Structure and Function of Starch and Resistant Starch from Corn with Different Doses of Mutant Amylose-Extender and Floury-1 Alleles

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Abstract
Four corn types with different doses of mutant amylose-extender (ae) and floury-1 (fl1) alleles, in the endosperm, including no. 1, aaea; no. 2, fl1fl1fl1; no. 3, aeaefl1; and no. 4, fl1fl1ae, were developed for use in making Hispanic food products with high resistant starch (RS) content. The RS percentages in the native starch (NS) of 1−4 were 55.2, 1.1, 5.7, and 1.1%, respectively. All NS were evaluated for pasting properties with a rapid viscoanalyzer (RVA) and for thermal properties with a differential scanning calorimeter (DSC). NS 1 had a low peak viscosity (PV) caused by incomplete gelatinization, whereas NS 3 had the greatest PV and breakdown of all four starch types. On the DSC, NS 2 had the lowest onset temperature and greatest enthalpy. NS 1 and 3 had similar onset and peak temperatures, both higher than those of NS 2 and 4. The gel strength of NS heated with a RVA was evaluated by using a texture analyzer immediately after RVA heating (fresh, RVA-F) and after the gel had been stored at 4 °C for 10 days (retrograded, RVA-R). NS 1 gel was watery and had the lowest strength (30 g) among starch gel types. NS 3 gel, although exhibiting syneresis, had greater gel strength than NS 2 and 4. The structures of the NS, the RS isolated from the NS (RS-NS), the RS isolated from RVA-F (RS-RVA-F), and the RS isolated from RVA-R (RS-RVA-R) were evaluated by using size exclusion chromatography. NS 1 had a greater percentage of amylose (AM) (58.3%) than the other NS (20.4–26.8%). The RS from all NS types (RS-NS) had a lower percentage of amylopectin (AP) and a greater percentage of low molecular weight (MW) AM than was present in the original NS materials. The RS-RVA-R from all starches had no AP or high MW AM. The percentages of longer chain lengths (DP 35–60) of NS were greater in 1 and 3 than in 2 and 4, and the percentages of smaller chain lengths (DP 10–20) were greater in 2 and 4 than in 1 and 3. In general, NS 3 seemed to have inherited some pasting, thermal, and structural characteristics from both NS 1 and 2, but was distinctly different from 4.

Keywords
Starch, resistant starch, corn, amylose-extender, floury-1

Disciplines
Food Chemistry | Food Science

Comments
Structure and Function of Starch and Resistant Starch from Corn with Different Doses of Mutant Amylose-Extender and Floury-1 Alleles

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Four corn types with different doses of mutant amylose-extender (ae) and floury-1 (fl1) alleles, in the endosperm, including no. 1, aaeaeae; no. 2, fl1fl1fl1; no. 3, aaeaf1; and no. 4, fl1fl1ae, were developed for use in making Hispanic food products with high resistant starch (RS) content. The RS percentages in the native starch (NS) of 1–4 were 55.2, 1.1, 5.7, and 1.1%, respectively. All NS were evaluated for pasting properties with a rapid viscoanalyzer (RVA) and for thermal properties with a differential scanning calorimeter (DSC). NS 1 had a low peak viscosity (PV) caused by incomplete gelatinization, whereas NS 3 had the greatest PV and breakdown of all four starch types. On the DSC, NS 2 had the lowest onset temperature and greatest enthalpy. NS 1 and 3 had similar onset and peak temperatures, both higher than those of NS 2 and 4. The gel strength of NS heated with a RVA was evaluated by using a texture analyzer immediately after RVA heating (fresh, RVA-F) and after the gel had been stored at 4 °C for 10 days (retrograded, RVA-R). NS 1 gel was watery and had the lowest strength (30 g) among starch gel types. NS 3 gel, although exhibiting syneresis, had greater gel strength than NS 2 and 4. The structures of the NS, the RS isolated from the NS (RS-NS), the RS isolated from RVA-F (RS-RVA-F), and the RS isolated from RVA-R (RS-RVA-R) were evaluated by using size exclusion chromatography. NS 1 had a greater percentage of amylose (AM) (58.3%) than the other NS (20.4–26.8%). The RS from all NS types (RS-NS) had a lower percentage of amyllopectin (AP) and a greater percentage of low molecular weight (MW) AM than was present in the original NS materials. The RS-RVA-R from all starches had no AP or high MW AM. The percentages of longer chain lengths (DP 35–60) of NS were greater in 1 and 3 than in 2 and 4, and the percentages of smaller chain lengths (DP 10–20) were greater in 2 and 4 than in 1 and 3. In general, NS 3 seemed to have inherited some pasting, thermal, and structural characteristics from both NS 1 and 2, but was distinctly different from 4.

KEYWORDS: Starch; resistant starch; corn; amylose-extender; floury-1

INTRODUCTION

A portion of dietary starch resists digestion by human pancreatic amylase in the small intestine and thus reaches the colon (7). The amount of this undigested starch that reaches the human large intestine is called resistant starch (RS) (2). RS is classified into four main types (3, 4). Type 1 includes starch trapped within whole plant cells and food matrices, thus preventing amyolysis. Milling (and chewing) can make these starches more accessible and less resistant. Type 2 is composed of starch granules from certain plants containing uncooked starch (e.g., in green banana) or starch that was gelatinized poorly and hydrolyzed slowly by α-amylases [e.g., high-amylose (AM) corn starches]. Type 3 refers to RS formed from retrogradation of starch after cooking. Type 4 includes chemically modified starches (e.g., ethers or esters) used by food manufacturers to alter the functional characteristics of the starch.

RS can be fermented by human gut microflora, providing a source of carbon and energy for the 400–500 species of bacteria present in this anaerobic environment (5, 6) and thus potentially altering the composition of the microflora and its metabolic activities. The fermentation of carbohydrates by anaerobic bacteria yields short-chain fatty acids, primarily composed of acetic, propionic, and butyric acids, which can lower the lumen pH, creating an environment less conducive to the formation of cancerous tumors (7, 8). Also, RS was shown to form nondigestible inclusion complexes with bile acids, attributed to the formation of a helical structure (9), and was demonstrated to be more effective than cholestyramine as a lipid-lowering agent in the rat (10). Furthermore, the fermentation end-products...
of RS could counteract the up-regulation of cholesterol and bile acid biosynthesis (10).

Floury endosperm corn (fl1fl1), native to the highlands of South America, is used to prepare traditional Hispanic foods requiring an easily milled kernel (11). The corn is used in foods prepared at the green corn stage, such as humitas, or as a mature grain to produce products such as tortillas. The fl1 genes from this specialty variety were introgressed by Genetic Enterprises International breeders by backcrossing and selection into Corn Belt germplasm to make hybrids. In an effort to increase the nutritional quality of the fl1 corn for RS in the resulting food products, an fl1fl1 and a high-amylase (aaee) corn hybrid, known to provide a good amount of RS (3), were used to produce grain with different levels of RS in the endosperm. Both the quantity of RS and the functional impact on the starch are important in evaluating specialty corn types for use in healthful food products. Thus, the aim of this study was to evaluate the structure and function of starch and RS from four corn types having different ratios of fl1 and ae alleles in the corn endosperm and to provide information for increasing the RS of corn used in Hispanic foods, such as tortillas and humitas, requiring easily milled kernels.

**MATERIALS AND METHODS**

**Corn with Modified Starch.** The fl1fl1 and aaee endosperm hybrids were developed by backcrossing the mutant endosperm genes, fl1 and ae, into Corn Belt germplasm. Two hybrids, one fl1fl1 and one aaee hybrid of similar maturities, were planted in Sheldon, IA, during summer 2007 in a breeding nursery. The two hybrids were selfed and/or crossed to produce grain of different doses of the endosperm mutants, fl1 and ae, in similar genetic backgrounds. Because the corn endosperm is a triploid tissue, where the female parent contributes a diploid set of chromosomes and the male parent contributes the other set of chromosomes, endosperm types with different doses of each gene were created using the following crossing scheme. The aaeeae (1) endosperm was obtained by selfing plants of the aaee hybrid; likewise, the fl1fl1fl1 (2) endosperm was obtained by selfing plants of the fl1fl1 hybrid. The aaeefl1 (3) endosperm was obtained by crossing the aaee hybrid as the female parent with pollen from the fl1fl1 hybrid. The fl1fl1ae (4) endosperm was produced by crossing the fl1fl1 hybrid as the female parent with pollen from the aaee hybrid. Two hundred ears of each endosperm type were harvested, dried, and shelled to obtain the grain bulks of each endosperm type to be used for the laboratory and food product evaluations. Corn was stored at 4 °C until needed.

**Starch Extraction.** Two hundred kernels of each corn type were soaked in a solution containing lactic acid (1%) and metabsulfite (0.3%) for 48 h at 42 °C (12–14). Germ and pericarp were then manually removed. Endosperms were homogenized at 13500 rpm for 30 s with a homogenizer (model Ultra-Turrax T25 S1, Janke & Kunkel GmbH & Co. KG) and then filtered in small portions at a time through a sieve with a pore size of 100 μm (N100C Cellmicsieves, Biodesign Inc. of New York) held in place by a Nalgene reusable nonsterile filter cylinder with intermittent stirring on a vortex mixer. The starch was cooled to room temperature, and a pancreatic α-amylase and amyloglucosidase (AMG) mixture was added, prepared at a concentration 3 times that indicated in the kit. Similarly, the enzymes and buffer addition for the RS measurement in RVA-F and RVA-R were adjusted according to the dry solids in these slurries. The RS was extracted from 0.4 g of NS, with the procedure described in the RS kit modified for this larger scale. The RS concentration in NS, heated starch, and RS-RVA-R was measured according to AACC method 32-40 (18) by using an RS kit from Megazyme (catalog no. K-RSTAR, Megazyme International Ltd., Bray, Ireland). All enzymes were purchased from Megazyme International Ltd. To prepare heated starch, 0.1 g was mixed with 2 mL of sodium maleate buffer (pH 6.0) and boiled for 6 min with intermittent stirring on a vortex mixer. The starch was cooled to room temperature at a rate of 1 °C/min and 0.1 mL of the pancreatic α-amylase and amyloglucosidase (AMG) mixture was added, prepared at a concentration 3 times that indicated in the kit. Two replicate starch gels were prepared for each starch type, with TA measurements also replicated and the values averaged for each starch gel.

**RS Measurement.** The RS concentration in NS, heated starch, and RVA-F and RVA-R gels was measured according to AACC method 32-40 (18) by using an RS kit from Megazyme (catalog no. K-RSTAR, Megazyme International Ltd., Bray, Ireland). All enzymes were purchased from Megazyme International Ltd. To prepare heated starch, 0.1 g was mixed with 2 mL of sodium maleate buffer (pH 6.0) and boiled for 6 min with intermittent stirring on a vortex mixer. The starch was cooled to room temperature at a rate of 1 °C/min and 0.1 mL of the pancreatic α-amylase and amyloglucosidase (AMG) mixture was added, prepared at a concentration 3 times that indicated in the kit. Two replicate starch gels were prepared for each starch type, with TA measurements also replicated and the values averaged for each starch gel.

**Viscosity of Starch Slurries in the Rapid Viscoanalyzer.** The rapid viscoanalyzer (RVA, model 4, Newport Scientific, Warriewood, Australia) was used to measure the viscosity profiles of NS/water slurries as a function of temperature, time, and stirring by using a standard pasting profile, STD1, included in the instrument software. NS slurries (8% dry solids) were stirred at 960 rpm for 10 s at 50 °C, followed by stirring at 160 rpm for the remainder of the test, as the temperature increased from 50 to 95 °C in 4 min and 42 s. Temperature was held at 95 °C for 2 min and 30 s and decreased to 50 °C in 3 min and 48 s. All tests were duplicated for each starch type and the resulting data averaged. The RVA parameters measured were maximum hot paste peak viscosity (PV), time to reach PV, holding strength as determined by the viscosity at the trough (trough), final viscosity after cooling to 50 °C and holding at this temperature (FV), breakdown (BD; calculated as PV – trough), and setback (SB; calculated as FV – trough). The pasting temperature of the samples was determined as the temperature at which viscosity increased by 24 cP in 0.1 min.

After NS/water slurries were heated in the RVA, the starch gels were tested for textural properties while still fresh (RVA-F) and after storage at 4 °C for 10 days to promote retrogradation (RVA-R). After storage, the RS concentration was measured and the RS dispersed as described under Preparation of Nongranular Starch for Gel Permeation Chromatography.

**Texture Analysis.** Texture of the RVA cooked NS gels was tested by using a TA.XT2i texture analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, U.K.) equipped with texture expert for Windows software version 1.11. Immediately after completion of the RVA test, the paddle of the RVA was removed from the canister and placed upside down to cover the canister to prevent moisture loss until further measurement. The test conditions for texture analysis were as follows: pre-speed, 10 mm/s; test speed, 1.2 mm/s; post speed, 2 mm/s; and press distance, 10 mm. The force (5 g) was measured by compression and cycled twice, with an acquisition rate of 5 points/s (pps). The peak at 10 mm (8.31 s) was reported as the firmness of the gel (15). The area of the positive force area during the second compression to that during the first compression was defined as cohesive ness (17). Two replicate starch gels were prepared for each starch type, with TA measurements also replicated and the values averaged for each starch gel.

**Preparation of Nongranular Starch for Gel Permeation Chromatography (GPC).** From NS, Granular starches (1 g, dry weight) extracted as described above were dispersed in 100 mL of 90% (v/v, in H2O) dimethyl sulfoxide (DMSO) by heating the mixture in a boiling water bath with constant stirring for 1 h then stirring at room temperature overnight (19).

From RS-NS. The RS was extracted from 0.4 g of NS, with the procedure described in the RS kit modified for this larger scale. The NS was placed in a 50 mL centrifuge tube and 16 mL of pancreatic α-amylase added, along with amyloglucosidase (AMG) at the concentration described in the kit (K-RSTAR, Megazyme International). The resulting RS was dispersed as described above for NS with the appropriate amount of DMSO.

From RS-RVA-F and RS-RVA-R. The starch gel made by using RVA was stirred with a spatula, and 15 g was placed into a 50 mL centrifuge
tube. Pancreatic α-amylase (1 g) was suspended in 71 mL of sodium maleate buffer (pH 6.0) and stirred for 5 min. The AMG (1.0 mL of 300 units/mL) was added and mixed well, followed by centrifugation at 2000 rpm for 10 min. The concentration of pancreatic α-amylase and AMG was adjusted so that the enzyme/substrate ratio and substrate concentration were the same as those suggested by the kit. After digestion with enzymes, the contents were transferred to a 200 mL bottle, and the same volume of 100% ethanol was added, with precipitation then occurring at room temperature for 1 h, followed by centrifugation at 2000 rpm for 10 min. The residual, that is, RS, was transferred back to the 50 mL centrifuge tube previously used with 20 mL of 50% ethanol.

For RS-NS, RS-RVA-F, and RS-RVA-R, the amount of DMSO (90%) added to make nongranular starch was adjusted to make the final concentration of RS to be 10 mg/mL for starches 1 and 3 on the basis of the %RS determined separately. For starches 2 and 4 and typical starch, because the %RS was low, it was difficult to achieve a dispersed starch concentration this high; thus, a lower concentration of 5 mg/mL was employed.

GPC. Nongranular starches made from NS, NS-NS, RS-RVA-F, and RS-RVA-R as described above were analyzed by GPC analysis to determine the distribution of carbohydrate according to the method of Li et al. (20). In brief, the starch was precipitated from the mixture of starch—DMSO by adding ethanol with 3 times the volume of the mixture and then centrifugation. The starch pellet was suspended in 5 mL of water and heated in a boiling water bath for 30 min with constant stirring with a small magnetic rod. Dissolved starches were filtered by using a 5 μm filter (GE Osmonics Labstore, Minnetonka, MN) before loading them onto a Sepharose CL2B column, with separations conducted by gravity with mobile phase as 10 mM sodium hydroxide mixture and then centrifugation. The starch pellet were suspended in 5 mL of water and heated in a boiling water bath for 30 min with constant stirring with a small magnetic rod. Dissolved starches were filtered by using a 5 μm filter (GE Osmonics Labstore, Minnetonka, MN) before loading them onto a Sepharose CL2B column, with separations conducted by gravity with mobile phase as 10 mM sodium hydroxide.

For RS-NS, RS-RVA-F, and RS-RVA-R, the amount of DMSO (90%) added to make nongranular starch was adjusted to make the final concentration of RS to be 10 mg/mL for starches 1 and 3 on the basis of the %RS determined separately. For starches 2 and 4 and typical starch, because the %RS was low, it was difficult to achieve a dispersed starch concentration this high; thus, a lower concentration of 5 mg/mL was employed.

The GPC analysis was performed using a high-performance liquid chromatography (HPLC) system (model 1200, Agilent Technologies). The eluent was 10 mM sodium hydroxide, and the flow rate was 1 mL/min. The column was a Sepharose CL2B, and the injection volume was 20 μL. The samples were analyzed at 200 nm for the BV by using a microplate spectrophotometer (SpectraMax 190, Molecular Devices Corp, Sunnyvale, CA). The solution was examined at 490 nm for the TCH analysis and at 630 nm for the BV analysis.

Statistical Analysis. Comparisons of means were conducted by the least significant difference (LSD) test at α = 0.05 by using SAS 9.1, where analysis of variance (ANOVA) found the responses significantly different (α = 0.05).

RESULTS AND DISCUSSION

RS Concentrations. In NS. The different corn types gave starch yields (dwg) of 47–60% (data not shown).

Yields of starch for ae corn types generally were lower than for typical corn, as also noted by Tziotis et al. (25), who reported a starch yield of 41.7% for ae25. NS from starch 1 yielded the greatest %RS of 55.2%, followed by starch 3 with %RS of 5.7%. The %RS of typical starch and starches 2 and 4 had the same lowest level of 1.1% (Table 1). The AM/AP ratio for starch 3 was the greatest among all starches, except for starch 1, possibly contributing to the higher RS concentration in starch 3 (26).

In Heated NS. Heating with water before digestion for starch 1 yielded less than half the RS obtained from its uncooked NS, whereas for starch 3 the yield was a bit more than half (Table 1). The reduced %RS in heated starches 1 and 3 was because gelatinized starch was more readily attacked by pancreatic α-amylase during the 16 h digestion. In contrast, the same treatment doubled the relatively small amount of RS found in starches 2 and 4 and in typical starch. This result may have occurred when depolymerized starch fragments created during gelatinization realigned during cooling to create RS. The %RS measured by AACC method 32-40 was designed to yield values in agreement with the amount of raw starch escaping digestion in the small intestine as determined in studies using ileostomy subjects (2, 4). Generally, cooked starch should have a lower %RS than uncooked raw starch; however, if the raw starch has a very low %RS, heating in water could actually slightly increase the %RS, as was found in the current study.

In RVA-F and RVA-R. The RS-RVA-F of all starch gels was greater than the RS-NS from heated or unheated starches, except for starch 1, which was the opposite. Possibly, the enhanced polymer alignment caused by the mechanical shear of the RVA resulted in more RS in starch 1. Storage of RVA-F gels for 10 days at 4 °C enhanced the development of RS through starch
retrogradation, increasing the %RS for all RVA-R (Table 1). The RS-RVA-F and RS-RVA-R of starch 1 were the greatest, followed by those of starch 3. The greater %RS in RS-RVA-R than RS-RVA-F was likely caused by retrogradation. Thus, by calculation, the %RS of the cooked gel from the RVA after storage for 10 days increased by 37, 71, 108, 70, and 106% for starches 1, 2, 3, and 4 and typical starch, respectively.

The RS-RVA-F can be formed by a number of factors. Retrogradation of gelatinized starch occurs almost immediately after gelatinization. Stable hydrogen bonding between linear segments of amylose occurs postgelatinization in most food processes (27). High density of a food matrix, including viscosity, and partial crystallinity of the starch molecules reduce enzyme susceptibility (28), creating type 3 RS. Englyst et al. (28) also determined that starch fractions trapped within a starch gel matrix and allowed to undergo retrogradation had reduced susceptibility to digestion by pancreatic amylose (EC 3.2.1.1). Although RS is enzyme-resistant, and chemical linkages can be formed during starch retrogradation, it can be fully digested after solubilization in 2 M potassium hydroxide or DMSO.

**Chain-Length Distribution of Amylopectin in Native Starch.** The AP of starch 1 had a lower percentage of chains with DP 6–25 than did starches 3 and 4 (Figure 1). Starch 2 had a greater percentage of chains with DP 6–35 than did starch 3, but was similar to that of starch 4. Starch 3 had a greater percentage of chains with DP 35 or above than did starch 4. Starch 1 contained the greatest percentage of longer chains, in agreement with another researcher (25), who found ae-type starch to have a high proportion of longer branch chains with DP 25 or above. They evaluated a wild-type corn, ae25, dull39, sugary2 (su2), and sugary1 (su1) corn mutants. The ae mutant corn reduces the activity of branching enzymes (BE) IIb, resulting in an apparent increase in the relative proportion of amylose to amylopectin in the endosperm (29). Fl1 encodes a transmembrane protein located in the protein body endoplasm, targeting the 22 kDa α-zein to a location at the interface between the γ-zein-rich periphery and the core of the protein body (30). The ae genes affected the protein composition (31); however, the effect of fl1 genes on the starch structure is not well known.

FACE analysis of the AP from NS 3 showed a much greater amount of DP 36–56 than in the AP from starches 2 and 4. These findings agree with those of other researchers, who also noted a higher proportion of longer chains (DP > 30) in the AP of starches from corn with an ae allele than in the AP from common corn starch (19, 32). The reason for the long chains in AP of starches with an ae gene contribution is a loss of the starch branching enzyme IIb activity with the introduction of the ae mutation alleles (33). Possibly, the greater percentage of AM and greater amount of these long chains in starch 3 contributed to a greater %RS than in starches 2 and 4.

**GPC of Starches.** **Total Carbohydrate Analyses of GPC Fractions.** (a) **NS and RS-NS.** Analysis of NS by Sepharose CL2B chromatography displayed two peaks, typically classified as AP (first peak, fractions 13–23) and AM (fractions 33–47) (Figure 2). An intermediate fraction was also observed between the AM and AP peaks (fractions 24–33), which was combined with AM when calculating %AM. The identification of these fractions was determined by comparing them to the chromatogram of a mixture of a waxy corn starch, peak at fractions 14–23, and glucose, peak at fractions 37–47. The average apparent AM concentrations of the NS starches from 1, 2, 3, and 4 and typical starch were 58.3, 20.4, 26.8, 23.1, and 23.7%, respectively (Table 1). The %AM in typical starch was consistent with that reported by other researchers (34–36).

The RS-NS extracted from uncooked NS had considerable amounts of AP. The RS-NS of starch 1 had the greatest %RS (Table 1), the lowest %AP, and the greatest %AM among all starches. The other four starch types had much lower %RS, %AP, and %AM. Starch 3 had only 5.7% of RS-NS, much lower than that of starch 1 (55.20%), but higher than that of the other three starches. The chromatogram indicated that the RS-NS of starch 3 had a greater percentage of AP and higher molecular weight AM fractions than the other three starches (Figure 2). The differences in the AM fraction of RS-NS between starch 3 and starches 2 and 4 and typical starch were greater than the corresponding differences among their NS as starch 3 contained a greater amount of RS. The distribution pattern of starch 4 was closer to that of 2 and typical starch than to that of starch 3 (Figure 2).

(b) **RS-RVA-F and RS-RVA-R.** In spite of distinctive differences in NS and RS-NS between starches 1, 3 and other starches, the distribution of RS-RVA-F and RS-RVA-R of these starches were only slightly different in peak shape and conformation (Figure 2). No AP fraction was observed for any starches, meaning that AP was hydrolyzed by α-amylase and AMG to lower MW molecules, which then did not appear in the AP fractions from GPC. Only one peak appeared for RS-RVA-F and RS-RVA-R starches at the position of glucose elution. However, the collected fractions were brown in color and had BV responses, suggesting the presence of materials with greater MW than glucose. Also, glucose should have been removed during RS extraction by repeated washings with ethanol. This result may be caused by a limitation of the Sepharose CL 2B separation. Although RS-RVA-F and RS-RVA-R of starch 1 had much higher %RS than the other starches, the TCH profiles (Figure 2) were surprisingly similar to those of the other starches. Eerlingen et al. (37) reported that the DPn of RS from retrograded potato starch was in the range of 19 and 26 and was independent of the chain length of the AM from which it was formed (DPn 40–610). The fine structures of the peaks present in RS-RVA-F and RS-RVA-R need to be further studied by using high-performance size exclusion chromatography, which has a better separation capacity than GPC.

**BV of GPC Fractions.** (a) **NS and RS-NS.** The AP of NS and RS-NS from starch 1 had λmax values greater than those of the other starches, indicating starch 1 had longer short chains in this AP fraction, a finding also revealed by FACE. There were no differences in λmax of the AM fractions among the starches.

Although AP and AM had different λmax values for BV, the BV for all fractions collected was determined at 630 nm, the wavelength generally used for BV measurement of AM. The AP from each NS and RS-NS starch had lower BV and λmax than did the AM fractions, indicating the presence of very more very short branch chains in the AP fractions than in the AM fractions. Long chains of AP can form a helical complex with iodine (37). In other work, the AM fraction of an ae-type NS did not have higher λmax than the AP fraction (19).

The RS-NS of starches 1 and 3 had greater λmax than the RS-NS from the other starches, but similar AM structures. On the other hand, the RS-NS from starches 2 and 4 were similar to each other in the λmax profile distribution. NS of starches 1 and 3 had greater λmax than the corresponding RS-NS, whereas NS of starches 2 and 4 had λmax similar to their NS, suggesting different iodine-binding capacities between NS and RS-NS among these starches.

(b) **RS-RVA-F and RS-RVA-R.** The RS-RVA-F of starch 2 had similar or greater BV and λmax values than those for the other starches, followed by starch 4. RS-RVA-F of starch 1 had
Figure 1. Amylopectin chain length distribution of starches 1–4 and typical starch and comparisons among the starch types as analyzed by FACE (1, aaeaeae; 2, fl1flflf1; 3 aaeaeft1; 4 flflf1ae; TS, a commercial typical corn starch).
Figure 2. Sepharose CL2B separation of starch of NS, RS-NS, RS-RVA-F, and RS-RVA-R (1, aaeaeae; 2, f11f1f1f1; 3 aeeelf1; 4 f1f1fae; TS, a commercial typical corn starch). The BV for each fraction was increased by 10 times to plot both TCH and BV, with BV on the left y axis and $\lambda_{max}$ on the secondary right y axis.
the lowest BV among all starch types. The RS-RVA-R of starch 3 had a BV closer to that of the RS-RVA-R of starch 1 than the corresponding RS-RVA-F values. No BV was revealed at the position of AP for all starches, which further indicated the hydrolysis of AP in starch by enzymes after starch was cooked using RVA.

Both AM and AP are involved in molecular ordering at the ultrastructural level by molecular association through intermolecular hydrogen bonding to form double helices. The considerable BV in RS-RVA-F and RS-RVA-R further confirmed that molecules with large branch chains existed to form helical complexes with iodine.

**Pasting Properties of Starches.** Starch 1, tested at 8% DS on the RVA, did not show an apparent peak (Figure 3). Starch 2 and typical starch were similar to each other in PV, with both being lower than those of starches 3 and 4 (Table 2). PV values of starches 3 and 4 were not different from each other, although starch 3 had a slightly greater PV of 1989 cP. The troughs of starches 2, 3, and 4 were not different from each other, but were all lower than that of typical starch. Both starches 3 and 4 had higher BD than starch 2 and ordinary starch; however, there were no differences in FV among starches 2 and 4 and ordinary starch. Typical starch took longer and needed a higher temperature to gelatinize than the other starches, except for starch 1 (Figure 3). The time to reach PV and pasting temperature for starches 2 and 4 were similar, with both being lower than that of starch 3. Overall, both starches 3 and 4 had some pasting properties similar to those of starch 2, but distinctly different from those of starch 1.

The pasting results revealed that by introducing one or two ae and fl1 alleles, the pasting properties can be manipulated greatly. Starch 2 had a lower AM (20.4%) than starches 3 and 4, which might explain the lower BD of starch 2 compared to the other two starches. The high PV and high BD of starches 3 and 4 indicated that these two starches had common attributes, although the textural properties of the resulting gels were distinctly different. Alleles with higher ae dosage effects may be able to be introduced for future development of corn lines with high RS content without also having a dramatic impact on pasting properties.

**Thermal Properties of Starches.** Gelatinization. Thermal properties of NS and starches retrograded for 10 days are shown in Table 3. Starch 1 had a $T_p$ similar to that of starch 3, and an enthalpy similar to those of starch 4 and typical starch. Starch 3 had a greater $T_c$ but a lower enthalpy than starch 2. The differences between starches 2 and 4 were not as great as those between starches 2 and 3. Starch 4 had similar $T_p$ and $T_c$ but slightly higher $T_s$ and lower enthalpy than starch 2. Starch 4 also was more similar to typical starch than was starch 3 in $T_c$ and enthalpy. The PHI of the starches did not differ among the starch types as much as other thermal properties. Starch 2 and typical starch had greater percentages of shorter chains, which might contribute to the lower $T_c$ of these two starches. The greater percentage of medium chain lengths (DP 15–35) in starch 2 than in typical starch may suggest a higher crystallite formation in starch 2 and, thus, a greater energy (higher enthalpy) to gelatinize.

Although starch 1 could not be fully gelatinized under RVA conditions, it was fully gelatinized after treatment on the DSC, as confirmed when the pan was opened after thermal analysis. However, starch 1 lost water much more rapidly than other starches and quickly formed a white fraction, whereas the other starch gels remained translucent. Starch 4 had a gelatinization range similar to that of typical starch. The $R$ and $T_c$ of typical starch were consistent with previously reported data (24).

Retrogradation. After storage at 4°C for 10 days, the enthalpy and $T_c$ of all starches greatly decreased. Starch 2 had similar to lower retrogradation enthalpies than the other starches. Starch 1 had much greater enthalpy than all retrograded starches, with a value of 76.6% retrogradation after 10 days at 4°C. Retrogradation of starch 4 was 16% greater than for starch 2. This can be explained by the difference in chain lengths of these two starches. AP in starch 4 had a greater percentage of long chains and fewer short chains than AP in starch 2, possibly explaining the greater retrogradation of starch 4 than of starch 2. For example, Shi and Seib (41) suggested that retrogradation rates of starch were inversely correlated with the proportion of short chains of DP 6–9. The branch chain length also affects gelatinization and retrogradation (41). The $T_c$ of the retrograded starches was consistent with a previously reported range (37).

**Textural Properties.** All retrograded gels had greater strength than fresh gels at both compression sites (Table 4; Figure 3). The difference in gel strength between starch 3 and other starches was greater following retrogradation: the firmness of gel 3 increased by 85.36 g, whereas other starches increased by 37.08–52.75 g after retrogradation.

Gels of starch 1 were not firm when fresh because of incomplete gelatinization; thus, they formed a very weak gel network after retrogradation for 10 days. Gel of starch 3 was similar in appearance to those of starches 2 and 4, but after retrogradation exhibited syneresis as noted by detachment of the starch gels from the interior wall of canister. Although the adhesiveness of most starch gels increased after retrogradation, starch 3 gel was less adhesive, likely a result of its greater syneresis during retrogradation (Table 4; Figure 3). Gel of starch 1 was the least cohesive, and gel of typical starch tended to be the most cohesive while fresh and after storage. In general, however, the differences in cohesiveness among starches were not as great as the differences in firmness and adhesiveness.

Starch gels obtained after the gelatinization of starch granules can be regarded as a composite in which swollen gelatinized granules enriched in AP reinforce an AM gel matrix (42). Jane and Chen (43) observed that the gel with the highest resistance to uniaxial compression was formed from a mixture containing AP from either ae maize, wx maize, or normal rice starch and AM from the ae starch. Thus, it is likely that starch gels from starch 1 would have had the highest gel strength if they had been fully gelatinized in the RVA. Given the heating conditions in the RVA, starch 3, with two doses of ae and one dose of fl1 alleles, had the highest gel strength. The results are reasonable.
Table 2. Pasting Properties Measured by the Rapid Viscoanalyzer a

<table>
<thead>
<tr>
<th>starch sample</th>
<th>peak viscosity (cP)</th>
<th>trough (cP)</th>
<th>breakdown (cP)</th>
<th>final viscosity (cP)</th>
<th>setback (cP)</th>
<th>peak time (min)</th>
<th>pasting temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86 d</td>
<td>66 c</td>
<td>21 c</td>
<td>88 b</td>
<td>22 c</td>
<td>6.8 a</td>
<td>not gelatinized</td>
</tr>
<tr>
<td>2</td>
<td>1739 c</td>
<td>1345 b</td>
<td>394 b</td>
<td>1654 a</td>
<td>509 ab</td>
<td>5.0 c</td>
<td>78.3 c</td>
</tr>
<tr>
<td>3</td>
<td>1809 a</td>
<td>1436 b</td>
<td>674 a</td>
<td>1905 a</td>
<td>568 a</td>
<td>4.9 c</td>
<td>81.2 b</td>
</tr>
<tr>
<td>4</td>
<td>1909 ab</td>
<td>1287 b</td>
<td>622 a</td>
<td>1909 a</td>
<td>622 a</td>
<td>4.9 c</td>
<td>79.1 c</td>
</tr>
<tr>
<td>typical starch</td>
<td>1865 b</td>
<td>1476 a</td>
<td>389 b</td>
<td>1872 a</td>
<td>396 b</td>
<td>5.6 b</td>
<td>85.2 a</td>
</tr>
</tbody>
</table>

 a Values within one column with common letters are not significantly different (P ≤ 0.05). 1, aaeae; 2, fl1fl1fl1; 3, aae6fl1; 4, fl1fl1ae.

Table 3. Thermal Properties of Starches a

<table>
<thead>
<tr>
<th>starch sample</th>
<th>T o (°C)</th>
<th>T p (°C)</th>
<th>T c (°C)</th>
<th>ΔH (J/g)</th>
<th>R (°C)</th>
<th>PHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68.7 a</td>
<td>76.7 a</td>
<td>110.5 a</td>
<td>41.8 a</td>
<td>1.6 b</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>64.1 c</td>
<td>71.4 cd</td>
<td>77.8 c</td>
<td>16.2 a</td>
<td>2.2 a</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>68.2 a</td>
<td>74.8 b</td>
<td>84.0 b</td>
<td>13.3 b</td>
<td>15.8 b</td>
<td>2.0 ab</td>
</tr>
<tr>
<td>4</td>
<td>65.9 b</td>
<td>72.2 c</td>
<td>78.9 bc</td>
<td>12.1 c</td>
<td>13.5 b</td>
<td>1.9 b</td>
</tr>
<tr>
<td>typical starch</td>
<td>64.3 c</td>
<td>70.5 d</td>
<td>79.0 bc</td>
<td>12.0 c</td>
<td>14.6 b</td>
<td>1.9 b</td>
</tr>
</tbody>
</table>

 a Values within one column with common letters are not significantly different (P ≤ 0.05). T o, onset temperature; T p, peak temperature; T c, completion temperature; ΔH, enthalpy change; R, gelatinization temperature range; PHI, peak height index. 1, aaeae; 2, fl1fl1fl1; 3, aae6fl1; 4, fl1fl1ae.

Table 4. Gel Strength of Starch Gels Made by RVA a

<table>
<thead>
<tr>
<th>starch gel b</th>
<th>0 days</th>
<th>10 days</th>
<th>0 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fresh gel</td>
<td>retrograded gel</td>
<td>fresh gel</td>
<td>retrograded gel</td>
</tr>
<tr>
<td>1</td>
<td>9.25 c</td>
<td>23.96 c</td>
<td>4.87 d</td>
<td>4.01 b</td>
</tr>
<tr>
<td>2</td>
<td>102.60 b</td>
<td>139.68 b</td>
<td>28.97 b</td>
<td>35.18 a</td>
</tr>
<tr>
<td>3</td>
<td>157.07 a</td>
<td>242.43 a</td>
<td>13.61 c</td>
<td>2.37 b</td>
</tr>
<tr>
<td>4</td>
<td>102.60 b</td>
<td>139.68 b</td>
<td>28.97 b</td>
<td>35.18 a</td>
</tr>
<tr>
<td>typical starch</td>
<td>106.37 b</td>
<td>148.64 b</td>
<td>44.10 a</td>
<td>0.59 a</td>
</tr>
</tbody>
</table>

 a Values within one column with common letters are not significantly different (P ≤ 0.05). 1, aaeae; 2, fl1fl1fl1; 3, aae6fl1; 4, fl1fl1ae. b Gels were retrograded at 4 °C for 10 days.

Figure 4. Compression force (texture analyzer) of starch gels after heating in the RVA from fresh (RVA-F) and retrograded (RVA-R) materials (1, aaeae; 2, fl1fl1fl1; 3, aae6fl1; 4, fl1fl1ae).

given that gels containing AP with longer branches, as was the case for starch 3, form stronger gels than those containing AP with shorter branches (43).

Overall, although starches 3 and 4 were different in structure and function from both corn parents 1 and 2, starch 3 was more similar to starch 2 than to starch 1 in starch structure, but with distinctly different pasting and gel properties from both parents. Further work will evaluate the use of the starches presented in this paper, along with additional starches containing enhanced amounts of RS and the fl1 genes, in Hispanic food formulations. The impact of these structural features on product quality will be examined.

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