Effect of Amylose Molecular Size and Amylopectin Branch Chain Length on Paste Properties of Starch[1]

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ABSTRACT

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Amyloses were fractionated from potato, normal, and high-amylose corn VII starches to yield large, intermediate, and small molecular weight amyloses, respectively. Amylopectins were fractionated from high-amylose corn V, waxy corn, and normal rice starches to yield long, intermediate, and short branch chain amylopectins, respectively. Reconstituted starches made with mixtures of these amyloses and amylopectins at different combinations and proportions were studied for their paste properties (viscosity, gel strength, and light transmittance). Synergistic effects on paste viscosities were observed when the amyloses and amylopectins were mixed. The long branch chain amylopectin and the intermediate molecular size amylose produce the greatest synergistic effect on viscosity.

Starches isolated from different botanical sources are known to have different functional properties. For example, normal corn starch produces an opaque and short paste (not stringy), which sets to a strong gel. Waxy corn and potato starches, on the other hand, produce clear and long pastes (sticky and stringy), which have less tendency to set to gels (Swinkels 1985). Traditionally, these differences have been attributed to the content of amylose and the presence of phosphate derivatives. However, chemical structures of these starches, such as amylose molecular size and amylopectin branch chain length also differ (Hizukuri 1985, Takeda and Hizukuri 1987). It is not known how these structural differences affect the paste properties. This study was conducted to reveal how amylose molecular sizes and amylopectin branch chain lengths affect paste properties. Correlations between these chemical structures and functional properties may provide a guideline for the genetic engineering of starch biosynthesis mechanisms that produce starches with tailored properties.

The molecular structures of many starches, including amylose molecular sizes and amylopectin branch chain lengths, have been reported (Hizukuri et al 1981, Hizukuri et al 1983, Hizukuri 1985, Takeda and Hizukuri 1987, Takeda et al 1988, Takeda et al 1989).

On the basis of this literature, we selected amyloses of potato (DP 6000) (Hizukuri et al 1981), normal corn (DP 980) (Takeda and Hizukuri 1987), and high-amylose corn VII (DP 700) (Takeda et al 1989) starches as large, intermediate, and small molecular weight amyloses, respectively. Amylopectins of high-amylose corn V (average branch chain length, 30.9 $\pm$ 0.9), waxy maize (18.6 $\pm$ 0.5), and normal rice (17.5 $\pm$ 0.6) starches (Hizukuri 1985), respectively, represented long, intermediate, and short branch chain amylopectins. Reconstituted starches were prepared with different combinations and proportions of these amyloses and amylopectins. Functional properties such as viscosity, gel strength, and clarity of the reconstituted starches were analyzed, and the effects of amylose and amylopectin structures on functional properties were determined.

MATERIALS AND METHODS

Normal corn, potato, and normal rice (Dutch variety) starches were purchased from Sigma Chemical Co. (St. Louis, MO). High-amylose corn V and VII starches were gifts of the National Starch and Chemical Co. (Bridgewater, NJ); waxy maize starch was a gift of A. E. Staley Mfg. Co. (Decatur, IL). *Pseudomonas* isoamylase was a product of Hayashibara Shoji Inc. (Okayama, Japan). Bio-gel P-6 and Sepharose CL-2B gels were products of Bio-Rad Laboratories (Richmond, CA) and Pharmacia Inc.

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(Piscataway, NJ), respectively. Other chemicals, all reagent grade, were used without further purification.

Fractionation of Starch

Fractionation of starch was carried out by following the general procedure of Schoch (1942). Waxy maize starch, containing about 99% amylopectin, was fractionated by the same procedure to be consistent with other samples. The procedure consisted of heating and stirring a starch suspension (1.33%, w/v, in water) in a water bath at 96°C until starch gelatinization. In high-amylose starch fractionation, 90% dimethyl sulfoxide (DMSO) was used to solubilize starch. The high-amylose starch was precipitated from solution by using methyl alcohol and then redissolved in distilled water to make a 1.33% aqueous solution. All the starch solutions were filtered to remove insoluble residues. The pH of the solution was adjusted to 5.9–6.3 with a phosphate buffer (Lansky et al 1949) and autoclaved at 121°C for 3 hr.

The flask containing the starch solution was stirred within a water bath at 96°C for 2 hr to disperse starch molecules. *n*-Butyl alcohol (20% by volume) was added, and the solution was stirred at 96°C for 1 hr. The mixture was transferred to a prewarmed dewar flask, sealed, and allowed to cool down to room temperature over 24–36 hr. An amylose-butyl-alcohol complex was formed during cooling.

The crude amylose-butyl-alcohol complex was separated by centrifuging (5°C, 8,700 × *g*, 30 min). Fractionated amylose was further purified by two recrystallizations. The amylopectin remaining in the supernatant was concentrated with a rotary evaporator and then treated twice with *n*-butyl alcohol to remove amylose residues. The solution was further concentrated and precipitated with methyl alcohol. Amylose and amylopectin samples were examined by gel-permeation column chromatography and iodine potentiometric titration to determine purity.

Amylose Purity Determined by Iodine Potentiometric Titration

The iodine potentiometric titration was performed following the procedure of Schoch (1964). Amylose (50 mg) in DMSO solution (5%, w/v) was precipitated with methyl alcohol. The precipitate was washed twice with methyl alcohol, centrifuged, and used for the titration. A digital pH/mV meter (Model 501, Orion Research Inc., Boston, MA) was used to measure iodine capacity. Amylose purity was calculated by dividing the iodine affinity values by 20%, the theoretical iodine affinity value of pure amylose (Banks and Greenwood 1975).

Debranching Amylopectin

Amylopectin (about 50 mg) in 1 ml of DMSO solution was precipitated with methyl alcohol, redissolved in distilled water (9 ml), and then stirred and heated in a water bath at 96°C for 1 hr. The solution was cooled to room temperature (25°C), and 1 ml of acetate buffer (0.1*M*, pH 3.5) and crystalline *Pseudomonas* isoamylase (9,000 U) were added. The mixture was incubated in a shaker bath (Model 236 Versa-bath S, Fisher Scientific) at 40°C for 48 hr to complete the debranching reaction. This was monitored by checking the average degree of polymerization (DP) in the digest and by gel-permeation column chromatography (Bio-Gel P-6 column).

Analysis of Amylopectin Branch Chain Length

The average chain length of debranched amylopectin was determined by dividing total carbohydrate by its reducing value. Total carbohydrate was analyzed by the phenol sulfuric acid method (Dubois et al 1956). The reducing value was measured by a modified Park-Johnson procedure (Hizukuri et al 1981). This consisted of mixing an aliquot (1 ml) with potassium cyanide reagent (0.5 ml). The reagent was prepared by dissolving potassium cyanide (0.65 g), Na_2CO_3 (4.8 g), and $NaHCO_3$ (9.2 g) in distilled water and diluted to 1 L. A ferricyanide reagent (1 ml) containing $K_3Fe(CN)_6$ (0.5 g) in 1 L of distilled water was then added. The mixture was heated for exactly 15 min in a vigorously boiling water bath and cooled with running tap water to 25°C. Ferric ammonium sulfate solution (2.5 ml) containing $NH_4Fe(SO_4)_2$ (3 g) in 1 L of 50 m*M* H_2SO_4 was added under a fume hood then held at 25°C for 20 min (Hizukuri et al 1981). Absorbance was measured at 715 nm using a Spectronic 21 spectrophotometer (Milton Roy Co., Rochester, NY). A standard curve was made with glucose solutions of 1–5 μg/ml. Amylopectin branch chain length distributions were analyzed by gel-permeation column chromatography (Bio-Gel P-6 column). Three fractions at the peak were pooled and used to determine the branch chain length.

Gel-Permeation Column Chromatography

Purity of amylose and amylopectin were determined using columns (2.6 i.d. × 80 cm) packed with Sepharose CL-2B gel. The columns were run in the ascending mode. A sample solution (5 ml) containing starch (about 15 mg) and glucose (0.75 mg as a marker) was injected into the column. The eluant was a NaCl aqueous solution (0.02%) with a flow rate of 30 ml/hr. Fractions of 4.8 ml each were collected and subjected to total carbohydrate and amylose content analyses with a dual-channel autoanalyzer (Bran and Lubbe, Elmsford, NY). Anthrone sulfuric acid (Wright and Gann 1966) and iodine staining reactions (Juliano 1971) were used for total carbohydrate and amylose content analyses, respectively. Percent purity of amylose was calculated by dividing the total carbohydrate peak area of the amylose peak by the sum of the area of the amylose and amylopectin peaks. Molecular sizes of the amyloses were determined by using pullulan standards (Shodex P-82 Standard, Waters, Milford, MA) and calculated following the method given by Pharmacia (1985).

Chain lengths of debranched amylopectin were determined using an Econo-column (1.5 i.d. × 80 cm) (Bio-Rad Laboratories, Richmond, CA) packed with Bio-Gel P-6 gel. The column was run in the descending mode with degassed and deionized distilled water as the eluant. The flow rate was 21 ml/hr, and fractions of 2.3 ml each were collected and analyzed for total carbohydrate content using the method previously described.

Preparation of Reconstituted Starches

Exact amounts of amylose and amylopectin (depending upon desired concentrations) in DMSO solutions (5%, w/v) were measured for total carbohydrate content and then mixed and stirred at 25°C for 8–12 hr. The mixture was precipitated with methyl alcohol, separated by centrifuging (8,700 × *g*, 15 min), and washed twice with methyl alcohol. The starch was redissolved in a KOH (0.5*M*) solution (30 ml) by stirring at 4°C for 1 hr, pH neutralized to 5.8–6.0 with HCl solution (6*N*), and diluted with water to the concentrations suitable for functional property studies.

Viscosity Measurements

Viscosity of the reconstituted starch paste (3%, w/w, in water) was measured with a Brookfield LV viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) at 30°C. The Brookfield U.L. adapter, consisting of a cylindrical spindle rotating inside a tube, was used for the analysis. The viscosity was recorded at a spindle speed of 30 rpm. The control was measured with a neutralized KOH solution.

Analysis of Gel Strength

Starch pastes (8%, w/w, in water) were prepared by heating and stirring the neutralized reconstituted starch dispersions in a water bath at 96°C for 20 min. Stirring rates and heating were kept consistent throughout the study. The paste was transferred into an aluminum pan and wrapped with aluminum foil around the wall to increase its depth (Takahashi et al 1989). The starch paste was stored in a sealed plastic bag and set at 25°C for 7 hr or at 4°C for 72 hr. Gel strength of the reconstituted starch paste (8%, w/w) was measured using a texture analyzer (Voland, Texture Technologies, Scarsdale, NY) with TA 53 punch probe at a test distance of 3 mm.

Light Transmittance

Light transmittance of reconstituted starch solutions (1%, w/w, in water) were measured by the method of Craig et al (1989). The solutions were prepared in screw-capped tubes and heated

in a water bath at 96°C for 30 min. The tubes were shaken every 5 min. After temperature equilibration at 25°C (5 min), transmittance at 650 nm was measured using the Spectronic 21.

RESULTS

Purities of the Isolated Amyloses and Amylopectins

Purities of the amyloses isolated from potato, normal corn, and high-amylose corn VII starches determined by gel-permeation column chromatography and iodine potentiometric titration are presented in Table I and Figure 1. Purities of the amylopectins isolated from high-amylose corn V, waxy maize, and normal rice starches are presented in Figure 2. Chromatograms of high-amylose corn and normal rice amylopectin (Fig. 2A and C, respectively) showed small blue-value peaks that had elution volumes close to that of the glucose marker. Total carbohydrate in each peak was negligible.

TABLE I
Purity of Amylose (%) Determined by Potentiometric Titration and Gel-Permeation Chromatography on Sepharose CL-2B Gel

Amylose	Potentiometric Titration		Gel-Permeation Chromatography
	Iodine Affinity	Purity[a]	
Potato	19.3	96.5	90.2
Normal corn	18.6	93.0	96.2
High-amylose corn VII	19.0	95.0	96.2

[a] The purity of amylose was calculated by dividing the iodine affinity of the sample by 20%.

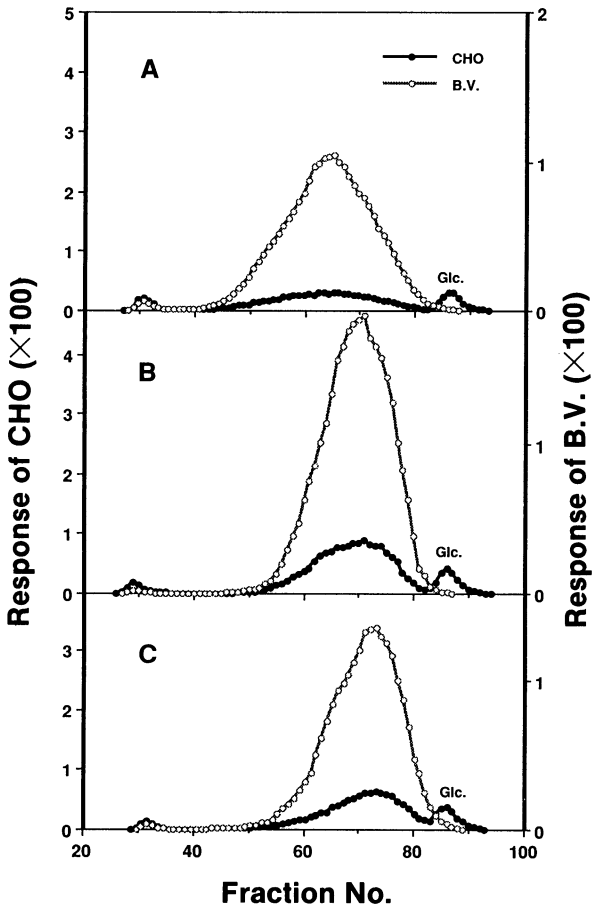

Fig. 1. Sepharose CL-2B gel permeation profiles of amyloses. **A,** potato amylose; **B,** normal corn amylose; **C,** high-amylose VII corn amylose. Glucose (Glc.) was used as a marker. CHO = total carbohydrate, B.V. = blue value.

Structural Studies of Amylose and Amylopectin

The differences between the molecular sizes of amyloses fractionated from potato, normal corn, and high-amylose corn VII starches were confirmed by gel-permeation column chromatograms (Fig. 1). Molecular sizes of the amyloses determined by using pullulan standards showed K_{av} values of 0.62, 0.73, and 0.76 (DP 1,500, 667, and 530) for potato, normal corn, and high-amylose corn VII amyloses, respectively. Gel-permeation profiles of debranched amylopectins of high-amylose corn VII, waxy maize, and normal rice starches are shown in Figure 3. The branch chain lengths and distributions of the amylopectins are summarized in Table II. The viscosity of amylose and amylopectin solutions increased with concentration (Fig. 4).

Functional Properties of Reconstituted Starch Pastes

Viscosities of the reconstituted starch solutions and the synergistic effects found after the mixing of amylose and amylopectin are shown in Table III. Gel strengths of the starch pastes containing 80% amylopectin and 20% amylose (set at 25°C for 7 hr and at 4°C for 72 hr) are presented in Table IV. Percent light transmittances of the starch solutions (1%, w/w, in water) are presented in Table V.

DISCUSSION

Results of gel-permeation column chromatography (Fig. 1) showed K_{av} values of 0.62, 0.73, and 0.76, which were calculated to be DP 1,500, 667, and 530 for amyloses of potato, normal corn, and high-amylose corn, respectively. The results were in the same order as those reported as 6,000 by Hizukuri et al (1981), 980 by Takeda and Hizukuri (1987), and 700 by Takeda et al (1989), respectively, for amyloses of potato, normal corn, and high-amylose corn. The discrepancy between the values obtained from two studies was attributed to different analytical methods

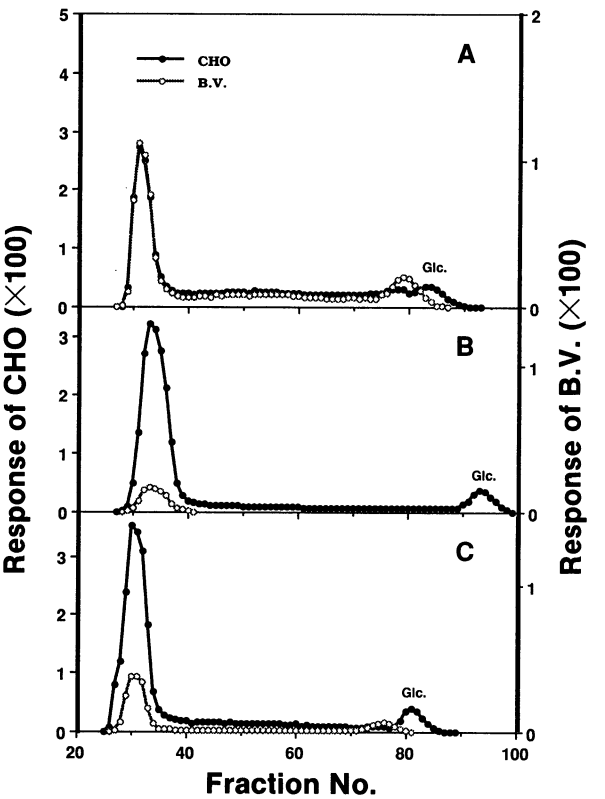

Fig. 2. Sepharose CL-2B gel permeation profiles of amylopectin. **A,** high-amylose V corn amylopectin; **B,** waxy maize amylopectin; **C,** rice amylopectin. Glucose (Glc.) was used as a marker. CHO = total carbohydrate, B.V. = blue value.

used for the analyses. Hizukuri et al (1981) determined average DP by a modified Park-Johnson method.

Purity of the amylose determined by iodine potentiometric titration and gel-permeation column chromatography were in agreement, ranging from 93 to 96% with the exception of potato amylose. The gel-permeation chromatogram of potato amylose showed a small peak at the void volume, containing 9.8% total carbohydrate (Fig. 1A). However, the peak also had a high proportion of blue value, suggesting the presence of large molecular amylose. On the basis of this analysis, the 96.5% purity of potato amylose determined by iodine titration seemed to be the more reliable value.

Both high-amylose corn V and rice amylopectin produced small blue-value peaks close to the glucose marker (Fig. 2A and C) resulting from amylodextrin contaminants. However, total carbohydrate content within the peaks was negligible. High-amylose corn amylopectin exhibited an exceptionally high blue value, which is attributed to the iodine complex of the long branch chains of the amylopectin.

Viscosities of all three amylose solutions increased linearly with concentration within the range of the study (Fig. 4A). Amyloses from normal corn had the greatest viscosity, followed by potato and high-amylose corn (Fig. 4A). Viscosities of amylopectin solutions also increased with concentration, although nonlinearly (Fig. 4B). This suggests interactions, such as entanglement, between the branched molecules. Among the three amylopectins, rice had the greatest viscosity, followed by high-amylose corn and waxy maize. The phosphorus content in rice starch (0.034%) was greater than that of high-amylose corn (0.028%) or waxy maize starches (0.004%), which might contribute to the high viscosity of rice amylopectin. Phosphorus content of potato amylose is reported as insignificant (Abe et al 1982, Takeda et al 1989). We based our investigations on these findings. No additional analysis was performed in the laboratory.

The viscosity difference between the reconstituted starch and the sum of the corresponding amylose and amylopectin solutions measured separately was attributed to the synergistic effect of the interaction between amylose and amylopectin molecules. The

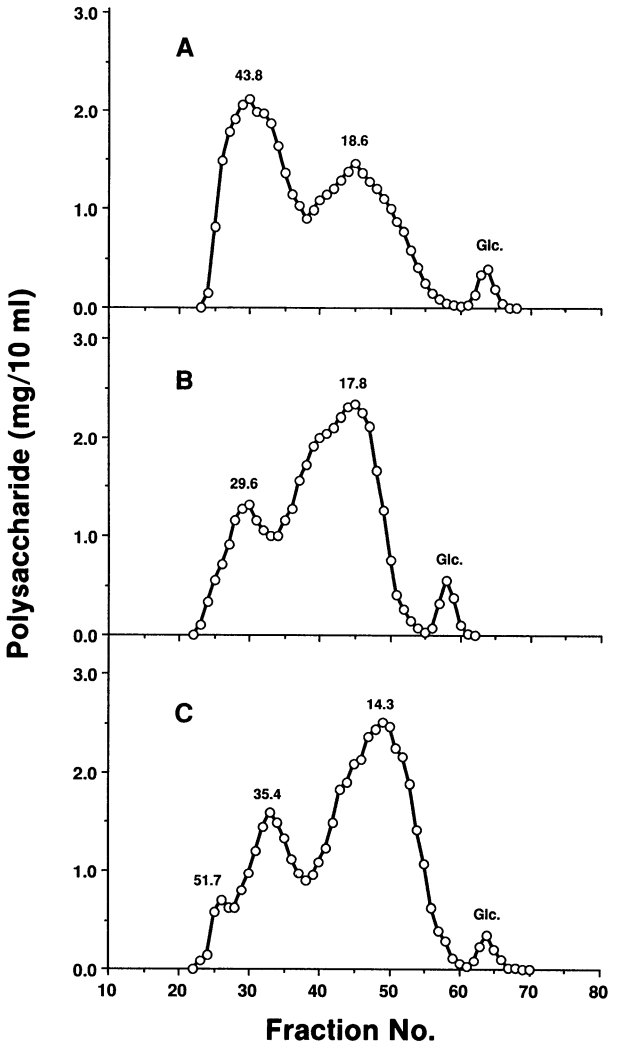

Fig. 3. Bio-gel P-6 gel-permeation profiles of amylopectins debranched by isoamylase. **A,** high-amylose V corn amylopectin; **B,** waxy maize amylopectin; **C,** rice amylopectin. Glucose (Glc.) was used as a marker. The number at each peak is the average DP of amylodextrin in three consecutive peak fractions.

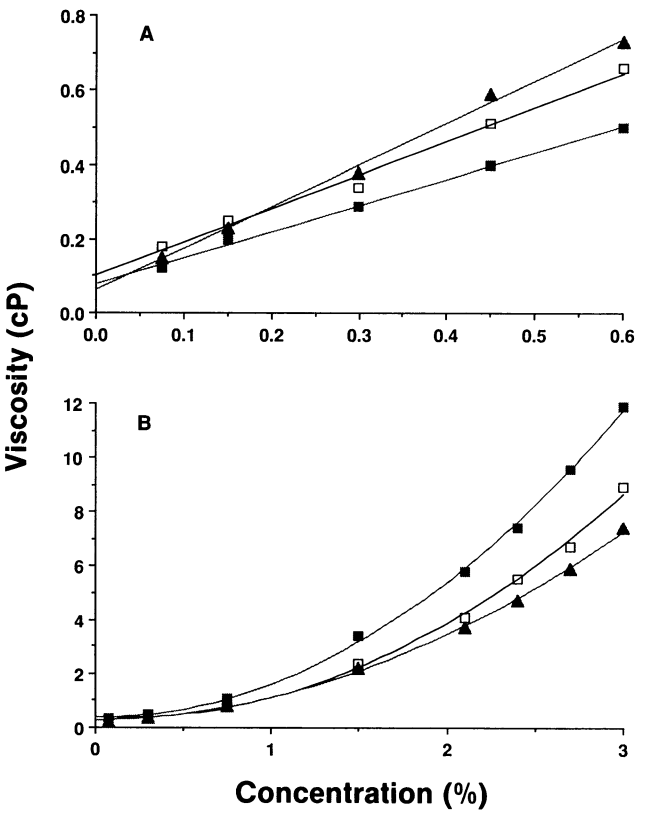

Fig. 4. Viscosity of amylose and amylopectin solutions measured by Brookfield viscometer with a Brookfield U.L. adapter at 30 rpm, 30°C. **A,** amylose solution (▲ = corn, □ = potato, ■ = high-amylose VII corn); **B,** amylopectin solution (▲ = waxy maize, □ = high-amylose V corn, ■ = rice).

TABLE II
Length and Distribution of Amylopectin Branch Chain Debranched with Isoamylase[a]

Amylopectin	Branch Chain Length,[b] DP		Distribution, %	
	Long	Short	Long	Short
High-amylose corn V	43.8 ± 2.5	18.6 ± 2.4	55.7	44.3
Waxy maize	29.6 ± 3.8	17.8 ± 2.0	23.9	76.1
Rice	51.7 ± 3.1, 35.4 ± 1.0	14.3 ± 0.3	5.8, 25	69.2

[a] Data reported are the averages of duplicate analyses.
[b] Determined with the peak fraction; DP = degrees of polymerization.

TABLE III
Viscosity of Amylopectin, Amylose, and Reconstituted Starch Solutions[a]

Amylopectin	Viscosity	Amylose	Viscosity	Viscosity of Reconstituted Starch (cp)	Synergistic Effect[b] (%)
90% High-amylose corn V	6.75 ± 0.09	10% Potato	0.35 ± 0.05	8.28 ± 0.08	16.62
		10% Corn	0.33 ± 0.06	8.87 ± 0.06	25.28
		10% High-amylose corn VII	0.28 ± 0.03	8.10 ± 0.26	15.22
90% Waxy maize	5.90 ± 0.01	10% Potato	0.35 ± 0.05	6.97 ± 0.06	11.52
		10% Corn	0.33 ± 0.06	7.03 ± 0.21	12.84
		10% High-amylose corn VII	0.28 ± 0.03	6.77 ± 0.06	9.55
90% Rice	9.57 ± 0.15	10% Potato	0.35 ± 0.05	10.53 ± 0.15	6.15
		10% Corn	0.33 ± 0.06	11.37 ± 0.15	14.05
		10% High-amylose corn VII	0.28 ± 0.03	10.95 ± 0.05	11.17
80% High-amylose corn V	5.52 ± 0.10	20% Potato	0.60 ± 0.05	8.02 ± 0.08	31.05
		20% Corn	0.70 ± 0.00	9.17 ± 0.42	47.49
		20% High-amylose corn VII	0.48 ± 0.03	8.53 ± 0.57	42.17
80% Waxy maize	4.75 ± 0.05	20% Potato	0.60 ± 0.05	6.53 ± 0.12	22.06
		20% Corn	0.70 ± 0.00	6.90 ± 0.00	26.61
		20% High-amylose corn VII	0.48 ± 0.03	6.45 ± 0.05	23.33
80% Rice	7.40 ± 0.00	20% Potato	0.60 ± 0.05	9.73 ± 0.12	21.63
		20% Corn	0.70 ± 0.00	10.68 ± 0.08	31.85
		20% High-amylose corn VII	0.48 ± 0.03	9.98 ± 0.05	26.65

[a] Data reported are the averages of triplicate analyses.

[b] $$\text{Synergistic effect (\%)} = \frac{\text{Viscosity of reconstituted starch} - (\text{viscosity of amylopectin} + \text{viscosity of amylose})}{\text{Viscosity of amylopectin} + \text{viscosity of amylose}} \times 100\%$$

TABLE IV
Gel Strength of Reconstituted Starch Paste with 80% Amylopectin and 20% Amylose[a]

Source		Gel Strength, g (at 25°C, 7 hr)	Gel Strength, g (at 4°C, 72 hr)
Amylopectin	Amylose		
High-amylose corn V	Potato	2.9 ± 0.2	8.7 ± 0.3
	Corn	7.8 ± 0.6	8.3 ± 0.6
	High-amylose corn VII	4.0 ± 0.5	13.9 ± 1.1
Waxy maize	Potato	...[b]	...
	Corn	...	...
	High-amylose corn VII	...	1.6 ± 0.3
Rice	Potato	...	0.8 ± 0.0
	Corn	1.0 ± 0.1	1.5 ± 0.1
	High-amylose corn VII	0.8 ± 0.1	7.2 ± 0.6

[a] Data reported are the averages of triplicate anlyses.
[b] No gel formed.

TABLE V
Light Transmittance of Reconstituted Starch Solution (1%)[a]

Source		Transmittance (%)
Amylopectin	Amylose	
High-amylose corn V	Potato	91.5 ± 0.6
	Corn	86.8 ± 1.0
	High-amylose corn VII	87.0 ± 1.3
Waxy maize	Potato	89.2 ± 0.8
	Corn	82.2 ± 0.3
	High-amylose corn VII	77.8 ± 1.0
Rice	Potato	84.3 ± 0.6
	Corn	81.2 ± 1.0
	High-amylose corn VII	68.5 ± 0.9

[a] Data reported are the averages of triplicate analyses.

synergistic effect was calculated by using the following equation:

$$\text{Synergistic effect (\%)} = \frac{V_{\text{reconstituted starch}} - (V_{am} + V_{ap})}{V_{am} + V_{ap}} \times 100\%$$

Results showed that normal-corn amylose, with an intermediate molecular size, had the greatest synergistic effect of the three amyloses tested. High-amylose corn amylose (smallest molecule) and potato amylose (largest molecule) were less effective (Table III). Synergistic effects among amylopectins decreased in the order of high-amylose corn, rice, and waxy maize (Table III). Structural studies showed that high-amylose corn amylopectin had the longest branch chain length (Table II). It is likely that the long branch chains of the amylopectin interact to a greater extent (entangling) with amylose than do the other amylopectins. Short branch chains of rice amylopectin have a peak DP of 14.3, which is shorter than that of waxy maize (DP 17.8) (Fig. 3B and C, Table II). However, long branch chains of rice amylopectin have peak DPs of 35.4 and 51.7, which are longer than the peak DP of waxy maize (29.6) (Fig. 3B and C, Table II). It is not clear how the different chain lengths affect the observed synergistic effects, and whether long branch chains of rice amylopectin contribute to those observed higher synergistic effects.

Gel strength analysis of the reconstituted starches showed that high-amylose corn amylopectin mixed with all three amyloses formed gel (Table IV). This indicated that the long branch chain amylopectin had a strong tendency to gel. A mixture of the amylopectin with normal corn amylose produced the greatest gel strength (25°C stored for 7 hr). However, when stored under different conditions (4°C for 72 hr), a mixture of long branch

chain amylopectin (from high-amylose corn V starch) and small molecular amylose (from high-amylose corn VII starch) had the greatest gel strength. This could be attributed to retrogradation of small amylose molecules. Rice amylopectin produced weak gels when mixed with amyloses, whereas waxy maize amylopectin gelled only when mixed with high-amylose corn amylose and stored at 4°C for 72 hr. These results support the synergistic effects observed with respect to viscosity.

Light transmittance of the reconstituted starches decreased with molecular size of amylose and branch chain length of amylopectin (Table V). Light transmittance of the reconstituted starch from 80% waxy maize and 20% potato amylose (89%) was greater than that made from 80% waxy maize and 20% corn amylose (82%). This may be related to the greater paste clarity of tapioca starch, which contains amylose of larger molecular size than that of corn.

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