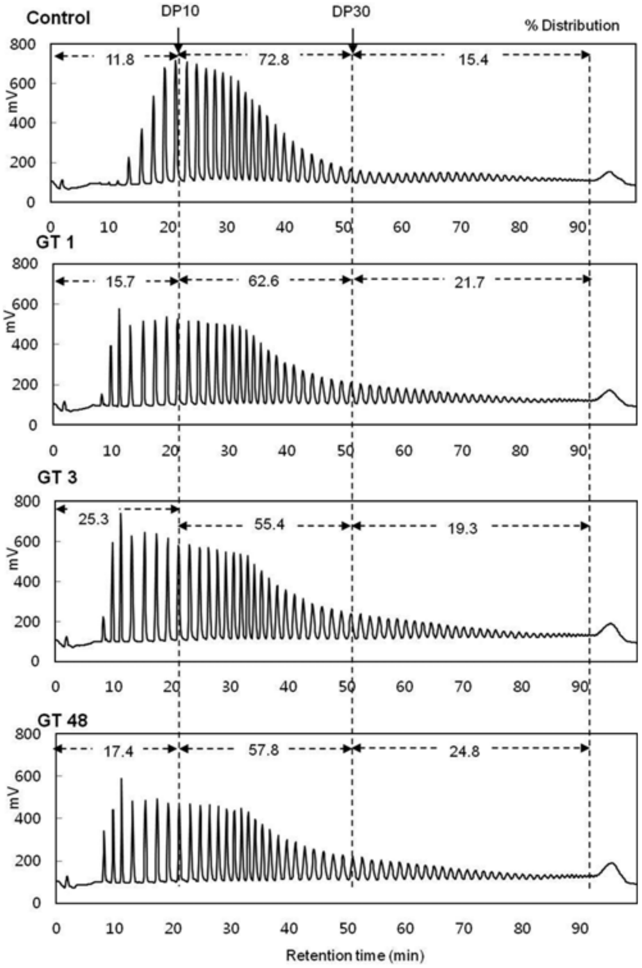

Fig. 2. High-performance anion-exchange chromatograms showing branched chain distributions of starches in control and GTase-treated rice flours. GT 1, GT 3, and GT 48 represent rice cakes containing 5% 4-α-glucanotransferase-treated rice flour for enzyme treatment time 1, 3, and 48 h, respectively.

Crystalline properties determined by X-ray diffractometry Crystalline structure of rice starches was changed by GTase modification (Fig. 3). X-ray diffraction patterns of raw rice starch displayed a typical A-type pattern, with diffraction peaks corresponding to Bragg angles of 15, 17, 18, and 23°, whereas 1 and 3 h-treated rice starches lost those peaks due to gelatinization before the enzyme reaction. However, the diagram of 48 h-treated rice starch had characteristics of B-type crystalline structure with main

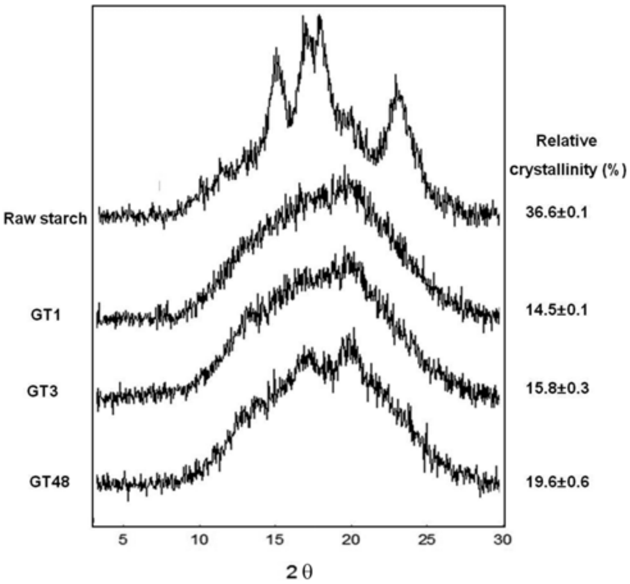

Fig. 3. X-ray diffractograms and relative crystallinities of starches in control and GTase-treated rice flour. GT 1, GT 3, and GT 48 represent rice cakes containing 5% 4-α-glucanotransferase-treated rice flour for enzyme treatment time 1, 3, and 48 h, respectively.

peaks emerging at 15, 17, 22, and 24°, even though the peak intensities were weak. The relative crystallinities of the GTase-modified starches were lower than that of the native starch (Fig. 3), but were higher than that of the gelatinized starch (data not shown). Typically, native starches lost most of the crystalline structures due to gelatinization before enzyme treatment, while the GTase-treated starches showed increased crystalline structure as the treatment time increased despite the fact that the treatments were done at 70°C. The higher crystallinity of the 48 h-modified starches resulted in denser packing of the crystalline structures than 1 and 3 h-modified starches. It has been reported that the amylopectin content has a positive correlation with the degree of crystallinity, although amylose disrupts the crystalline packing of amylopectin (16,17). The difference in peak intensity of native and modified starches could be induced by the increase in shorter and longer branch chains and the rearrangement of amylopectin, leading to a decrease in the amorphous region and an increase in the crystalline region.

TPA Texture parameters in rice cakes containing GTase-treated rice flour with different treatment times were significantly changed ($p<0.05$, Table 1). Hardness, chewiness, and resilience gradually increased at both day 0 and day 1 as the treatment time of the substituted rice flour increased. Cohesiveness significantly increased in the samples containing 48 h-treated rice flour. Adhesiveness was higher in the samples containing GTase-treated flour, but no significant difference linked to treatment time was observed in day 0 samples. However, in the samples stored for 1 day, adhesiveness decreased as GTase-treatment time increased. Overall, the hardness and chewiness of GT 48-containing rice cake were 1.5-fold higher than those of control samples at both storage periods with only 5% substitution with 48 h-treated rice flour. These results did not coincide with those of Seo *et al.* (12), who reported that the hardness of GTase-treated rice cakes increased more slowly and that adhesiveness remained comparable to the control samples during storage at 4°C for 24 h. It could be explained by the difference in the process of enzymatic treatment and substitution level of modified starch. They used the incubated solution and modified starch as a whole in making rice cake so that higher amount of mono-, di-, and oligosaccharides remaining in the rice cake compared to this study might delay hardening of rice cake (18). However, in our experiment, modified flour substituted only 5% of the rice flour. These might cause the difference in instrumental texture properties between their results and ours.

Sensory descriptive analysis MANOVA and ANOVA showed that the intensities of all the texture attributes were significantly different among the rice cake samples ($p<0.05$). The mean attribute intensities for the rice cakes stored for 3 h are listed in Table 1. The sensory descriptive results of 3 h-stored rice cake (day 0) revealed that the rice cakes containing 48 h-treated rice flour (GT 48) represented significantly increased springiness, hardness, toughness, roughness of surface, adhesiveness, chewiness, and cohesiveness compared with the rice cakes made without treated flour. Rice cakes containing 3 h-treated rice flour (GT 3) were similar to control in all of the sensory attributes evaluated except for the chewiness. When compared with control, the rice cakes containing 1 h-treated rice flour (GT 1) demonstrated notably lower hardness, toughness, chewiness, and cohesiveness, but higher residual starch-like attribute. When the rice cakes were stored for 21 h (day 1), they lost most of the toughness attribute and showed a tendency to crumble, induced by retrogradation. As a result, ‘crumbliness’ was added to the sensory terms instead of toughness. After 1 day of storage, GT 48 still exhibited the highest levels of sensory springiness, hardness, roughness of surface, adhesiveness, chewiness, and cohesiveness, while showing the lowest starch-like attribute and crumbliness. GT 1 and GT 3 showed higher starch-like property and crumbliness, whereas they displayed lower springiness, hardness, adhesiveness, chewiness, and cohesiveness compared with control and GT 48 rice cakes.

From the sensory analysis results of day 0 rice cakes, GT 1 and GT 3 showed no significant differences in sensory texture properties; however, GT 48 showed significant

Table 1. Instrumental texture parameters and sensory texture properties of rice cakes containing GTase-treated rice flour stored for 0 and 1 day

	0 day				1 day			
	Control	GT 1$^{1)}$	GT 3	GT 48	Control	GT 1	GT 3	GT 48
Texture parameters								
Hardness	11,628$^{c2)}$	15,660^{b}	16,494^{b}	18,999^{a}	19,978^{c}	21,003^{c}	23,264^{b}	29,949^{a}
Adhesiveness	-1,456^{b}	-1,999^{ab}	-2,056^{a}	-2,319^{a}	-692^{a}	-480^{b}	-362^{b}	-357^{b}
Cohesiveness	0.56^{b}	0.56^{b}	0.57^{b}	0.61^{a}	0.45^{b}	0.44^{b}	0.51^{a}	0.51^{a}
Springiness	0.77^{a}	0.71^{a}	0.80^{a}	0.76^{a}	0.81^{a}	0.80^{a}	0.81^{a}	0.78^{a}
Chewiness	5,028^{d}	6,195^{c}	7,634^{b}	8,729^{a}	7,225^{c}	7,488^{c}	9,489^{b}	11,899^{a}
Resilience	0.46^{b}	0.48^{ab}	0.50^{ab}	0.51^{a}	0.59^{bc}	0.58^{c}	0.61^{b}	0.63^{a}
Sensory properties								
Moistness of surface	7.52^{a}	5.93^{b}	6.78^{ab}	6.37^{ab}	6.41^{a}	6.07^{a}	5.48^{a}	5.93^{a}
Springiness	6.15^{b}	5.70^{b}	5.59^{b}	8.96^{a}	7.48^{b}	4.93^{c}	6.63^{b}	9.56^{a}
Hardness	6.30^{b}	3.30^{c}	6.19^{b}	10.00^{a}	7.22^{b}	3.26^{d}	4.78^{c}	9.81^{a}
Toughness/Crumbliness	6.30^{b}	3.22^{c}	5.85^{b}	9.78^{a}	-	-	-	-
Roughness of surface	5.41^{b}	5.67^{b}	6.26^{b}	8.15^{a}	4.74^{b}	5.59^{b}	8.81^{a}	9.26^{a}
Crumbliness	-	-	-	-	6.37^{c}	10.26^{a}	7.93^{b}	4.56^{d}
Adhesiveness	6.19^{b}	5.89^{b}	5.59^{b}	8.30^{a}	6.93^{a}	4.96^{b}	6.44^{ab}	7.52^{a}
Chewiness	7.78^{b}	5.85^{c}	6.19^{c}	9.33^{a}	8.07^{b}	6.00^{c}	5.96^{c}	9.96^{a}
Cohesiveness	7.15^{b}	5.33^{c}	6.11^{bc}	8.52^{a}	6.78^{b}	4.74^{c}	4.85^{c}	9.30^{a}
Starch-like	6.33^{b}	9.19^{a}	6.41^{b}	5.59^{b}	6.70^{b}	9.11^{a}	8.26^{a}	5.04^{c}
Steamed rice flavor	4.56^{a}	4.33^{a}	4.41^{a}	4.26^{a}	3.26^{a}	2.96^{a}	3.56^{a}	3.44^{a}
Sweetness	3.63^{a}	3.74^{a}	3.41^{a}	3.74^{a}	3.44^{a}	3.37^{a}	3.63^{a}	3.33^{a}
Off flavor	1.26^{a}	1.37^{a}	1.22^{a}	1.19^{a}	0.81^{a}	0.78^{a}	0.70^{a}	0.74^{a}

1) GT 1, GT 3, and GT 48 represent rice cakes containing 5% 4-α-glucanotransferase-treated rice flour for enzyme treatment time 1, 3, and 48 h, respectively.

2) Means within a row not sharing the same superscript are significantly different ($p<0.05$, Duncan's multiple range test).

changes compared with control samples. After 1 day of storage, GT 48 rice cakes displayed less retrograded properties. The retrograded rice cakes usually have properties of crumbliness due to loss of moisture (19). However, the rice cakes containing 48 h-treated rice flour maintained better structure and showed much less crumbliness compared with the control, GT 1, and GT 3. Moreover, they displayed firmer, springier, chewier, more cohesive, but less starch-like attributes compared with the control rice cakes. In contrast, GT 1 and GT 3 exhibited more retrograded sensory properties at both storage periods. GTase catalyzes the transfer of a sugar moiety to an acceptor by forming a new α-1,4-glucosidic bond (9). It has been reported that this process reduces the amount of amylose, resulting in the formation of amylopectin with a broader chain-length distribution (12). The presence of longer chains can promote the retrogradation rate of starch, and conversely, short chains are thought to retard starch retrogradation (15). The essential mechanism by which starch-hydrolyzing enzymes retard starch retrogradation has not been fully explained. However, it has been suggested that these enzymes modify amylopectin molecules so that their reassociation becomes less effective, thereby increasing rigidity during storage (20). The sensory analysis results suggest that the GTase treatment can effectively reduce the degree of retrogradation of rice cakes depending on the extent of treatment. Meanwhile, it seems that the moisture in the rice cakes was less distributed on the surface of rice cakes with GTase-treated flour.

Relationship between structural parameters and instrumental and sensory texture attributes PCA indicated that 80 and 78% of the data were explained by the first 2 PCs for day 0 and day 1, respectively (Fig. 4). As for the day 0 plot (Fig. 4A), PC 1 dimension mainly separated the sample according to the GTase treatment time and the structural parameters such as peak Mw, side chain distribution and crystallinity. The control samples were loaded negatively on the PC 1 dimension, whereas all the GTase-treated samples were loaded positively, which introduced the difference in structural and textural properties given by the GTase treatment. Most sensory attributes were loaded in the negative direction for PC 1, while roughness of surface and starch-like attribute were loaded in the positive direction on PC 1. On the other hand, all the TPA parameters were loaded positively along the PC 1 dimension, showing their poor correlation with the sensory properties in fresh rice cake samples (Fig. 4A). PC 2

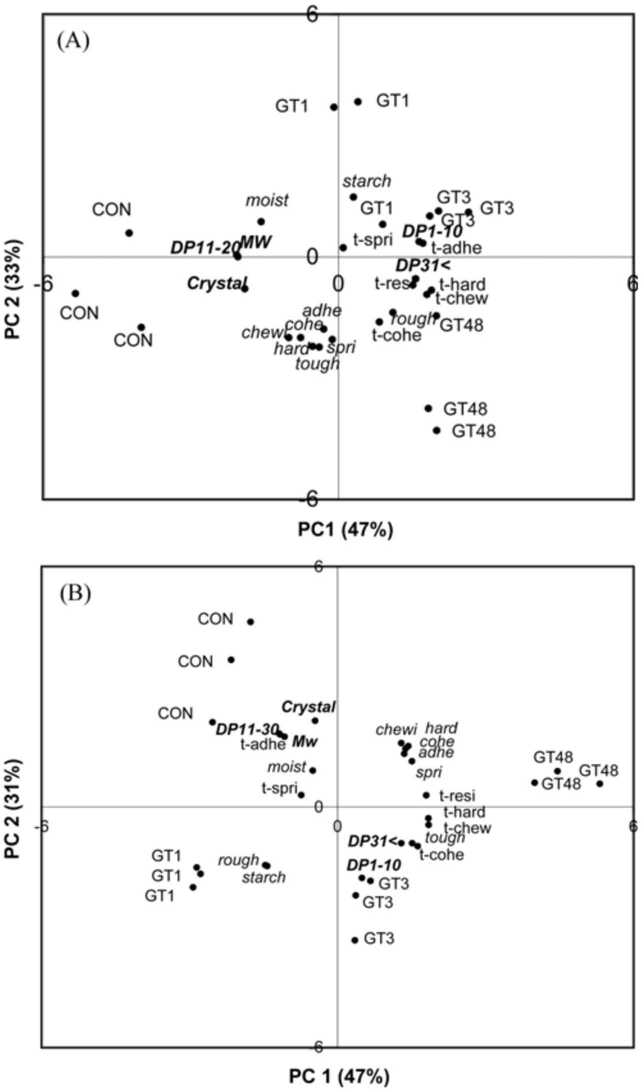

Fig. 4. Principal component plots showing the relationships between molecular structural properties[1], instrumental[2] and sensory texture properties[3] of rice cakes containing GTase-treated rice flour stored for 0 (A) and 1 day (B). [1]Mw, weight average Mw; DP, degree of polymerization. Crystal, relative crystallinity. [2]t-adhe, adhesiveness; t-chew, chewiness, t-cohe, cohesiveness; t-hard, hardness; t-spri, springiness; t-resi, resilience. [3]adhe, adhesiveness; chew, chewiness; cohe, cohesiveness of mass; crumb, crumbliness; hard, hardness; moist, moistness of surface; spri, springiness; star, starch-like (residual); tough, toughness; rough, roughness of surface

mainly separated the samples based on their sensory properties. Control and GT 48 were loaded negatively on PC 2 representing higher sensory hard, tough, springy, cohesive, chewy, and adhesive properties, while GT 1 and GT 3 were loaded positively showing more starch-like texture. As for the plot of 1 day stored samples, PC 1 mainly separated the samples based on their sensory and instrumental texture properties, and PC 2 loaded the samples according to their Mw, branched chain distribution, and crystallinity. Interestingly, sensory and instrumental parameters showed strong significant correlations in 1 day stored samples (Fig. 4B). More surprisingly, instrumental resilience exhibited significant correlations with all the sensory properties presented (Fig. 4B). Correlation coefficients (Person's r) between resilience and sensory attributes were; hardness 0.75 ($p<0.01$), springiness 0.69 ($p<0.05$), roughness 0.77 ($p<0.01$), crumbliness –0.80 ($p<0.01$), chewiness 0.66 ($p<0.05$), cohesiveness 0.74 ($p<0.01$), and starch-like attribute –0.76 ($p<0.01$), respectively. It has been reported that sensory perception and instrumental determination of hardness are highly correlated (21,22). More specifically, significant correlations between sensory chewiness and instrumental hardness, cohesiveness, and springiness have been reported in diverse food categories (22,23). In the rice cake system used in this study, none of instrumental parameters correlated with its sensory counterpart in fresh samples, even in hardness, due to very complicated textural properties of rice cakes. However, in more retrograded samples, TPA values were more useful in explaining sensory attributes than in fresh samples. Hardness and resilience were significantly correlated to sensory hardness, springiness, cohesiveness, and chewiness ($p<0.05$). Thus, there seems to be possibilities for predicting the textural properties of retrograded samples by applying mathematical models on the instrumental hardness and resilience values.

The mechanism of starch-hydrolyzing enzymes' retardation of starch retrogradation is not fully understood. A positive effect of oligosaccharides including cyclodextrin and maltotetrose on the retardation of starch retrogradation has been established (18,24-26).

Researches on texture properties of rice cakes have mainly used instrumental means of measurement or have focused on a few major sensory attributes such as hardness, softness, and cohesiveness. A strong negative correlation between instrumental hardness and sensory softness was reported in oligosaccharides-containing rice cake system, which confirmed the tendency shown in other food systems (18). However, rice cakes containing GTase-treated rice flour in this study displayed no correlation in a fresh state, which indicates that this may have been derived from the specific molecular properties of GTase-treated rice flour; the differences in molecular weight profile, branched chain distribution, and relative crystallinity could be possible explanations. Reduced amylose content and rearrangement in amylopectin molecules accompanied by the enzymatic reaction of GTase could also be the reasons for effective retardation of starch retrogradation in rice cakes containing GTase-treated rice flour (12). The synergistic effects of these components on the staling process in rice cake system require further study.

In conclusion, GTase treatment caused significant changes in the structure of rice starch and in the textural properties of rice cakes containing GTase-treated rice flour during storage. The results clearly demonstrated that rice flour can be treated with amylolytic enzymes like GTase for application to rice cake production. For effective prevention of staling of rice cakes, thorough investigations on physicochemical properties of GTase-treated starch should be undertaken. For more practical applications, it also seems to be crucial to examine the consumer acceptance of rice cakes containing GTase-treated rice flour.

Acknowledgments This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-005-J0470) and the Technical Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

References

1. Bao J, Bergman CJ. The functionality of rice starch. pp. 258-294. In: Starch in Food. Eliasson AC (ed). CRC Press, Inc., Boca Raton, FL, USA (2004)
2. Biliaderis CG. Thermal analysis of food carbohydrates. pp. 168-220. In: Thermal Analysis of Foods. Harwalkar VR, Ma CY (eds). Elsevier, New York, NY, USA (1990)
3. BeMiller JN. Carbohydrate Chemistry for Food Scientists. 2nd ed. AACC International, Inc., St. Paul, MN, USA. pp. 195-196 (2007)
4. Lee CH, Maeng YS. A literature review on Korean rice-cakes. Korean J. Dietary Culture 2: 117-132 (1987)
5. Kim KO, Youn KH. Effects of hydrocolloids on quality of *backseolgi*. Korean J. Food Sci. Technol. 16: 159-164 (1984)
6. Lee SY, Kim KO. Sensory characteristics of *backseolgie s* containing various sweetening agents. Korean J. Food Sci. Technol. 16: 325-328 (1986)
7. Alexander RJ. New starches for food applications. Cereal Food. World 41: 796-798 (1996)
8. Lee SH, Kim YW, Lee S, Auh JH, Yoo SS, Kim TJ, Kim JW, Kim ST, Rho HJ, Choi JH, Kim YB, Park KH. Modulation of cyclizing activity and thermostability of cyclodextrin glucanotransferase and its application as an antistaling enzyme. J. Agr. Food Chem. 50: 1411-1415 (2002)
9. Park JH, Kim HJ, Kim YH, Cha HJ, Kim YW, Kim TJ, Kim YR, Park KH. The action mode of *Thermus aquaticus* YT-1 4-α-glucanotransferase and its chimeric enzymes introduced with starch-binding domain on amylose and amylopectin. Carbohyd. Polym. 67: 164-173 (2007)
10. Kuriki T, Imanaka T. The concept of the α-amylase family: Structural similarity and common catalytic mechanism. J. Biosci. Bioeng. 87: 557-565 (1999)
11. Svensson B. Protein engineering in the α-amylase family: Catalytic mechanism, substrate specificity, and stability. Plant Mol. Biol. 25: 141-157 (1994)
12. Seo NS, Roh SA, Auh JH, Park JH, Kim YR, Park KH. Structural characterization of rice starch in rice cake modified by 4-α-glucanotransferase (TSαGTase) *Thermus scotoductus*. J. Food Sci. 72: 331-336 (2007)
13. Takaha T, Yanase M, Takata H, Okada S, Smith SM. Cyclic glucans produced by intramolecular transglycosylation activity by potato D-enzyme on amylopectin. Biochem. Bioph. Res. Co. 247: 493-497 (1998)
14. Lee SH, Choi SJ, Shin SI, Park KH, Moon TW. Structural and rheological properties of sweet potato starch modified with 4-α-glucanotransferase from *Thermus aquaticus*. Food Sci. Biotechnol. 27: 705-712 (2008)
15. Levine H, Slade L. A polymer physicochemical approach to study of commercial starch hydrolysis products (SHP's). Carbohyd. Polym. 6: 213-244 (1986)
16. Cheetham NWH, Tao L. Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study. Carbohyd. Polym. 36: 277-284 (1998)
17. Jenkins PJ, Donald AM. The influence of amylose on starch granule structure. Int. J. Biol. Macromol. 17: 315-321 (1995)
18. Yoo JN, Kim YA. Effect of oligosaccharides addition on gelatinization and retrogradation of *backseolgies*. Korean J. Soc. Food Cookery Sci. 17: 157-164 (2001)
19. Cho K. Quality characteristics of *backseolgi* with germinated brown rice flour. Korean J. Food Nutr. 20: 185-194 (2007)
20. Hug-Iten S, Escher F, Conde-Petit B. Staling of bread: Role of amylose and amylopectin and influence of starch-degrading enzymes. Cereal Chem. 80: 654-661 (2003)
21. Meullenet JF, Carpenter JA, Lyon BG, Lyon CE. Bicyclical instrument for assessing texture profile parameters and its relationship to sensory evaluation of texture. J. Texture Stud. 28: 101-118 (1997)
22. Meullenet JF, Lyon BG, Carpenter JA, Lyon CE. Relationship between sensory and instrumental texture profile attributes. J. Sens. Stud. 13: 77-93 (1998)
23. Montejano JG, Hamann DD, Lanier TC. Comparison of two instrumental methods with sensory texture of protein gels. J. Texture Stud. 16: 403-424 (1985)
24. Durán E, León A, Barber B, Benedito de Barber C. Effect of low molecular weight dextrins on gelatinization and retrogradation of starch. Eur. Food Res. Technol. 212: 203-207 (2001)
25. Smits ALM, Kruiskamp PH, van Soest JJG, Vliegenthart JFG. The influence of various small plasticisers and malto-oligosaccharides on the retrogradation of (partly) gelatinised starch. Carbohyd. Polym. 51: 417-424 (2003)
26. Auh JH, Lee SY, Yoo SS, Son HJ, Lee JW, Lee SJ, Kim YB, Park KH. A novel maltopentaose-producing amylase as a bread antistaling agent. Food Sci. Biotechnol. 14: 681-684 (2005)