α-グルコシダーゼとシクロデキストリン生成酵素の共反応による非還元性末端にα-1,3結合およびα-1,6結合したグルコシル基を有する二つのα-1,4-グルカンの生成
Production of Two $\alpha$-1,4-Glucans Having Glucosyl Residues Linked by $\alpha$-1,3- and $\alpha$-1,6-Linkages at the Non-reducing End by the Coupled Reaction of $\alpha$-Glucosidase and Cyclodextrin Glucanotransferase

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Abstract: The isomalto- and nigeroligosaccharides are usually produced from starch by the combination of $\alpha$-glucosidase and starch degrading enzymes, such as $\alpha$- and $\beta$-amylases. In this study, a new reaction system for the production from starch of two $\alpha$-1,4 glucans having $\alpha$-1,6- and $\alpha$-1,3-linked glucosyl residues at or near the non-reducing end was established. These glucans were efficiently produced by the coupled reaction of $\alpha$-glucosidase and cyclodextrin glucanotransferase (CGTase) with negligible cyclodextrin production. The produced glucans underwent very little hydrolyzation by $\beta$-amylase but $\alpha$-amylase clearly enhanced the digestion of glucan. This indicated that glucosidic linkage other than $\alpha$-1,4-linkage was introduced at or near the non-reducing end of the glucan and the reducing end part of the glucan was mainly composed of $\alpha$-1,4-linkages.

The glucosidic linkage introduced was dependent on the specificity of $\alpha$-glucosidase for glucosidic linkage. $\alpha$-Glucosidases from Aspergillus niger (ANG) and Acremonium strictum (ASG) produced $\alpha$-1,6- and $\alpha$-1,3-glucosidic linkages, respectively. The chain length distribution also varied according to the specificity of $\alpha$-glucosidases for substrate chain length. The major DP of the glucans produced by ANG and ASG were 4–6 and 6–10, respectively. The glucan produced by the coupled reaction was highly resistant to retrogradation. The syrup including this glucan maintained transparency despite the lower content of long-chain glucan. This indicates that the glucosyl residue linked by $\alpha$-1,3-linkage present at or near the non-reducing end of glucan strongly inhibits aggregation of glucan and provides retrogradation tolerance.

Key words: transglucosylation, resistance to retrogradation, $\alpha$-glucosidase, cyclodextrin glucanotransferase

Starch-derived oligosaccharides such as isomalto- and nigeroligosaccharides are usually produced from starch by the combination of hydrolysis and transglucosylation.1,2 As a first step, starch is liquefied by thermostable $\alpha$-amylase at around 100°C. This gelatinized starch is then cooled to around 50–65°C and hydrolyzed to maltose and maltotriose by $\beta$-amylase and starch debranching enzymes. $\alpha$-Glucosidase is added and catalyzes the transglucosylation toward maltose and maltotriose to produce glucosidic linkage other than $\alpha$-1,4-linkage at the non-reducing end of maltooligosaccharides. The type of linkage produced is dependent on the specificity of $\alpha$-glucosidase for the glucosidic linkage. For example, $\alpha$-glucosidases from Aspergillus niger (ANG) and Acremonium strictum (ASG) produce isomalto-($\alpha$-1,6-linkage) and nigeroligosaccharides ($\alpha$-1,3-linkage) oligosaccharides, respectively.3,4 These enzymes are utilized in industrial oligosaccharide production.

The degree of polymerization (DP) of these oligosaccharides is usually 2–4. This is because the substrate for transglucosylation is mainly maltose and maltotriose produced from starch by $\beta$-amylase. It is very difficult to introduce glucosidic linkage other than $\alpha$-1,4-linkage at the non-reducing end of longer maltooligosaccharides by the usual hydrolysis-transglucosylation process because long maltooligosaccharides are immediately insoluble in water due to retrogradation. In addition, it is very difficult to produce long maltooligosaccharides efficiently with no production of short maltooligosaccharides by $\alpha$-amylase since this enzyme produces short maltooligosaccharides even in the initial stages of starch degradation. Cyclodextrin glucanotransferase (CGTase), which is a type of glycosyl transferase belonging to glycoside hydrolase family 13,5,6 produces CDs from starch via intramolecular transglycosylation.7 This enzyme is known to catalyze four reactions: (i) CD production, (ii) transglycosylation of maltooligosaccharides to other maltooligosaccharides (disproportionation), (iii) the opening of circular CDs and the subsequent transfer to maltooligosaccharides (coupling), and (iv) hydrolysis.8 The hydrolytic activity of CGTase is generally lower than the transglycosylation activity.9 CGTase are thought to degrade starch more mildly than $\alpha$-amylase and CGTase is an appropriate enzyme to produce long maltooligosaccharides from starch. The sweetness of the starch-derived glucan generally depends on its chain length. Shorter-chain length glucans have a stronger sweetness than longer-chain glucans. Although the sweetness of long-chain glucans is weak, these substances are capable of masking the bitter tastes of foods and beverages and providing a rich taste.10 Long glucans such as dextrin are added to food and beverages

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as a substance to improve flavor based on the above properties. In addition, long-chain glucans exhibit lower osmotic pressure than short-chain glucans, and are more gently absorbed by humans.80

In this study, we established a novel reaction system to produce long-chain α-1,4-glucans having α-1,3- and α-1,6-linkage at the non reducing end from starch with CGTase and α-glucosidase. The characteristics of this reaction system and the obtained glucans are presented.

MATERIALS AND METHODS

Enzymes. All enzymes used in this study were purchased from the enzyme suppliers. α-Glucosidases from ANG and ASG were purchased from Amano Enzyme Inc. (Nagoya, Japan) and Kirin Kyowa Foods Co., Ltd. (Tokyo, Japan), respectively. One unit of ANG was defined as the amount of enzyme hydrolyzing 1 μmol of maltose (substrate concentration was 0.2%) in 40 mM sodium acetate buffer (pH 4.2) in 1 min at 37°C. In the case of ASG, 1% maltose and 25 mM sodium phosphate buffer (pH 7.0) were used as substrate and reaction buffer, respectively.

CGTases from Bacillus sp. No. 38-2 and Bacillus coagulans were from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan), and those from Geobacillus stea rotherophilus and B. macerans were from Hayashibara Biochemical Laboratories Inc. (Okayama, Japan) and Amano Enzyme Inc., respectively. One unit of CGTase was defined as the enzyme amount producing 1 mg of β-CD from 0.9% soluble starch in 36 mM potassium phosphate buffer (pH 6.0) in 1 min at 40°C.

Starch debranching enzymes, isoamylase from Flavobacterium odoratum KU and pullulanase from Klebsiella pneumoniae were purchased from Nihon Shokuhin Kako Co., Ltd. and Amano Enzyme Inc., respectively. One unit of isoamylase was defined as the amount of enzyme necessary to increase A560 by 0.01 in 1 min under following conditions. A reaction mixture consisting of 0.1 mL of enzyme solution, 0.35 mL of 0.5% waxy corn starch, and 0.1 mL of 50 mM sodium acetate buffer containing 20 mM CaCl2 (pH 6.0) was incubated for 15 min at 45°C. Then 0.5 mL of 1.66% KI and 0.127% I2 in 0.08 M HCl was added and A560 was measured after addition of 10 mL of water. One unit of pullulanase was the amount of enzyme that produced reducing sugar equivalent to 1 μmol glucose from 1% pullulan in 1 min at 40°C in 0.1 M sodium acetate buffer (pH 5.0).

Soybean β-amylase was from Nagase Chemtex Co. (Osaka, Japan). One unit of β-amylase was defined as the amount of enzyme releasing reducing sugar equivalent to 100 μg of glucose from 1% of soluble starch in 50 mM sodium acetate buffer (pH 4.5) in 1 min at 40°C.

α-Amylase from B. amylogeiquefaciens and that from Microbacterium imperiale was from Daiwa Kasei K.K. (Shiga, Japan) and Amano Enzyme Inc. One unit of α-amylase from B. amylogeiquefaciens was defined as the amount of enzyme needed to reduce the kinetic viscosity of 91 g/L gelatinized potato starch to 250 × 10^-4 m^2/s in 0.1 M sodium acetate buffer (pH 6.0) at 65°C in 15 min. One unit of α-amylase from M. imperiale was defined as the amount of enzyme which released reducing sugar equivalent to 1 μmol glucose from 0.5% (w/v) soluble starch in 40 mM sodium acetate buffer (pH 6.0) in 1 min at 40°C.

HPLC analysis of the reaction mixture. The chain length distribution of the glucan produced by the coupled reaction was analyzed by HPLC. HPLC conditions were as follows: Column, MCI GEL CKO2AS (ϕ 20 x 250 mm, Mitsubishi Chemical Co., Tokyo, Japan); column temperature, 80°C; eluent, water; flow rate, 1.0 mL/min; detection, refractive index; injection volume, 50 μL (concentrations of the samples were 50 mg/mL).

The content of β-amylase resistant glucan in the reaction products of the coupled reaction was determined as follows. Fifty microliters of 150 U/mL soybean β-amylase in 1 mM sodium acetate buffer (pH 5.5) was added to 1 mL of the reaction mixture diluted to 5% (w/v) with water. This mixture was then incubated for 30 min at 55°C and boiled for 10 min. Then HPLC analysis was performed as described above. The content of the β-amylase resistant glucan was determined from the total content of non-hydrolyzed sugar longer than a tetrasaccharide. Maltooligosaccharides longer than maltotetraose were completely hydrolyzed to maltose and maltotriose under these conditions.

DP distribution of α- and β-amylase digested glucans was analyzed by HPLC under following conditions: Column, Aminex HPX-42A (ϕ 7.8 × 300 mm, Bio-Rad, Hercules, USA); column temperature, 75°C; eluent, water; flow rate, 0.5 mL/min; detection, refractive index; injection volume, 10 μL (sample concentration was 50 mg/mL).

The coupled reaction of CGTase and α-glucosidase. The coupled reaction of α-glucosidase and CGTase was examined using maltodextrin with a dextrose equivalent of 4 (Pinedex No. 100, Matsutani Chemical Industry, Co., Ltd., Itami, Japan) as a substrate. The reaction mixtures of 5 mL containing 5–30% (w/v) Pinedex No. 100, 10 mM sodium acetate buffer (pH 6.0), 2.5 mM CaCl2, Bacillus sp. No. 38-2 CGTase (13 U per 1 g of substrate), ANG or ASG (50 U or 20 U per 1 g of substrate, respectively), isoamylase (4000 U per 1 g of substrate) and pullulanase (12 U per 1 g of substrate) were incubated at 37°C for 6 h. The reaction mixtures were then boiled to terminate the reaction and applied to the HPLC for analysis. To evaluate the effect of the enzymes, reactions without α-glucosidase, pullulanase and isoamylase were also tested.

The coupled reactions using various CGTases were also examined. The CGTase from Bacillus sp. No. 38-2 was replaced with an enzyme from other origins, G. stea rotherophilus, B. macerans or B. coagulans.

The coupled reaction of CGTase and α-glucosidase with gelatinized corn starch and the preparation of the syrup. The coupled reaction of CGTase and α-glucosidase towards gelatinized corn starch was tested using ANG and ASG. Ten liters of 30% (w/v) gelatinized corn starch obtained from the maltose syrup factory of Nihon Shokuhin Kako Co., Ltd. (dextrose equivalent is 6.5) was cooled to 53°C in a water bath. The pH was then adjusted to 6.0 with 2 N HCl or NaOH. Bacillus sp. No. 38-2 CGTase (0.65 U per 1 g of substrate), ANG or ASG (2.5 U or 1 U per 1 g of substrate, respectively),
isoamylase (200 U per 1 g of substrate) and pullulanase (0.6 U per 1 g of substrate) were incubated at 53°C for 48 h.

The syrups containing β-amylase resistant glucans produced by ANG and ASG were prepared by the following procedure. Several grams of active carbon (Kiris Kyowa Foods Co., Ltd.) was added to the reaction mixture and heated at 80°C for 30 min. The mixture was then filtered with a diatomite filter and deionized by ion exchange column chromatography, where Amberlite MB-4 (Rohm and Haas Co., Philadelphia, USA) was used. The deionized mixture was filtered using a 0.45 μm membrane and concentrated in vacuo up to 70% (w/v).

**Digestion of β-amylase resistant glucan by α- and β-amylase.** β-Amylase resistant glucans produced by the coupled reaction using ANG and ASG described above were digested by α- and β-amylase. In the β-amylase digestion, 15 U soybean β-amylase per 1 g of solid material and 5 mL of 1 M sodium acetate buffer (pH 5.5) were added to 1 L of the 10% (w/v) respective glucan, and the mixture was incubated at 53°C for 5 h. In the α- and β-amylase digestion, 55 U M. imperiale α-amylase per 1 g of solid material was added to 1 L of the 10% (w/v) respective glucan of 1 L in addition to the β-amylase and buffer described above, and the reaction mixture was incubated at 53°C for 5 h. Then, the mixture was boiled for 10 min to stop the reaction.

The glucan digested by α- and β-amylases was concentrated in vacuo up to 50% (w/v) and a sample of 10 mL was subjected to carbon-celite column chromatography. Equal weight of active carbon (Kiris Kyowa Foods Co., Ltd.) and celite 545 (Kanto Kagaku Co., Inc., Tokyo, Japan) were mixed and suspended in water. Then, the slurry was packed in the glass column and the chromatography was performed under the following conditions: Column size, φ 5.0 × 45 cm (880 mL), column temperature, room temperature; flow rate, 3.75 mL/min; elution, linear gradient of 1-butanol (from 0 to 3%); fraction size, 20 mL. Sugar contents of the fractions were measured by the phenol sulfuric acid method and the purity of the fraction was checked by HPLC analysis using Aminex HPX-42A as described above. The fractions containing oligosaccharide with DP-5 were collected and concentrated in vacuo. Then, the sample was deionized as described above and freeze-dried.

**Methylation analysis.** The oligosaccharide with DP-5 purified from the hydrolysate of the glucan produced by the coupled reaction was subjected to methylation analysis to examine the composition of the glucosidic linkage. A 5 mg solid of purified oligosaccharide was freeze-dried and methylated by the method of Hakomori. Following extraction with chloroform, a methylated sample was hydrolyzed by 4 M trifluoroacetic acid at 100°C for 3 h. The hydrolysate was then reduced with 1% NaBH₄ at room temperature for 18 h and acetylated with acetic anhydride at 100°C for 4 h. The partially methylated alditol acetates were analyzed with a GC under the following conditions: Column, TC-17 (φ 0.5 mm × 30 m; GL Science Inc., Tokyo, Japan); injection volume, 1 μL; injection temperature, 300°C; column temperature profile, 1 min at 50°C and increased to 280°C at a rate of 10°C/min; flow rate, 2.5 mL/min; detection, flame ionization detector; detection temperature, 300°C.

**Comparison of the resistance to retrogradation between β-amylase resistant glucan and corn syrup.** The resistance to retrogradation of the syrup including the glucan produced by the coupled reaction of CGTase and ASG was compared against the retrogradation of corn syrup. The corn syrup including the hydrolysate of gelatinized corn starch by α-amylase and starch debranching enzymes was prepared as follows. One liter of 22.5% (w/v) gelatinized corn starch was cooled to 53°C and the pH adjusted to 6.0 as described above. B. amyloliquefaciens α-amylase (3.6 U per 1 g of substrate), isoamylase (400 U per 1 g of substrate) and pullulanase (1.2 U per 1 g of substrate) were then added and incubated for 38 h. The syrup was prepared from this reaction mixture as described above. The resistance to retrogradation was evaluated by apparent transparency of the syrup after storage at room temperature for 1 month.

**RESULTS AND DISCUSSION**

**The coupled reaction of CGTase and α-glucosidase.**

The coupled reaction of CGTase and α-glucosidase toward dextrin, Pinedex No. 100, in the presence of starch debranching enzymes, pullulanase and isoamylase was examined. The reaction mixture with no α-glucosidase immediately clouded due to the retrogradation of amylose released from the substrate by the starch debranching enzymes, isoamylase and pullulanase. In contrast, the mixture including α-glucosidase remained transparent throughout the reaction (Fig. 1). This result indicated that the glucan produced by the coupled reaction of CGTase and α-glucosidase was resistant to retrogradation.

The sugar composition of the reaction mixture of the coupled reaction was analyzed by HPLC. In the case of the reaction with no α-glucosidase, production of β-CD and γ-CD was observed (Figs. 2(c) and (f)). However, when CGTase was coupled with α-glucosidase, which transfers a glucose moiety toward the non-reducing end of glucan, very small amounts of CDs were produced in the

**Fig. 1.** The appearance of the reaction mixture of the coupled reaction of CGTase and α-glucosidase.

The appearance of the mixtures reacted for 6 h is shown. (a), (b) and (c) indicate the reaction mixtures in which CGTase, isoamylase and pullulanase were coupled with ANG, ASG and no α-glucosidase, respectively.
Initial stages of the reaction. The produced CDs were completely degraded in the later stages of the reaction by CGTase (Figs. 2(a), (b), (d) and (e)). The glucan produced in the absence of α-glucosidase was efficiently digested by β-amylase to maltose and maltotriose (after digestion, content of maltose and maltotriose in the solid material was 66 and 9%, respectively), which efficiently hydrolyzes α-1,4-glucosidic bonds at the non-reducing end of glucans such as starch and dextrin longer than maltotetraose (Fig. 2(f)). In contrast, the glucan produced in the presence of α-glucosidase was not fully digested (Figs. 2(d) and (e)). The content of disaccharide in the solid material of the glucan produced by the coupled reaction with ANG before and after β-amylase treatment was 13.7 and 15.2%, respectively, and that with ASG before and after β-amylase treatment was 3.7 and 7.7%, respectively. This observation indicates that the glucan produced by the coupled reaction of CGTase and α-glucosidase has glucosidic linkage other than α-1,4-linkage positioned at or near the non-reducing end of the glucan, and this linkage was clearly produced by the transglucosylation activity of α-glucosidase. It was assumed that this β-amylase resistant glucan inhibited CD production by CGTase due to the presence of the glucosidic linkage other than α-1,4-linkage at or near the non-reducing ends. This implied that the linear structure comprised by only α-1,4-glucosidic linkage at the non-reducing ends is essential for the production of CD by CGTase.

The DP of the β-amylase resistant glucan produced by the coupled reaction was clearly longer than the isomalto- and nigero-oligosaccharides produced by the usual processes: Starch degradation by α-amylase and β-amylase, followed by the transglucosylation with α-glucosidase. Although the chain length of the isomalto- and nigero-oligosaccharides produced by the usual process was mainly DP 2-4, very long glucan with ≥ DP-10 (megalo-sugar) was included in the reaction product by the coupled reaction (Figs. 2(d) and (e)). The chain length of the β-amylase resistant glucan produced by the coupled reaction varied depending on the α-glucosidase used (Figs. 2(d) and (e)). The glucan produced by the reaction using ASG was longer than that of the reaction employing ANG. The majority of the glucans produced with ASG and ANG were glucans with DP 6-10 and 4-6, respectively. This result was explained by the specificities of these α-glucosidases towards substrate chain length. ASG shows higher preference for long-chain substrates than ANG. Therefore, ASG was able to transfer the glucose moiety toward longer-chain glucans than ANG. It was thought that the chain length of the produced glucan is controllable by selecting α-glucosidases based on the specificity for substrate chain length.

The yield of β-amylase resistant glucan (≥ DP 4) was changed by the substrate (Pinedex No. 100) concentration and the α-glucosidase used. A higher yield of β-amylase resistant glucan was obtained by the reaction with higher concentrations of substrate (Fig. 3). ASG gave higher yields of β-amylase resistant glucan than ANG, indicating that ASG has stronger transglucosylation activity than ANG. Similar yields of β-amylase resistant glucan were obtained in the reaction involving the coupling of the enzymes with CGTase from various origins; however, the...
The purified oligosaccharides were subjected to methylation analysis. Three peaks were detected in the GC analysis of the derivatives of both oligosaccharides from BRGI (BRGI-5) and BRGII (BRGII-5). The retention times of the three peaks of the derivatives of BRGI-5 corresponded to those of 2,3,4,6-tetramethyl glucose, 2,3,6-trimethyl glucose, and 2,4,6-trimethyl glucose, and the ratio of peak area of 2,3,6-trimethyl glucose and 2,4,6-trimethyl glucose was around 1 to 1. This indicated that BRGI-5 had 2 of α-1,4- and α-1,3-glucosidic linkages on average. Therefore, the structure of typical

![Graph](image1.png)

**Fig. 4.** The yield of the β-amylase resistant glucan by the coupled reaction with various CGTases.

The yield of the β-amylase resistant glucan reacted for 6 h is shown. The results of the reaction with ASG and ANG are presented in panel (a) and (b), respectively. A, B, C and D indicate the reaction with *Bacillus* sp. No. 38-2, *B. coagulans*, *B. maserans* and *G. stearothermophilus*, respectively.

![Graph](image2.png)

**Fig. 5.** The effects of the starch debranching enzymes in the coupled reaction of CGTase and α-glucosidase.

The HPLC profile of the mixture of the coupled reaction using ANG and *Bacillus* sp. No. 38-2 CGTase with or without starch debranching enzymes for 6 h is shown. The long-chain sugars eluted earlier than shorter-chain sugars. The numbers above the peaks indicate the degree of polymerization of the glucan. (a) The coupled reaction of ANG and *Bacillus* sp. No. 38-2 CGTase with pullulanase and isoamylase. (b) That with only isoamylase. (c) That with only pullulanase. (d) That with no starch debranching enzymes.
The peaks of the derivative of BRGII-5 corresponded to those of the reducing end. The reaction mixtures with ASG and ANG are shown in panels (a) and (b), respectively.

The HPLC profile of the mixtures reacted for 48 h at 53°C is shown. Long-chain sugars eluted earlier than short ones. The numbers above the peaks indicate the degree of polymerization of the glucan. The reaction mixtures with ASG and ANG are shown in panels (a) and (b), respectively.

**Fig. 6.** Digestion of BRG I and BRGII by α- and β-amylases.

BRGI and BRGII were digested by α- and β-amylases, and the DP distribution was analyzed by HPLC. Black, gray and white bars indicate untreated, β-amylase digested, and α- and β-amylase digested samples, respectively.

**Fig. 7.** Digestion of BRGI and BRGII by α- and β-amylases.

BRGI-5 was predicted to be 41-α-nigerotriosyl-maltose, 41-α-glucosyl-4-α-nigerotriosyl-glucose, 3-α-glucosyl-31-α-maltosyl-maltose, 31-α-maltotriosyl-nigerose, 31-α-maltotriosyl-3-α-maltotriosyl-glucose and 31-α-glucosyl-3-α-maltotriosyl-glucose, and these oligosaccharide units were bound at the non-reducing end of BRGI. It is likely that BRGI is mixture of the glucans having above structures at the non-reducing end.

In the case of BRGII-5, the retention times of the three peaks of the derivative of BRGII-5 corresponded to those of 2,3,4,6-tetramethyl glucose, 2,3,6-trimethyl glucose and 2,3,4,6-trimethyl glucose, and the ratio of peak area of 2,3,6-trimethyl glucose and 2,3,4-trimethyl glucose was around 3 to 1, indicating that BRGII-5 had 3 α-1,4-glucosidic linkages and 1 α-1,6-glucosidic linkage on average. The possible structures of BRGII-5 were thought to be 61-α-glucosyl-maltotetraose, 61-α-maltosyl-maltotriose, 61-α-maltotriosyl-maltose, and 6-α-maltotetraosyl-glucose. However, it is possible for a maltooligosaccharide moiety linked by α-1,6-linkage at the non-reducing end of the glucan to be digested by starch debranching enzymes, pullulanase or isoamylase, added in the reaction mixture of the coupled reaction of α-glucosidase and CGTase. Therefore, these structures were not thought to remain after the reaction and the BRGII-5 is predicted to be 61-α-glucosyl-maltotetraose. It was assumed that BRGII has the α-1,6-glucosyl residue at the non reducing end.

**The resistance to retrogradation of BRGI.**

The β-amylase resistant glucan produced by the coupled reactions was judged to be resistant to retrogradation. We focused on this characteristic and evaluated the resistance to retrogradation by preservation stability of the prepared syrup including BRGI. The preservation stability of the syrup following storage for 1 month at room temperature. The appearances of the prepared syrups including β-amylase resistant glucan produced by the coupled reaction (a) and starch hydrolysate (b) are shown.

**Table 1.** The chain-length distribution of the BRGI syrup and the control syrup.

<table>
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<th>DP</th>
<th>BRGI syrup (%)</th>
<th>Corn syrup (%)</th>
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<tr>
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</tr>
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</tr>
<tr>
<td>≥10</td>
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<td>18.1</td>
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the BRGI syrup was evaluated by the apparent transparency following storage at room temperature for 1 month and was compared with that of corn syrup, which was a hydrolysate of gelatinized corn starch by α-amylase, iso-amylase and pullulanase. The transparency of the control syrup was clouded after 1 month of storage (Fig. 8, Table 1). The white turbidity of corn syrup was thought to arise from the retrogradation of amylose present in small amounts within the syrup leading to the aggregation of the amylose chains. Therefore, branched structures at the non-reducing end of BRGI strongly inhibited aggregation of glucan branched structures at the non-reducing end.

REFERENCES