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# Physicochemical Properties of Corn Starch-derived Branched Dextrin Produced by a Branching Enzyme

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**Abstract** The optimal conditions for the production of branched dextrin from corn starch (CSBD) using branching enzyme (BE) were established by investigating the degree of retrogradation of the gelatinized starch. The physicochemical properties of CSBD prepared using the established process were evaluated. It was found that physicochemical properties of corn starch were greatly modified by BE treatment. CSBD had a higher dextrose-equivalent value and water solubility than the corresponding control. On the other hand, the viscosities in gelatinized solution and amylose contents of CSBD were lower than those of the control. A high-performance size-exclusion chromatography/multiangle laser light scattering/refractive index (HPSEC/MALLS/RI) system showed that the average molecular weight of CSBD was lower than that of the control. The pasting viscosities of CSBD were stable during the entire temperature cycle. In general, the BE treatment resulted in the retrogradation during storage being lower for CSBD than for the control.

**Keywords:** branching enzyme, branched dextrin, corn starch, physicochemical property

## Introduction

Starch constitutes the major reserve of polysaccharides for plants, and consists of two components: amylose and amylopectin. Amylose is an essentially linear glucan linked with α-1,4 glucosidic linkages, while amylopectin is highly branched. The hydrolysis of starch results in dextrin, which is widely used in food, paper, and other industries (1). This degradation of starch increases its reducing power and solubility, while the viscosity of the paste solution decreases, and the starch gradually loses the blue staining that occurs with iodine (2). Dextrins of various sizes can be produced by treating starch with α-amylase (EC 3.2.1.1) or acid under appropriate conditions (3). The action of pancreatic α-amylase on amylopectin or glycogen eventually produces a series of dextrins that contain α-1,6 linkages (4,5).

Branching enzyme (BE) is a transferase that acts on some α-1,4 linkages of linear glucan (e.g., amylose) to produce the branched glucan in α-1,6 linkages as found in amylopectin or glycogen. BE enzymatically converts the α-1,4 linkages into α-1,6 linkages to form a new branched structure (6). It has been demonstrated that BE also catalyzes the cyclization of amylose and amylopectin (7,8). Takata *et al.* (8) also produced cyclic glucan from amylopectin by *Bacillus stearothermophilus* BE, and analyzed the structure of cyclic glucan and weight-averaged degree of polymerization. Takata *et al.* (9) reported that amylopectin was converted to dextrin by the cyclization of BE. The enzyme efficiently degraded amylopectin even when the substrate concentration was as high as 20-30%. These results suggested that BE would be useful as a new starch-degrading enzyme. The products were highly soluble in water, giving a highly stable, clear solution. The results also suggested that the product reduced the tendency of other dextrins to undergo retrogradation. However, the production of branched dextrin (BD) using BE has not been reported.

The objective of this study was to determine the optimal conditions and process for producing BD using BE from *Rhodothermus obamensis*, and evaluated the physicochemical properties of BD from corn starch (CSBD).

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## Materials and Methods

**Carbohydrate samples** Rice starch was purchased from Sigma-Aldrich (St. Louis, MO, USA). Corn starch, waxy corn starch, and maltodextrin (DE 1.67) were obtained from Daesang (Seoul, Korea). Branching enzyme (BE) from *Rhodothermus obamensis* was kindly donated by Novozymes (Chiba, Japan).

**Production of branched dextrin from corn starch (CSBD)** Corn starch slurries with the concentration of 10%(w/w) were prepared with distilled water, adjusted to pH 7.5 with 4%(w/w) NaOH and preheated at 60°C for 30 min. Then the slurries were autoclaved at 125°C for 20 min and cooled to 65°C. BE was added to each slurry with the amount of 800 U/g starch and the mixtures were incubated at 65°C for 24 hr. The reaction mixtures were boiled for 15 min and cooled to room temperature. Branched dextrin (BD) was acquired by spray drying.

**Estimation of CSBD retrogradation** Turbidity of solution was estimated with slight modifications (10). CSBD slurry was prepared with the concentration of 4%(w/w) and autoclaved at 125°C for 20 min. The solution was cooled at

80°C water bath for 30 min and absorbance at 660 nm was measured. The absorbance was used for absorbance at To. Then the solution was stored at 4°C, and absorbance at 660 nm was measured at each sampling time. Blank test was performed with distilled water. Degree of retrogradation was calculated using following equation.

Degree of retrogradation
= (ΔAbs at 660 nm)Ti − (ΔAbs at 660 nm)To

Ti: sampling time
To: time when the temperature of solution was at 80°C

**Dextrose equivalent (DE)** The DE value was determined according to the Somogyi method (11). A sample (7-8 g) was transferred to a 200-mL mass flask and diluted to 200 mL with distilled water. The flask was shaken well, and 25°C to 50 mL of the sample solution was run through a 25-mL burette before the burette was refilled with the sample solution. Fehling solution (25 mL) was pipetted into a 250-mL flask and brought to a boil over an open flame. [The Fehling solution was prepared by mixing equal volumes of solution A (34.64 g of crystalline copper sulfate dissolved in distilled water to a final volume of 500 mL) and solution B (173 g of potassium sodium tartrate and 50 g of NaOH was dissolved in distilled water to a final volume of 500 mL) immediately prior to use.] The Fehling solution was titrated with the sample solution from the burette to within 0.5 mL of the expected end point. The flask was swirled while its contents were boiled for 2 min. Two drops of methylene blue indicator and 2 drops of sample solution were added to the solution. This solution was brought to a boil again, and the brick-red cuprous oxide was allowed to settle to the bottom of the flask. The sample solution was added dropwise until the blue color completely disappeared from the supernatant liquid. DE was calculated using following equation:

% Reducing sugar (K)

$$= \frac{200 \times \text{Fehling factor} \times 100}{\text{sample titer (mL)} \times \text{sample wt. (g)}}$$

$$\text{DE} = \frac{\text{K} \times 100}{\% \text{ dry solids}}$$

Fehling factor: 1.45 for glucose

**Water solubility** Water solubility was determined by the methods described by Chang and Cho (12). Substrate (12 g) was added to 20 mL of water and stirred vigorously at room temperature for 24 hr, followed by centrifugation at 4,032×g for 30 min. Then 3 volumes of ethanol were added to 8 mL of the supernatant followed by centrifugation at 11,200×g for 30 min. The precipitate was taken, dried at 50°C under vacuum for 24 hr, and weighed, from which the solubility was calculated.

**Viscosity** Viscosity was measured with a Brookfield viscometer (model LVF; Brookfield Engineering, Middleboro, MA, USA) after the sample was gelatinized and cooled with an automated gelatinization apparatus (Eunhye Electric, Bucheon, Korea) to a 3% substrate suspension at 50°C. The temperature was raised from 50 to 95°C at a rate of 1.5°C/min, maintained at 95°C for 30 min, and then lowered to 50°C instantaneously.

**Amylose content** The amylose content was determined by measuring the absorbance at 660 nm resulting from the reaction with an iodine solution. A sample (0.1 g) was transferred into a 100-mL volumetric flask and combined with 1 mL of 99%(v/v) ethanol, 2 mL of 10%(w/w) NaOH, and 10 mL of distilled water. The mixture was heated at 70°C for 20 min, cooled, and the volume was increased to 100 mL with distilled water. Ten mL of the mixture was pipetted into a 1,000-mL volumetric flask, combined with 3 drops of 6 N HCl and 5 mL of 0.1 N $I_2$ solution, increased to 1,000 mL with distilled water, shaken, and then allowed to stand for 20 min. The absorbance of the final solution was measured at 660 nm using distilled water as a blank, and was converted to the amylose content using a standard curve.

**Scanning electron microscopy (SEM)** The particle size and surface image of corn starch, CSBD, and maltodextrin were evaluated by SEM (UV1201; Shimadzu, Kyoto, Japan) at a magnification of 1,500 times.

**Determining the average molecular weight (Mw)** A high-performance size-exclusion chromatography/multiangle laser light scattering/refractive index (HPSEC/MALLS/RI) system was used to determine Mw values of corn starch, waxy corn starch, and CSBD, as described by You and Lim (13). Samples were dissolved in 90% dimethyl sulfoxide and precipitated by ethanol according to the procedures of Jane and Chen (14). Ethanol (100 μL) was added to the purified sample. After 5 min, 1 mL of 2 M NaOH was added, and the sample was then stirred at 70°C for 5 min. The sample was diluted with 18 mL of 0.15 M $NaNO_3$ and neutralized by 2 M HCl. The alkaline-dissolved sample solution was autoclaved at 121°C for 20 min.

The mobile phase used for HPSEC was aqueous $NaNO_3$ solution (0.15 M) that had been filtered through a 0.1-μm cellulose nitrate filter (7181-004; Whatman, Maidstone, UK) and degassed by a vacuum pump for 2 hr before use. The HPSEC/MALLS/RI system consisted of a pump (model P2000; Spectra System, San Jose, CA, USA), an injector valve with a 500-μL loop (model 7072; Rheodyne, Cotati, CA, USA), a guard column (TSK PWH, Tosoh, Tokyo, Japan), a SEC column (7.8×6,000 mm, TSK G5000PW; Tosoh), a MALLS scattering detector (632.8 nm, DAWN DSP-F; Wyatt Technology, Santa Barbara, CA, USA), and an RI detector (model SE71; Shodex, Tokyo, Japan). The column temperature and flow rate were 60°C, and 0.4 mL/min, respectively. The sample solution was filtered through a 3.0-μm cellulose nitrate filter (7193-002; Whatman) at 70°C before being injected into the HPSEC system.

**Pasting properties** The pasting properties of corn starch, CSBD, and corn starch replaced with CSBD and maltodextrin were analyzed using a rapid visco-analyzer (RVA, model 3D-plus; Newport Scientific, Narrabeen, Australia). The temperature-time conditions for the samples were as follows: an equilibration phase at 50°C for 1 min, a heating step from 50 to 95°C at 6°C/min, a holding phase at 95°C for 5 min, a cooling step from 95 to 50°C at 6°C/min, and a holding phase at 50°C for 5 min.

**Statistical analysis** Statistical analyses comprising analysis of variance and pairwise comparisons (Student-Newman-Keuls method) were performed using SigmaStat for Windows (version 1.0, Jandel, CA, USA).

## Results and Discussion

**Production of CSBD** The effects of some of the production parameters on the retrogradation of BD were evaluated by measuring the turbidity of solution. We focused on retrogradation because we considered it to be the most important factor when BD is used for the production of starchy food.

The effect of corn starch concentration on retrogradation of BD is showed in Fig. 1. Degree of retrogradation increased as substrate increased. Degree of retrogradation was greatly decreased when enzyme was added with the amount of 800 U/g substrate in compared to the amount of 400 U/g substrate. There were little differences in the degree of retrogradation when substrates were treated with more than 800 U/g substrate (Fig. 2). Figure 3 shows the influence of reaction time on retrogradation of BD. The prolonged reaction time resulted in decrement of degree of retrogradation of BD. In case of substrate concentration, when substrate concentration was more than 15%(w/w), the viscosity of gelatinized substrate solution was very high, so it would be difficult to produce BD in large scale. BD obtained from 400 U/g substrate underwent rapid retrogradation, compared to those from more than 800 U/g substrate. This suggests that it is necessary to use 800 U/g substrate enzyme dosage to produce BD. In addition, the more the reaction is done, the less the retrogradation of BD occurs (Fig. 3). From the results described above, it is concluded that the optimal conditions for production of BD are 10%(w/w), 800 U/g substrate, and 24 hr for substrate concentration, enzyme dosage, and reaction time, respectively.

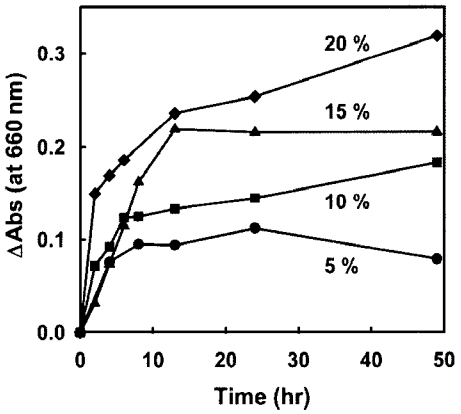


**Fig. 1. Effect of substrate concentration (w/w) on retrogradation of gelatinized branched dextrin (BD) solution.**

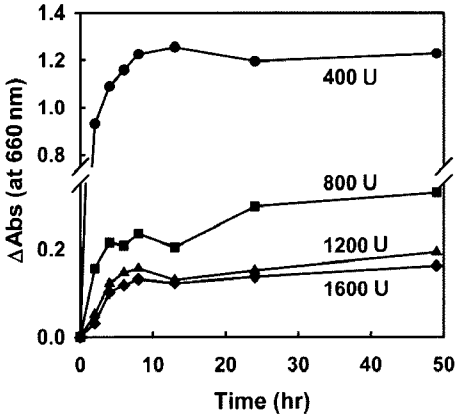


**Fig. 2. Effect of enzyme dosage on retrogradation of gelatinized branched dextrin (BD) solution.** Enzyme dosage was U/g substrate.

**Dextrose equivalent (DE)** The DE is widely used in the corn syrup industry: it is defined as the reducing sugars present expressed as dextrose, and is calculated as a percentage of the dry substance. Maltodextrin or hydrolyzed cereal solids are usually classified by their DE values. The DEs of CSBD, maltodextrin, and soluble starch were 0.49, 1.67, and 0.28, respectively (Table 1). The DE of CSBD was lower than that of maltodextrin. In general, a higher DE of maltodextrin and a lower Mw are associated with a higher reducing capacity relative to starch (15,16). Heating a maltodextrin with a higher DE in the presence of protein, amino acids, etc., produces a dark brown color due to the Maillard reaction. This also restricts their useful applications.

The above DE values indicate that the reactivity of CSBD is significantly lower than that of maltodextrin, and hence that CSBD would not readily color compared to conventional dextrins.

**Water solubility** The solubility of CSBD, corn starch, waxy corn starch, and maltodextrin are given in Table 1. The solubility of CSBD and maltodextrin were 11.24 and 9.52%, respectively. The water solubility of the native starch has been reported as zero due to the strong bonding forces between the starch molecules (17), while the water solubility of CSBD was higher than that of maltodextrin. The increased water solubility of CSBD is due to the formation of short-chained α-1,6 branched linkages.

**Viscosity** The Brookfield viscosities of corn starch, CSBD, maltodextrin, and soluble starch were 122.0, 2.7, 3.1, and 2.8 cp, respectively, for a 3% substrate concentration (Table 1). Leman *et al.* (18) reported that the viscosity of starch was

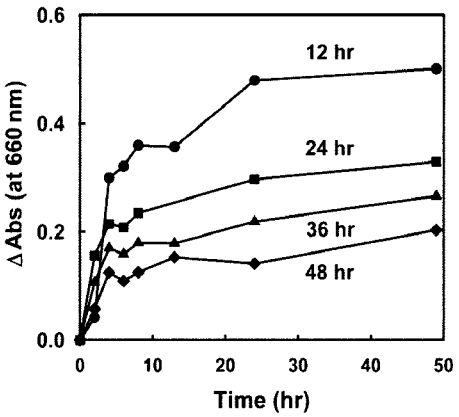


**Fig. 3. Effect of reaction time on retrogradation of gelatinized branched dextrin (BD) solution.** Substrate concentration and enzyme dosage were 10%(w/w) and 800 U/g starch, respectively.

**Table 1. Dextrose equivalents (DEs), solubility, viscosity, and amylase contents of corn starch, branched dextrin from corn starch (CSBD), maltodextrin, and soluble starch**

| Source | DE[1] | Solubility (%, w/v) | Viscosity (cp)[2] | Amylose (%)[3] |
|---|---|---|---|---|
| Corn starch | ND[4] | ND | 122.0 | 24.7 |
| CSBD | 0.49 | 11.24 | 2.7 | 4.0 |
| Maltodextrin | 1.67 | 9.52 | 3.1 | 15.3 |
| Soluble starch | 0.28 | - | 2.8 | - |
| Waxy corn starch | -[5] | ND | - | 2.6 |

[1]Measured by the Somogyi method (30).
[2]Measured with a Brookfield viscometer using a gelatinized solution (3%) at 50°C.
[3]Measured by an iodine affinity method (30).
[4]Not detected.
[5]Not analyzed.

affected by various factors, including Mw, the amylose/amylopectin ratio, and the degree of branching. Thus, the lower viscosity of CSBD may be attributable to the formation of branching and the degradation of starch molecules.

**Amylose content** It has long been recognized that starch can be separated into 2 fractions (amylose and amylopectin) with widely differing physical properties. The amylose content has been determined quantitatively for many years, commonly by measuring the blue amylose-iodine complex. In this study, the amylose contents were determined by measuring the intensity of the blue color produced when stained with iodine.

The amylose contents of CSBD, corn starch, waxy corn starch, and maltodextrin are listed in Table 1. The amylose content of corn starch was 24.7%, which is consistent with Chrastil (19). Amylose content of CSBD was lower than that of corn starch, similar to that of waxy corn starch. This decrement in amylose content may be due to the BE reaction increasing the α-1,6 branch point on corn starch or by the degradation of the amylose. However, BE could not hydrolyze amylose as described previously, suggesting that BE converts amylose into amylopectin by synthesis of the α-1,6 branch point.

**Surface image of CSBD** Figure 4 shows SEM micrographs of corn starch, CSBD, and maltodextrin obtained at a magnification of 1,500 times. Corn starch comprised a mixture of rounded and angular granules with diameters ranging from 5 to 25 μm, and its surface featured crater-like shapes. The more spherical granules usually had smooth or more regular surfaces compared to those of the angular granules, which were often grooved or dimpled. A starch granule is greatly weakened when damaged or eroded by enzymatic treatment, resulting in many granules cracking open (20,21).

The reactions of CSBD and maltodextrin with BE and α-amylase, respectively, resulted in many surface pits that lead to a granular interior and small granules. There were more small granules in CSBD than in maltodextrin, which may have resulted in decrease of Mw of CSBD due to the action of BE. The surface structures of CSBD and maltodextrin were swollen and dented, which were due to disruption of the crystalline structure of the starch granule during the reaction.

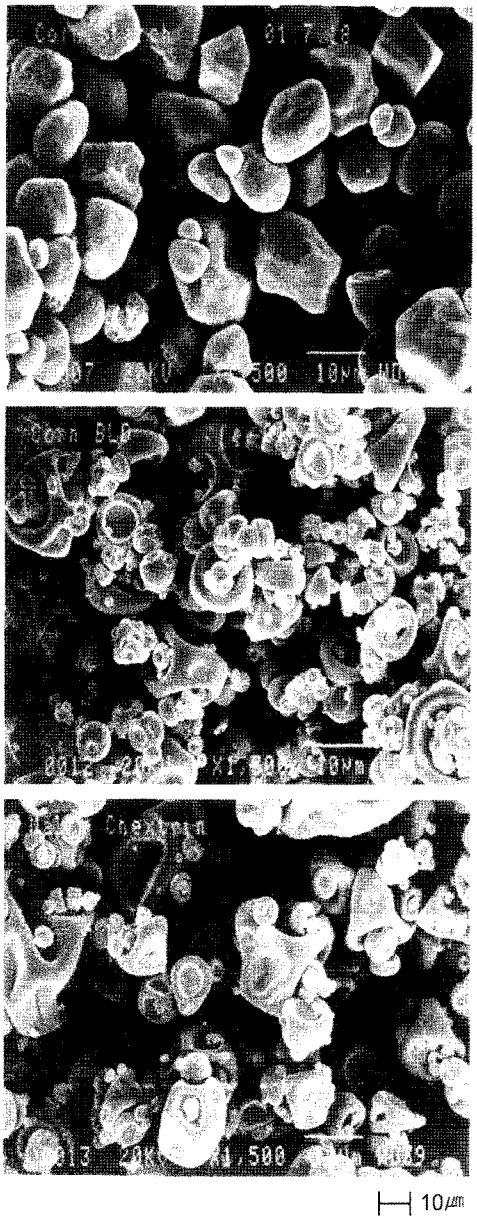


**Fig. 4. Scanning electron micrographs of (a) corn starch, (b) CSBD, and (c) maltodextrin.**

**Molecular weight of CSBD** The macromolecules constituting starch granules are normally categorized as amylopectin and amylose, based on their linearity. The molecular structures of both fractions determine the

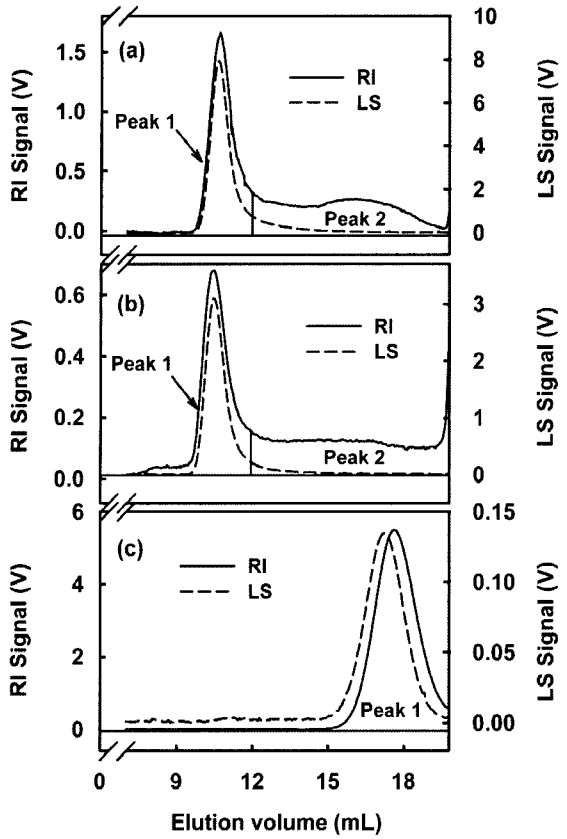


**Fig. 5. Light scattering (LS) and refractive index (RI) chromatograms for (a) corn starch, (b) waxy corn starch, and (c) CSBD.**

**Table 2. Average molecular weight (Mw) values of corn starch, waxy corn starch, and branched dextrin from corn starch (CSBD)[1)]**

| Source | Peak 1 | Peak 2 |
|---|---|---|
| Corn starch | $9.400\times10^7$ | $2.463\times10^6$ |
| Waxy corn starch | $1.271\times10^8$ | $6.575\times10^6$ |
| CSBD | $1.274\times10^5$ | |

[1)]The temperature and flow rate of the TSK G5000 column were 60°C and 0.4 mL/min, respectively.

physical properties of starch in both a dry state and an aqueous medium. The light scattering properties of natural and synthetic macromolecules have recently been used in the molecular characterization and solution behavior analysis of macromolecules (13).

The HPSEC/MALLS/RI chromatograms are shown in Fig. 5, and the Mw of each peak is listed in Table 2. You and Lim (13) reported that the Mw values of amylopectin and amylose from corn starch were $(60\text{-}232)\times10^6$ and $(1.56\text{-}16.9)\times10^6$, respectively, depending on the dissolution and analytical conditions. Hanselmann *et al.* (22,23) and Klavons *et al.* (24) reported that the Mw of amylopectin from waxy corn starch was $(37.5\text{-}360)\times10^6$ and $(20\text{-}224)\times10^6$, respectively. Aberle *et al.* (25) tested starches from various sources, and reported Mw values for amylopectin and amylose of $(55.3\text{-}112)\times10^6$ and $(2.09\text{-}19.6)\times10^6$, respectively.

The results differ with the reaction conditions, but it can be assumed from comparisons with previously reported

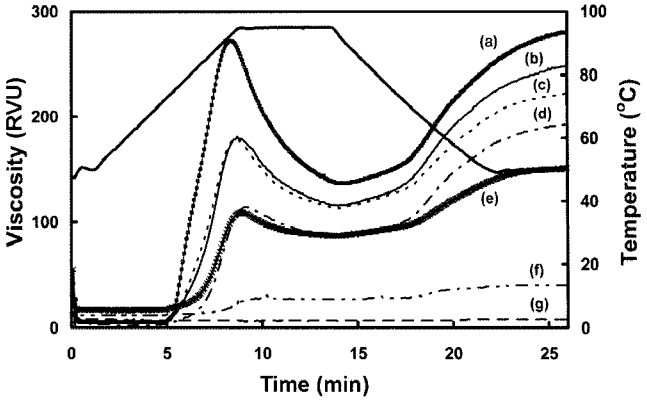


**Fig. 6. RVA profiles of corn starch replaced with CSBD and maltodextrin for (a) 0% CSBD, (b) 10% CSBD, (c) 10% maltodextrin, (d) 20% CSBD, (e) 20% maltodextrin, (f) 50% CSBD, and (g) 100% CSBD.** Solid line indicates temperature. The concentration of corn starch replaced with CSBD was 20%.

data that peak 1 in Fig. 5 for corn starch and waxy corn starch is amylopectin. It is generally known that waxy corn starch is composed of amylopectin only. Therefore, peak 2 for waxy corn starch may indicate the degradation of some component during sample treatment or when it is passed through the pressurized column (26). The Mw of CSBD was very low compared to the other values, which indicates that BE hydrolyzed the corn starch during the reaction. It is reported that this BE may hydrolyze amylopectin as well as form α-1,6 linkages. This result also coincides with previously reported SEM data (17). The data on the amylose content and from the iodine test indicate that the amylose content of CSBD was low. It can therefore be assumed that during the reaction with corn starch, BE catalyzes the branching of amylose and the hydrolysis and branching of amylopectin.

**Pasting properties** Starch pastes are widely used in food products for thickening, filling, and texture control. The properties of pastes made from starches of different botanical sources vary greatly. The use of an RVA to investigate pasting characteristics was first reported by Walker *et al.* (27). The advantages of the RVA are the requirement for a small sample only, a complete analysis being performed in less than 30 min, the durability of the machine and its ease of operation, the versatility of the test procedures, and the direct demonstration of starch applications in foods. Moreover, the pasting characteristics of all-purpose flour as measured by the RVA have been shown to be similar to those measured by the Brabender visco-amylograph (28,29).

The pasting profiles of corn starch replaced with CSBD measured in the RVA are shown in Fig. 6, and the pasting characteristics are summarized in Table 3. The pasting properties of corn starch and CSBD differed significantly. Corn starch exhibited a characteristic type of curve, while a clear peak was not evident for CSBD. The viscosity of CSBD did not vary during the entire temperature cycle. The initial pasting and peak temperatures were increased as the amount of CSBD increased, while the initial pasting and peak temperatures in 50 and 100% CSBD were not obscure.

The peak viscosity was highest in corn starch, and

**Table 3. RVA characteristics of corn starch replaced with branched dextrin from corn starch (CSBD) and maltodextrin (MD)**

| CSBD or MD | Temperature (°C) | | Viscosity (cp) | | |
|---|---|---|---|---|---|
| | Initial pasting | Peak | Peak (P) | Hot paste (H) | Cold paste (C) |
| 0 | 74.2 | 92.4 | 272 | 139 | 280 |
| 10% CSBD | 74.6 | 94.8 | 181 | 116 | 249 |
| 20% CSBD | 78.5 | 94.9 | 114 | 86 | 193 |
| 50% CSBD | ND[1)] | ND | ND | ND | ND |
| 100% CSBD | ND | ND | ND | ND | ND |
| 10% MD | 74.0 | 94.2 | 179 | 114 | 222 |
| 20% MD | 75.7 | 94.8 | 109 | 87 | 151 |

[1)] Not detected.

decreased notably as the amount of CSBD increased. This was expected since the Brabender viscosity of CSBD was much lower than that of corn starch.

The amylose content and Mw were markedly lower for CSBD than for corn starch. Gelatinized starch is crystallized as the temperature of its paste is decreased below its gelatinization temperature. Due to the crystallization of starch, the viscosity of the paste under the gelatinization temperature is increased. This phenomenon is known as retrogradation. The cooling step from 95 to 50°C in RVA analysis as well as Brabender viscometer analysis showed retrogradation of the starch. As the ratio of CSBD to starch increased, the viscosity of the mixture paste of cooling step is decreased. It is notable that CSBD hardly retrograded.

The RVA profiles of corn starch partially replaced with maltodextrin are also shown in Fig. 6. The peak and hot-paste viscosities of corn starch partially replaced with CSBD and maltodextrin were similar, but the cold-paste viscosity of corn starch partially replaced with CSBD was higher slightly than that of corn starch partially replaced with maltodextrin (Table 3). In general, the lower DE has it, the more similar to starch it is. DE of maltodextrin is higher than that of CSBD (Table 1), so lower cold-paste viscosity of maltodextrin compared with that of CSBD means that starch replaced with maltodextrin retrograded less than starch replaced with CSBD and may means that CSBD is more similar to starch than maltodextrin in terms of pasting properties.

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