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Relationship of the channels of normal maize starch to the properties of its modified products

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ABSTRACT

Starches from 5 inbred lines of normal maize with different relative average degrees of channelization (RADC) that could be divided into two groups (2 with RADC values of 1.49–1.52 and 3 with RADC values of 0.10–0.17) were reacted with 4 highly reactive reagents. No consistent correlations between RADC and the effects of derivatization with the 4 reagents on physical properties, either without or after surface protein removal, were found. Reaction with propylene oxide, a slowly reacting reagent whose reaction should be independent of RADC, resulted in an inverse relationship between several physical properties and RADC. The results indicate that there are inherent granular and molecular differences in the maize starches that control reactivity that are more influential than RADC (at least with the degrees of modification used), that the differences carry through chemical derivatization, and that different reagents react differently with different starches.

1. Introduction

First surface pores were discovered in maize/corn, sorghum, and millet starch granules (Fannon, Hauber, & BeMiller, 1992). Then the hypothesized channels that connect those surface openings with the central cavity were confirmed in maize and sorghum starch granules (Fannon, Shull, & BeMiller, 1993; Huber & BeMiller, 1997). Studies of channels, including proof that they are natural structural features of starch granules, their characterization, and preliminary information about their influence on chemical modification, were reviewed in three publications (Fannon, Gray, Gunawan, Huber, & BeMiller, 2003, 2004; Han, Gray, Huber, & BeMiller, 2006). Since the writing of those reviews, it has been determined that crosslinking of granules with both $POCl_3$ and STMP appeared to be more effective in commercial maize starches than it was in commercial maize starches that had been treated with a proteolytic enzyme to remove external and channel surface protein (Han & BeMiller, 2008). Han and BeMiller (2008) hypothesized that protein molecules were, at least in part, involved in crosslinking reactions (either protein molecule to protein molecule or protein molecule to starch molecule), while also recognizing that it was possible that the differences in properties of the modified starches might be only indirectly related to the presence of protein.

It had previously been observed that the presence of pores and channels affects both the rate and pattern of digestion of raw starch granules with amylases, with those having surface pores being more accessible to enzyme molecules. When granules containing pores and channels are treated with an amylase, corrosion canals form and the internal cavity is enlarged as granules rather rapidly digest from the inside out (for the many references, see Fannon et al., 2003, 2004; Widya, Gunawan, & BeMiller, 2010). On the other hand, potato starch granules, which do not contain pores, slowly undergo surface exocorrosion upon α-amylolysis (for references, see BeMiller, 1997).

Earlier, Han, Benmoussa, Gray, BeMiller, and Hamaker (2005) had provided evidence that channels of normal maize, waxy maize, and sorghum starches were lined with protein molecules. Han et al. (2005) and Benmoussa, Hamaker, Huang, Sherman, Weil, and BeMiller (2010) identified some of the proteins lining channels and provided evidence of a microtubular origin. Widya et al. (2010) presented several ways to determine the relative average degrees of channelization (RADC) of maize starch granules. Results presented indicated that RADC was determined by the genetic makeup of the plant. Used in this method-development research were 6 inbred lines of maize. Five of those inbred lines were used in the research reported here.

Based on the findings of Huber and BeMiller (2001) and of Gray and BeMiller (2004) that $POCl_3$ reacts at granule surfaces, i.e., with the first molecules it encounters, including, if not especially, those at the surfaces of channels, it was logical to hypothesize that the RADC would affect granule modification with it and other rapidly

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reacting reagents and properties of the resulting products. This project was a test of that hypothesis. The five starches were also reacted with a slowly reacting reagent, propylene oxide, that has time to penetrate throughout the granule matrix before reacting (Gray & BeMiller, 2005; Huber & BeMiller, 2001), with the expectation that the RADC would not make a difference in this case.

2. Materials and methods

2.1. Materials

The five inbred lines of maize/corn (B73, Mo17, Oh43, W22, W23) used in this investigation were grown for us by Professor C.E. Weil on the Agronomy Center for Research and Education, Purdue University, West Lafayette, IN USA. Thermolysin (from *Bacillus thermoproteolyticus, p1512*), phosphoryl chloride, adipic acid, acetic anhydride, 2-octen-1-ylsuccinic anhydride, and propylene oxide were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA).

2.2. Laboratory-isolated starch

Starch was isolated from whole maize kernels using the method described by Wongsagonsup, Varavinit, and BeMiller (2008) and Sui, Shah, and BeMiller (2011).

2.3. Crosslinking with phosphoryl chloride ($POCl_3$)

The starches (20.0 g, db) were dispersed in ROD water (40 mL) with stirring at rt. The pH of the slurry was adjusted to 11.3 with 1 M NaOH. Phosphoryl chloride (0.01% of the weight of starch) was added dropwise (as a 3% solution in 1,4-dioxane) to the rapidly stirred slurry. The reaction was maintained at pH 11.3 for 1 h using a pH-Stat auto-titrator (Radiometer Analytical, Copenhagen, Denmark). After reaction, the reaction mixture was neutralized to pH 6.5 with dilute HCl. The modified starch was collected by centrifugation, washed three times with ROD water and once with 100% ethanol, and air-dried.

2.4. Crosslinking with acetic–adipic mixed anhydride (AAMA)

After adipic acid (0.20 g) was clearly dissolved in acetic anhydride (5.6 mL), the acetic–adipic mixed anhydride was diluted with 28 mL of 1,4-dioxane. The starches (25.0 g, db) were dispersed in ROD water (46 mL) with stirring at rt. The pH of the slurry was adjusted 9.0 with 1 M NaOH, The diluted acetic–adipic mixed anhydride solution (6.1 mL) was added dropwise to the rapidly stirred slurry. The reaction was maintained at pH 9.0 for 2 h using a pH-stat auto-titrator. After reaction, the reaction mixture was neutralized to pH 6.0 with dilute HCl. The modified starch was collected by centrifugation, washed three times with ROD water and once with 100% ethanol, and air dried. The dried preparations were stored in a refrigerator.

2.5. Esterification with acetic anhydride (AA)

The starches (20.0 g, db) were dispersed in ROD water (50 mL) with stirring at rt. The pH of the slurry was adjusted to 8.2 with 1 M NaOH. Acetic anhydride (1.7 mL) was added dropwise to the rapidly stirred slurry. The reaction was maintained at pH 8.2 for 6 h using a pH-stat auto-titrator. After reaction, the reaction mixture was neutralized to pH 6.5 with dilute HCl. The modified starch was collected by centrifugation, washed three times with ROD water and once with 100% ethanol, and air-dried.

2.6. Esterification with octenylsuccinic anhydride (OSA)

The starches (20.0 g, db) were dispersed in ROD water (45 mL) with stirring at rt. The pH of the slurry was adjusted to 8.5 with 1 M NaOH. 2-Octen-1-ylsuccinic anhydride (0.6 mL) was added dropwise to the rapidly stirred slurry. The reaction was maintained at pH 8.5 for 6 h using an auto-titrator. After reaction, the reaction mixture was neutralized to pH 6.5 with dilute HCl. The modified starch was collected by centrifugation, washed three times with ROD water and once with 100% ethanol, and air-dried.

2.7. Hydroxypropylation

The starches (33 g, db) were mixed with 100 mL of 0.527 m Na_2SO_4 and stirred for 5 min. With continued stirring, the pH was adjusted to pH 11.2 using 1 M NaOH. The reaction vessel was sealed tightly with a septum, and propylene oxide (2.6 mL) was added via a syringe. The flask was placed in a water bath at 49 °C, and the sample was stirred on a submergible magnetic stir plate for 24 h. After that, the starch slurry was stirred magnetically for 2 h at room temperature. The starch slurry was neutralized, and the starch was recovered in a Büchner funnel, washed several times with 50% acetone (~50 mL), and allowed to air-dry.

2.8. Pasting characteristics

Pasting characteristics of the modified starches were determined with a Rapid Visco-Analyzer (Model 4, Newport Scientific, Warriewood, Australia) using standard profile 1. Modified starch (1.96 g, db) and distilled water (26.04 g) (sample + water = 28.00 g) (7.0% starch; OSA products were measured at 5.0% starch) were mixed and stirred within the analyzer's aluminum sample canister. A 13-min analysis was used with a programmed heating and cooling cycle. The sample was heated to and held at 50° for 1 min; the temperature was raised to 95 °C within 3.7 min and held there for 2.5 min, then cooled to 50 °C within 3.8 min, and finally held there a further 2 min. Triplicate analyses were performed on each sample, and the results were averaged.

2.9. Differential scanning calorimetry (DSC)

The thermal properties of the starch samples were investigated with a differential scanning calorimeter (DSC; TA 2910, TA Instruments, Wilmington, DE, USA). The starch sample (5.0 mg, db) was placed in a pan; ROD water was added to a total of 20.0 mg (starch to water ratio = 1:3, w/w). The pan was sealed and equilibrated at room temperature for at least 2 h before the scan. The samples were heated from 20 °C to 120 °C at a heating rate of 10 °C/min. A sealed empty pan was used as a reference, and indium was used as a calibration standard. Triplicate analyses were performed on each sample, and the results were averaged.

After being stored 7 days at 4 °C, the gelatinized samples were rescanned from 20 °C to 140 °C at a heating rate of 10 °C/min. Thermal transitions for gelatinization and retrogradation were characterized by T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature), and ΔH (enthalpy). Triplicate analyses were performed on each sample, and the results were averaged.

2.10. Degree of substitution of OSA-derivatized starch

The modified starch samples (1 g, db) were weighed accurately and wetted with a few mL of reagent-grade 2-propanol. The samples were then dispersed in 25 mL of 2.5 M HCl in 2-propanol and stirred for 30 min. Aqueous 2-propanol (90%, v/v, 100 mL) was added to the starch slurry, which was then stirred for 10 min. The suspension was filtered through a glass filter and the residue was washed with 90% 2-propanol until no Cl^- could be detected by 0.1 M $AgNO_3$. The starch was dispersed in 300 mL of ROD water, and the slurry was heated 15 min in a boiling water bath. The starch solution was titrated while hot with standardized 0.1 M NaOH to pH 8.6 using an auto-titrator. The degree of substitution (DS) was expressed as

$$\mathrm{DS}\cdot\mathrm{OSA} = \frac{0.162 \times (A \times M)/W}{1 - 0.210 \times (A \times M)/W}$$

where 162 is the molecular weight of a glucosyl unit; 210 is the molecular weight of an octenylsuccinyl group; A is the titration volume of standardized NaOH solution; M is the molarity of NaOH solution; and W is the dry weight of starch.

2.11. Degree of substitution of AA- and AAMA-derivatized starch

The modified starch samples (1 g, db) were weighed accurately and suspended in 50 mL of ROD water. The starch slurry was adjusted to pH 8.6 with standardized 0.1 M NaOH to neutralize excess acid. After adding 25.0 mL of standardized 0.45 M NaOH, the flask was stoppered with a septum and kept in a 40 °C water bath with vigorous stirring for 3.5 h. The excess alkali was titrated with standardized 0.2 M HCl to pH 8.6 using a auto-tritator. A blank was titrated at the same time. The degree of substitution (DS) of AA- or AAMA-derivatized starch was expressed as

$$\mathrm{DS}\cdot\mathrm{AA} = \frac{0.162 \times (B - A) \times M/W}{1 - 0.043 \times (B - A) \times M/W}$$

$$\mathrm{DS}\cdot\mathrm{AAMA} = \frac{0.162 \times (B - A) \times 0.5M/W}{1 - 0.112 \times (B - A) \times 0.5 \times M/W}$$

where 162 is the molecular weight of a glucosyl unit; 43 is the molecular weight of an acetyl group; 112 is the molecular weight of an adipyl group; B is the volume of standardized 0.2 M HCl used to titrate the blank; A is the volume of standardized 0.2 M HCl used to titrate the sample; M is the molarity of HCl solution; W is the dry weight of starch, with the understanding that AAMA-modified starch contains acetyl groups and probably monostarch adipate groups in addition to distarch adipate groups.

2.12. Phosphorus analysis

Analysis of the $POCl_3$ and STMP reaction products for phosphorus was done in duplicate using ICP-MS by the Analytical Sciences Laboratory, University of Idaho, Moscow, ID, USA.

2.13. Incubation of starch with thermolysin

Starch granules were treated with thermolysin as described by Mu-Forster and Wasserman (1998), using 1 mg enzyme/g starch and 30 min incubation.

3. Results and discussion

In developing methods to measure RADC, Widya et al. (2010) compared 5 methods using starch from 6 inbred lines of maize (wild types) and starch isolated in the laboratory from a commercial maize hybrid using the same method. They concluded that measurement of the relative amounts of actin, a channel protein, was the preferred method. Using that method, Widya et al. (2010) found that the rank order of the inbred lines used in this research was as follows (RADC values based on the relative amount of actin present in parentheses): Oh43 (1.52), W23 (1.49), Mo17 (0.17), W22 (0.10), B73 (0.10). These data indicate 2 groups of starches with respect to RADC, with Oh43 and W23 being in the group with a higher degree of channelization and Mo17, W22, and B73 being in the group with a lower degree of channelization. An important point is that, using all three methods deemed to be reliable, viz., measurement of the relative amounts of actin, a 42 kDa protein, and total surface protein (which includes channel protein), Oh43 gave the highest value and B73 the lowest value, with the value for Oh43 being 3.75–15 times greater than that for B73 (Widya et al., 2010), so starches from these two inbred lines could be used as indicators of any correlations between RADC and granule reactivity.

It had previously been found that phosphoryl chloride ($POCl_3$), a reagent presumed to react very rapidly with starch granules, reacts primarily in granule channels, when they are present, such as in maize starch granules, and at the outer granule surface, especially when no pores or channels are present, such as in potato starch granules (Huber & BeMiller, 2001; Gray & BeMiller, 2004), so this reagent was chosen. Also chosen as derivatizing reagents were carboxylic acid anhydrides, viz., acetic anhydride (AA), acetic–adipic mixed anhydride (AAMA), and 2-octen-1-ylsuccinic anhydride (OSA), which were also assumed to be rather reactive, although requiring a longer reaction time at a lower pH (to minimize saponification).

Related to the hypothesis tested is the report of Hellman and Melvin (1950) that the ratio of the surface area of starch granules as determined from nitrogen sorption to that calculated from photomicrographs was 1.5:1.0 for maize starch granules and 0.73:1.0 for potato starch granules, which do not have pores or channels (Fannon et al., 1992), indicating that perhaps as much as one-third of the surface area of maize starch granules is in their channels and cavity.

It was assumed that, if there were differences in the reactivity of starch granules that were related to the average number of channels in them, the differences would (1) be manifested only when using rapidly reacting reagents because they react at channel surfaces, and (2) be most evident in the amount of derivatization and RVA parameter values. The first evidence that cast doubt on these assumptions was that values for the RVA parameters of the native starches themselves varied considerably and were not correlated to the RADC (Table 1). Therefore, in analyzing data, comparisons were made using the differences between the values given by the chemically modified starch and those given by the native/unmodified starch.

An examination of the RVA data in Table 1 indicates no consistent correlation between the RADC and the effects of derivatization with known or assumed to be relatively rapidly reacting reagents, i.e., for products of reaction with $POCl_3$ or the carboxylic acid anhydrides (AAMA, AA, OSA). All difference values for peak and final viscosities, breakdown, and setback increased and pasting temperatures decreased (as compared to the values given for the native starches) with 5 exceptions, viz., values for the differences in breakdown for Mo17 and Oh43 starches reacted with AA and for Mo17 and W23 starches reacted with OSA decreased, and peak viscosity values for 4 of the 5 starches decreased after reaction with AA. (Decreases in peak viscosity values following acetylation shown by 4 of the 5 starches, which were greater for the three starches with the higher RADC than were the values for the two starches with the lower RADC, were unexpected, because in recent years (in order) Gonzalez and Perez (2002) (rice starch), Wilkens, Wang, Xu, Niu, Tumbleson, and Rausch (2003a) (commercial normal maize starch), Wilkens, Wang, Xu, Niu, Tumbleson, and Rausch (2003b) (commercial waxy maize starch), Adebowale and Lawal (2003) (mucuna bean starch), Van Hung and Morita (2005) (wheat starch), Adebowale, Afolabi, and Olu-Owalabi (2006) (sword bean starch), and Nunez-Santiago, Garcia-Suarez, Roman-Gutierrez, and Bello-Perez (2010) (barley starch) found that low degrees of acetylation increased peak viscosity of the starch. However, Ali and Hasnain (2011) found that a low degree of acetylation

Table 1
RVA data and differences between values for derivatized and unmodified starches for 5 inbred lines of maize with different relative average degrees of channelization (RADC).[a]

Reagent[b]	Inbred	Peak η (mPa s)[c]	Breakdown (mPa s)[c]	Final η (mPa s)[c]	Setback (mPa s)[c]	Pasting temp. (°C)[d]
None[e,f]	B73	1190 ± 13	152.0 ± 4.6	1370 ± 16	331.3 ± 6.8	83.4 ± 0.9
	W22	1064 ± 8	129.0 ± 27.7	1388 ± 10	452.3 ± 33.0	78.5 ± 1.2
	Mo17	1259 ± 4	333.3 ± 15.0	1453 ± 5	527.7 ± 13.4	77.5 ± 0.8
	W23	1162 ± 12	156.3 ± 6.0	1283 ± 47	277.3 ± 61.7	78.3 ± 0.8
	Oh43	1145 ± 13	314.0 ± 7.8	1259 ± 12	428.0 ± 6.1	76.8 ± 0.1
$POCl_3$	B73	1366 ± 3	232.7 ± 45.5	1801 ± 49	667.7 ± 17.8	75.4 ± 0.4
	Difference[g]	+176	+81	+431	+336	−8.0
	W22	1324 ± 17	366.7 ± 11.6	1672 ± 17	715.7 ± 21.2	74.3 ± 0.0
	Difference[g]	+260	+238	+284	+263	−4.2
	Mo17	1550 ± 1	487.0 ± 7.2	1813 ± 16	749.0 ± 21.6	75.7 ± 0.4
	Difference[g]	+291	+154	+360	+221	−1.8
	W23	1374 ± 12	450.0 ± 23.5	1654 ± 41	729.3 ± 54.0	74.9 ± 1.0
	Difference[g]	+212	+294	+371	+452	−3.4
	Oh43	1353 ± 9	385.7 ± 41.3	1439 ± 28	471.4 ± 25.0	76.2 ± 0.5
	Difference[g]	+208	+72	+180	+44	−0.6
AAMA	B73	1597 ± 6	603.3 ± 7.0	1787 ± 10	793.3 ± 9.9	76.1 ± 1.2
	Difference[g]	+407	+451	+480	+462	−7.3
	W22	1556 ± 2	609.5 ± 16.3	1675 ± 17	729.0 ± 2.8	74.7 ± 0.6
	Difference[g]	+492	+481	+287	+277	−3.8
	Mo17	1411 ± 4	489.3 ± 32.7	1610 ± 2	687.7 ± 31.5	77.0 ± 0.4
	Difference[g]	+152	+156	+157	+160	−0.5
	W23	1437 ± 12	904.7 ± 4.9	1665 ± 19	760.0 ± 13.8	75.3 ± 0.5
	Difference[g]	+275	+748	+382	+483	−3.0
	Oh43	1425 ± 18	580.7 ± 12.5	1384 ± 24	539.7 ± 21.4	75.4 ± 0.4
	Difference[g]	+307	+267	+125	+11	−1.4
AA	B73	1167 ± 17	259.7 ± 22.5	2231 ± 29	1323 ± 12	74.0 ± 0.9
	Difference[g]	−23	+108	+861	+992	−9.4
	W22	1084 ± 7	221.0 ± 2.7	1914 ± 28	1050 ± 20	71.0 ± 1.4
	Difference[g]	+20	+92	+524	+598	−7.5
	Mo17	989.0 ± 4.6	200.3 ± 12.7	1856 ± 39	1067 ± 28	73.0 ± 0.4
	Difference[g]	−270	−133	+403	+539	−4.5
	W23	988.3 ± 14.0	261.3 ± 10.2	1807 ± 13	1080 ± 9	71.6 ± 0.5
	Difference[g]	−174	+105	+524	+803	−6.7
	Oh43	961.0 ± 9.6	208.0 ± 15.1	1586 ± 22	833.3 ± 27.3	72.7 ± 0.8
	Difference[g]	−184	−106	+327	+405	−4.1
OSA	B73[h]	1682 ± 17	110.3 ± 23.9	2387 ± 30	815.0 ± 23.1	73.8 ± 0.9
	Control[f,h]	418.7 ± 5.0	57.33 ± 0.58	394.7 ± 6.1	33.33 ± 1.53	92.6 ± 0.4
	Difference[g]	+1263	+53	+1992	+782	−18.8
	W22[h]	1564 ± 8	40.67 ± 7.37	2236 ± 14	713.3 ± 15.0	72.0 ± 0.1
	Control[f,h]	374.0 ± 11.4	20.67 ± 3.21	404.7 ± 23.8	51.33 ± 9.24	90.9 ± 0.9
	Difference[g]	+1190	+20	+1831	+662	−18.9
	Mo17[h]	1482 ± 9	17.33 ± 7.77	2233 ± 30	768.0 ± 29.8	73.8 ± 0.5
	Control [f,h]	441.7 ± 1.5	66.00 ± 4.00	425.7 ± 5.5	50.00 ± 5.00	89.6 ± 0.7
	Difference[g]	+1040	−49	+1807	+718	−15.8
	W23[h]	1386 ± 15	28.67 ± 13.20	1955 ± 46	597.7 ± 24.7	74.1 ± 0.5
	Control[f,h]	399.3 ± 11.2	41.33 ± 2.08	396.3 ± 16.1	38.33 ± 3.79	91.0 ± 0.5
	Difference[g]	+987	−13	+1559	+560	−16.9
	Oh43[h]	1398 ± 14	266.7 ± 16.0	1710 ± 15	578.7 ± 16.5	73.5 ± 0.1
	Control [f,h]	417.7 ± 14.6	54.00 ± 2.65	418.3 ± 9.7	54.67 ± 8.74	84.5 ± 6.8
	Difference[g]	+980	+213	+1292	+524	−11.0
PO	B73	1327 ± 16	439.0 ± 39.2	1946 ± 70	1058 ± 36	70.8 ± 0.5
	Difference[g]	+137	+287	+576	+727	−12.6
	W22	1319 ± 13	488.7 ± 18.6	1787 ± 33	956.3 ± 22.8	71.6 ± 1.0
	Difference[g]	+255	+360	+399	+504	−6.9
	Mo17	1299 ± 25	557.3 ± 19.1	1641 ± 11	898.7 ± 20.8	73.0 ± 0.5
	Difference[g]	+40	+224	+188	+371	−4.5
	W23	1252 ± 9	458.3 ± 11.9	1632 ± 30	865.3 ± 31.3	73.3 ± 0.5
	Difference[g]	+90	+302	+349	+588	−5.0
	Oh43	1234 ± 14	458.3 ± 10.0	1542 ± 28	767.3 ± 21.5	73.2 ± 0.4
	Difference[g]	+89	+144	+283	+339	−3.6

[a] Ordered from the lowest RADC value to the highest RADC value based on actin content (Widya et al., 2010).
[b] $POCl_3$, phosphoryl chloride; AAMA, acetic–adipic mixed anhydride; AA, acetic anhydride; OSA, ocetenylsuccinic anhydride; PO, propylene oxide.
[c] Values are means of triplicate analyses reported to 4 significant figures.
[d] Values are means of triplicate analyses reported to 3 significant figures.
[e] 7.00% concentrations (controls for all products except those derivatized with OSA).
[f] Non-derivatized native starch from inbred line.
[g] Change from value for control/unmodified starch.
[h] 5.00% concentration.

increased peak viscosity of sorghum starch only slightly, Nunez-Santiago et al. (2010) found that a low degree of acetylation made almost no change in the peak viscosity of normal maize starch, and Lawal and Adebowale (2005) (jack bean starch) and Bello-Perez, Agama-Acevedo, Zamudio-Flores, Mendez-Mentealvo, and Rodriguez-Ambriz (2010) (barely starch) found that a low degree of acetylation reduced the peak viscosity of barley starch. The decrease in peak viscosity is probably the result of increased granule swelling and disintegration, the evidence for which is in the increased breakdown observed by Wilkens et al. (2003a) (commercial normal maize starch), Lawal and Adebowale (2005) (jack bean starch) and Adebowale et al. (2006) (sword bean starch); however, Wilkens et al. (2003b) (commercial waxy maize starch) and Adebowale and Lawal (2003) (mucuna bean starch) found less breakdown after low degrees of acetylation. Therefore, decreases in breakdown shown by 2 of the 5 starches was also somewhat surprising. When comparing difference results for products of native starches from the 3 inbred lines with the lower RADC (B73, W22, Mo17) with those from the 2 inbred lines with the higher RADC (W23, Oh43), it was found that only after octenylsuccinylation did peak and final viscosities of the modified starches increase in the same order as RADC, which was not the case with the native starches.

In comparing actual values of the RVA parameters for only unmodified Oh43 starch (the highest RADC) and unmodified B73 starch (the lowest RADC), it was found that native Oh43 starch gave lower peak and final viscosity values and greater breakdown and setback values than did native B73 starch. After derivatization with AAMA or AA, Oh43 starch gave lower values for all four RVA parameters than did B73 starch, which may be an indication that acetylation was more important than crosslinking after reaction with the AAMA reagent, which effects both reactions. After crosslinking with $POCl_3$, Oh43 starch gave lower values for only peak and final viscosities and setback as compared to B73 starch. After derivatization with OSA, the products were analyzed at 5.00% rather than 7.00% (for products of the other three reactions) concentrations, and a different pattern was observed. Like the products of reaction with $POCl_3$, the products of reaction of Oh43 starch with OSA gave lower values for peak and final viscosities and setback and a higher value for breakdown than did B73 starch. However, at the 5.00% concentration used for RVA analysis, native Oh43 starch had the same peak viscosity as the B73 starch, a lower breakdown, and higher values for final viscosity and setback. Together, the lack of congruity between RADC and RVA parameters indicates that there are inherent differences in maize starches with different genetic makeups that carry through chemical derivatization and indicate that the average number of channels per granule is not a major factor in determining the changes effected by derivatization. However, in addition to the possibility that there are differences in the granular or molecular structures of the different maize starches of different genotypes other than the degrees of channelization, it is possible that, because of the low level of $POCl_3$ used, the amounts of external and/or channel surfaces in the granules is not the limiting factor.

Because there is a correlation between channel protein content and RADC (Widya et al., 2010), because the protein molecules should contain functional groups that are more reactive than the hydroxyl groups of starch (Han & BeMiller, 2008), and because protein content is related to granule swelling (Debet & Gidley, 2006), while swelling is related to granule reactivity (Gray & BeMiller, 2005), protein was removed from the channels and the exterior surface of native starch granules before derivatization, using an enzymic method described by Mu-Forster and Wasserman (1998) that had previously been shown to remove most or all of the protein from surfaces of channels (Gray & BeMiller, 2004; Han et al., 2005; Han & BeMiller, 2008), using the minimal concentration of thermolysin required (1 mg/g starch) to minimize the already insignificant degree of starch hydrolysis (Han & BeMiller, 2008). The overall RVA parameter patterns for starches derivatized after surface protein removal (Table 2) were compared to the patterns in Table 1. Again, values for peak and final viscosities, breakdown, and setback increased and pasting temperatures decreased over the values for the native starches, but now, with only two exceptions, viz., again the peak viscosity of all five starches and the breakdown of Mo17 starch decreased after acetylation. Only for final viscosity did the OSA-modified starches give a pattern in which there was a steady decrease in the increase in values for the modified starches minus the values for the unmodified starches as the RADC increased. All RVA viscosity parameter values for the modified, protease-treated (PT) starches were less than the corresponding values for the modified native starches, with a few exceptions. For W22 starch, peak viscosity and setback increased after reaction with $POCl_3$, final viscosity increased after reaction with AAMA, and all RVA viscosity parameter values increased after reaction with AA. Breakdown increased after acetylation of Oh43 starch. After reaction with OSA, increases were found in breakdown for all five starches; values for all RVA viscosity parameters increased for B73 starch, and all RVA viscosity parameter values, except setback, increased for Oh43 starch. After protein removal (Table 2), peak and final viscosities of the 3 starches with the lower RADC (B73, W22, Mo17) crosslinked with $POCl_3$ were greater than those of the 2 starches with the higher RADC (Oh43, W23) (but the same pattern was found with the native starches). Final viscosities of the 3 starches with lower RADC reacted with $POCl_3$ and OSA were greater than (or, in the case of products of reaction with AA, greater than or equal to) those of the 2 starches with the higher RADC (but once again, the same pattern was observed with the native starches), and setback values for products of reaction of the 3 starches with the lower RADC with $POCl_3$ and OSA were greater than those of the 2 starches with the higher RADC. More important, derivatization of the PT starches with OSA also resulted in greater changes in final viscosities and setback values for the 3 starches with the lower RADC values (as compared to the native starches) than did the same modification of the 2 starches with the higher RADC values. Another thing seen in Table 2 is that in the case of all 4 modifications, the PT-B73 products had greater peak and final viscosities than did the PT-Oh43 products (as did the native starches). Also, in the case of all 4 derivatizations, the changes in final viscosity were greater for the PT-B73 products as compared to the control starches than were the changes for the PT-Oh43 products. However, the tested hypothesis was that there would be greater changes in the starches with the higher RADC. Overall, some changes in the pasting and paste properties of the products were effected via protein removal from the 5 starches, but again no real correlation with RADC was observed.

Removal of protein has been reported to facilitate granule swelling and disintegration (Debet & Gidley, 2006). For the unmodified PT starches, reductions in peak viscosity and breakdown were exhibited only by PT-Mo17, -W23, and -Oh43 starches, the starches with the greatest amounts of channels and, therefore, channel protein. For these starches, a greater degree of swelling and easier disintegration, resulting in a lowering of the peak viscosity and less breakdown because more granule disintegration occurred before the peak viscosity was reached, may be indicated. Reductions in RVA viscosity parameter values after reaction with the two crosslinking reagents is contrary to the finding of Han and BeMiller (2008) that removal of remaining protein from commercial normal and waxy maize starches resulted in less effective croslinking, but is consistent with the hypothesis that removal of protein with which the reagent can react should result in more effective crosslinking. Reductions in RVA viscosity parameter values after reaction with AA (except for the W22 starch) are consistent with greater acetylation of the starch polymer molecules. It might indicate that, when

Table 2
RVA data for starches that had been treated with a protease to remove surface protein and differences between values for derivatized and unmodified starches for 5 inbred lines of maize with different relative average degrees of channelization (RADC).[a]

Reagent[b]	Inbred	Peak η (mPa s)[c]	Breakdown (mPa s)[c]	Final η (mPa s)[c]	Setback (mPa s)[c]	Pasting temp. (°C)[d]
None[e,f]	B73	1234 ± 7	95.00 ± 31.43	1431 ± 20	291.7 ± 20.2	84.5 ± 0.5
	W22	1190 ± 46	136.7 ± 10.7	1480 ± 57	426.3 ± 25.8	79.7 ± 0.5
	Mo17	1177 ± 1	214.0 ± 30.1	1326 ± 8	362.7 ± 30.8	78.1 ± 1.2
	W23	1073 ± 9	89.7 ± 12.7	1179 ± 6	195.3 ± 10.1	78.9 ± 0.5
	Oh43	1077 ± 31	141.7 ± 21.1	1252 ± 9	316.3 ± 4.5	77.2 ± 0.5
POCl$_3$	B73	1264 ± 16	199.3 ± 8.0	1616 ± 30	550.7 ± 21.4	76.9 ± 0.4
	Difference[g]	+30	+24	+185	+259	−7.6
	W22	1396 ± 6	347.0 ± 9.5	1820 ± 10	771.0 ± 9.5	74.3 ± 0.1
	Difference[g]	+206	+210	+340	+345	−5.4
	Mo17	1324 ± 9	334.3 ± 21.5	1618 ± 25	628.3 ± 35.3	75.4 ± 0.5
	Difference[g]	+147	+120	+292	+266	−2.7
	W23	1238 ± 8	277.7 ± 16.0	1437 ± 12	476.7 ± 20.2	75.7 ± 0.4
	Difference[g]	+165	+188	+258	+281	−3.2
	Oh43	1168 ± 6	335.3 ± 24.5	1263 ± 7	430.3 ± 18.6	71.9 ± 7.0
	Difference[g]	+91	+194	+11	+114	−5.3
AAMA	B73	1448 ± 17	452.7 ± 94.5	1731 ± 60	735.7 ± 24.3	74.8 ± 0.5
	Difference[g]	+214	+358	+300	+444	−9.7
	W22	1435 ± 3	447.5 ± 5.0	1691 ± 0	703.5 ± 2.1	73.6 ± 0.0
	Difference[g]	+245	+311	+211	+277	−6.1
	Mo17	1213 ± 15	386.0 ± 22.5	1471 ± 6	644.7 ± 11.2	76.3 ± 0.4
	Difference[g]	+36	+172	+145	+282	−1.8
	W23	1293 ± 7	399.3 ± 33.5	1571 ± 20	677.7 ± 20.6	75.6 ± 0.5
	Difference[g]	+220	+310	+392	+482	−3.3
	Oh43	1304 ± 10	488.7 ± 37.9	1343 ± 31	528.0 ± 29.5	75.2 ± 0.9
	Difference[g]	+227	+347	+91	+212	−2.0
AA	B73	1103 ± 8	200.7 ± 16.6	2069 ± 15	1167 ± 23	73.7 ± 0.4
	Difference[g]	−131	+106	+638	+875	−10.8
	W22	1105 ± 9	276.7 ± 12.4	2015 ± 20	1186 ± 17	71.6 ± 0.5
	Difference[g]	−85	+140	+535	+760	−8.1
	Mo17	941.7 ± 8.5	194.7 ± 11.7	1775 ± 29	1028 ± 14	72.2 ± 0.5
	Difference[g]	−235	−19	+449	+665	−5.9
	W23	988.3 ± 0.6	252.0 ± 19.9	1784 ± 4	1048 ± 14	71.9 ± 0.0
	Difference[g]	−85	+162	+605	+853	−7.0
	Oh43	940.0 ± 20.1	224.7 ± 31.3	1480 ± 29	764.7 ± 42.5	71.5 ± 0.6
	Difference[g]	−137	+83	+228	+449	−5.7
OSA	B73[h]	1716 ± 28	150.5 ± 14.9	2498 ± 100	889.0 ± 108.5	72.2 ± 0.4
	Control[f,h]	425.3 ± 11.9	54.67 ± 11.24	412.7 ± 5.1	42.00 ± 1.00	91.8 ± 1.0
	Difference[g]	+1291	+96	+2085	+847	−19.6
	W22[h]	1545 ± 10	134.0 ± 6.6	2064 ± 10	653.3 ± 12.7	70.8 ± 0.6
	Control[f,h]	405.7 ± 3.5	42.00 ± 4.00	411.7 ± 2.9	48.00 ± 6.24	90.1 ± 0.4
	Difference[g]	+1139	+92	+1652	+605	−19.3
	Mo17[h]	1451 ± 15	96.00 ± 45.21	1993 ± 37	637.3 ± 23.1	74.1 ± 0.5
	Control[f,h]	444.3 ± 14.2	66.33 ± 3.06	446.3 ± 21.8	68.33 ± 5.69	89.3 ± 0.5
	Difference[g]	+1007	+30	+1547	+569	−15.2
	W23[h]	1380 ± 6	42.67 ± 32.08	1872 ± 4	534.3 ± 23.0	72.2 ± 0.4
	Control[f,h]	380.3 ± 11.4	43.00 ± 1.73	380.7 ± 17.0	43.33 ± 4.04	91.0 ± 1.9
	Difference[g]	+1000	0	+1491	+491	−18.8
	Oh43[h]	1469 ± 59	301.0 ± 60.3	1723 ± 41	554.3 ± 41.7	72.8 ± 0.0
	Control[f,h]	382.0 ± 3.6	42.00 ± 5.29	381.3 ± 2.5	41.33 ± 5.69	89.3 ± 0.9
	Difference[g]	+1087	+259	+1342	+513	−16.5

[a] Ordered from the lowest RADC value to the highest RADC value based on actin content (Widya et al., 2010).
[b] POCl$_3$, phosphoryl chloride; AAMA, acetic–adipic mixed anhydride; AA, acetic anhydride; OSA, ocetenylsuccinic anhydride.
[c] Values are means of triplicate analyses reported to 4 significant figures.
[d] Values are means of triplicate analyses reported to 3 significant figures.
[e] 7.00% concentrations (controls for all products except those derivatized with OSA).
[f] Native starch of inbred line treated with protease only.
[g] Change from value for control/unmodified starch.
[h] 5.00% concentration.

surface protein is present, some reagent reacts with it, leaving less to react with the starch. However, the greater increases in setback found with acetylation of PT starches (except PT-B73 starch) as compared to the acetylated native starches do not support this idea.

Only after modification with OSA was any pattern to changes evident. After modification of the native starch, the changes in setback values for the OSA reaction products of the 3 starches with the lower RADC values were greater than those of the 2 starches with the higher RADC values (Table 1). For products made after protein removal from the native starches, both final viscosities and setback values were greater for the 3 starches with the lower RADC values (as compared to the controls) than they were for the 2 starches with the higher RADC values (Table 2). However, the starting hypothesis was that, because channels increase the surface area of granules, there should be greater changes after reaction with rapidly reacting reagents in the starches with the higher RADC values as compared to the starches with the lower RADC values. If any relationship was found, it was the opposite. We do not believe that the results

Table 3

Gelatinization (DSC) data and differences between values of derivatized and unmodified starches for 5 inbred lines of maize with different relative average degrees of channelization (RADC).[a]

Reagent[b]	Inbred	T_p (°C)[c]	Temp. range (°C)[d]	Enthalpy (J/g)
None[e]	B73	69.63 ± 0.0	6.98 ± 0.08	15.60 ± 0.21
	W22	69.70 ± 0.01	10.0 ± 0.32	16.09 ± 0.40
	Mo17	72.63 ± 0.74	9.48 ± 0.21	16.96 ± 0.23
	W23	71.98 ± 0.23	9.05 ± 0.62	16.47 ± 1.38
	Oh43	72.19 ± 0.05	9.82 ± 0.43	17.10 ± 0.35
$POCl_3$	B73	69.17 ± 0.10	7.94 ± 0.42	17.64 ± 1.69
	Difference[f]	−0.46	+0.96	+2.04
	W22	69.06 ± 0.23	10.35 ± 0.07	15.86 ± 0.07
	Difference[f]	−0.64	+0.35	−0.23
	Mo17	71.33 ± 0.25	9.21 ± 0.33	16.75 ± 0.25
	Difference[f]	−0.90	−0.27	−0.21
	W23	71.53 ± 0.06	9.57 ± 0.15	16.69 ± 0.37
	Difference[f]	−0.45	+0.52	+0.22
	Oh43	71.48 ± 0.08	11.08 ± 0.29	16.67 ± 0.54
	Difference[f]	−0.71	+1.26	−0.43
AAMA	B73	66.76 ± 0.11	8.63 ± 0.18	15.73 ± 0.78
	Difference[f]	−2.87	+1.65	−0.13
	W22	66.58 ± 0.11	10.25 ± 0.72	15.53 ± 0.25
	Difference[f]	−3.12	+0.25	−0.56
	Mo17	69.05 ± 0.17	9.21 ± 0.60	15.47 ± 0.66
	Difference[f]	−3.58	−0.27	−1.49
	W23	68.73 ± 0.02	9.93 ± 0.14	14.78 ± 0.07
	Difference[f]	−3.25	+0.88	−1.69
	Oh43	69.02 ± 0.21	10.04 ± 0.34	15.06 ± 1.27
	Difference[f]	−3.17	+0.22	−2.04
AA	B73	63.87 ± 0.27	9.82 ± 0.84	15.10 ± 0.02
	Difference[f]	−5.76	+2.84	−0.50
	W22	64.20 ± 0.41	11.21 ± 0.64	11.22 ± 1.00
	Difference[f]	−5.50	+1.21	−4.87
	Mo17	66.22 ± 0.21	10.50 ± 0.39	16.62 ± 0.88
	Difference[f]	−6.41	+1.02	−0.34
	W23	68.10 ± 0.64	12.81 ± 0.18	10.32 ± 0.33
	Difference[f]	−3.88	+3.76	−6.15
	Oh43	66.41 ± 0.14	10.85 ± 0.37	16.82 ± 0.37
	Difference[f]	−5.78	+1.03	−0.28
OSA	B73	68.27 ± 0.04	8.72 ± 0.40	14.26 ± 0.27
	Difference[f]	−1.36	+1.74	−1.34
	W22	68.33 ± 0.10	11.00 ± 0.27	13.83 ± 0.62
	Difference[f]	−1.37	+1.00	−2.26
	Mo17	70.01 ± 0.30	10.08 ± 0.11	13.51 ± 0.11
	Difference[f]	−2.62	+0.60	−3.45
	W23	69.93 ± 0.02	10.44 ± 0.30	13.06 ± 0.49
	Difference[f]	−2.05	+1.39	−3.41
	Oh43	70.25 ± 0.01	10.89 ± 0.08	14.20 ± 0.71
	Difference[f]	−1.94	+1.07	−2.90

[a] Ordered from the lowest RADC value to the highest RADC value based on actin content (Widya et al., 2010).

[b] $POCl_3$, phosphoryl chloride; AAMA, acetic–adipic mixed anhydride; AA, acetic anhydride; OSA, octenylsuccinic anhydride.

[c] T_p = peak temperature. Values are means of triplicate measurements.

[d] Conclusion temperature–onset temperature. Values are means of triplicate measurements.

[e] Non-derivatized native starch from inbred line (controls).

[f] Change from value for control/unmodified starch.

indicate that the external surface of channels is more reactive than their channel surfaces because it does not seem to be congruent with visual information (Gray & BeMiller, 2004), so again, it seemed to be obvious that there were differences in the starches controlling reactivity that were more influential than were RADC values. It was also indicated that the different reagents reacted differently with the different starches.

Few correlations were found with DSC parameters for the unmodified and derivatized starches (Table 3). After derivatization with any of the four reagents, T_p and enthalpy of gelatinization (ΔH_G) (with two exceptions) decreased, the phase transition temperature range ($T_c - T_o$) increased (with two exceptions), and T_p of the two starches with the higher RADC was greater than T_p of the two starches with the lower RADC, but the latter was also true of the native starches. After reaction with AAMA or OSA, there was a greater (negative) difference in T_p and ΔH_G values for the two starches with the higher RADC than for the two starches with the lower RADC. After removal of protein before derivatization (Table 4), some of the same correlations were found, but there were fewer of them. The main changes resulting from protein removal were the following changes in actual (not difference) values: slight reductions in T_p for both the native and modified starches with two exceptions (T_p values for B73 and W22 starches reacted with AAMA were unchanged), increases in $T_c - T_o$ with several exceptions (values for unmodified Mo17 and B73 starches and for W22 starch reacted with AAMA were unchanged; B73, W22, Mo17, and Oh43 starches reacted with $POCl_3$ and W23 starch reacted with AA were reduced), and ΔH_G increased with a few exceptions (the value

Table 4

Gelatinization (DSC) data for starches that had been treated with a protease to remove surface protein and differences between values for derivatized and unmodified starches for 5 inbred lines of maize with different relative average degrees of channelization (RADC).[a]

Reagent[b]	Inbred	T_p (°C)[c]	Temp. range (°C)[d]	Enthalpy (J/g)
None[e]	B73	68.94 ± 0.05	8.21 ± 0.29	16.01 ± 0.40
	W22	68.90 ± 0.15	10.21 ± 0.16	16.76 ± 0.46
	Mo17	71.65 ± 0.21	9.24 ± 0.41	15.32 ± 0.17
	W23	70.49 ± 0.12	9.43 ± 0.06	18.12 ± 1.35
	Oh43	71.81 ± 0.15	10.83 ± 0.07	17.00 ± 0.08
$POCl_3$	B73	68.83 ± 0.07	7.71 ± 0.13	18.85 ± 2.09
	Difference[f]	−0.11	−0.50	+2.84
	W22	68.86 ± 0.28	10.15 ± 0.67	16.79 ± 0.05
	Difference[f]	−0.04	−0.06	+0.03
	Mo17	70.97 ± 0.01	9.14 ± 0.12	18.35 ± 0.97
	Difference[f]	−0.68	−0.10	+3.03
	W23	71.01 ± 0.02	9.91 ± 0.08	17.57 ± 0.72
	Difference[f]	+0.52	+0.48	−0.55
	Oh43	71.33 ± 0.06	10.75 ± 0.45	17.89 ± 1.22
	Difference[f]	−0.48	−0.08	+0.89
AAMA	B73	66.44 ± 0.28	8.65 ± 0.01	15.14 ± 0.78
	Difference[f]	−2.50	+0.44	−1.27
	W22	66.53 ± 0.14	10.61 ± 0.04	15.31 ± 0.53
	Difference[f]	−2.37	+0.40	−1.45
	Mo17	68.74 ± 0.05	9.51 ± 0.01	14.55 ± 0.25
	Difference[f]	−2.91	+0.27	−0.77
	W23	68.20 ± 0.04	10.52 ± 0.04	15.11 ± 0.27
	Difference[f]	−2.29	+1.09	−3.01
	Oh43	68.85 ± 0.02	10.50 ± 0.16	14.55 ± 0.09
	Difference[f]	−2.96	−0.33	−2.45
AA	B73	62.98 ± 0.0	10.49 ± 0.07	15.95 ± 0.22
	Difference[f]	−5.96	+2.28	−0.06
	W22	63.40 ± 0.10	11.50 ± 0.93	15.88 ± 0.12
	Difference[f]	−5.50	+1.29	−0.88
	Mo17	65.26 ± 0.06	10.86 ± 0.62	15.46 ± 0.59
	Difference[f]	−6.39	+2.28	+0.14
	W23	64.54 ± 0.45	11.91 ± 2.28	14.85 ± 0.31
	Difference[f]	−5.95	+2.48	−3.27
	Oh43	65.69 ± 0.08	11.43 ± 0.37	17.38 ± 1.02
	Difference[f]	−6.12	+0.60	+0.38
OSA	B73	67.76 ± 0.02	9.75 ± 0.54	18.34 ± 1.30
	Difference[f]	−1.18	+1.54	+2.33
	W22	68.09 ± 0.13	12.41 ± 0.52	16.92 ± 0.47
	Difference[f]	−0.81	+2.20	+0.16
	Mo17	69.94 ± 0.09	10.60 ± 0.04	18.24 ± 0.08
	Difference[f]	−1.71	+1.36	+2.92
	W23	69.58 ± 0.28	11.90 ± 0.21	17.84 ± 0.69
	Difference[f]	−0.91	+2.47	−0.28
	Oh43	69.88 ± 0.01	12.54 ± 0.18	17.53 ± 0.45
	Difference[f]	−1.93	+1.71	+0.53

[a] Ordered from the lowest RADC value to the highest RADC value based on actin content (Widya et al., 2010).

[b] $POCl_3$, phosphoryl chloride; AAMA, acetic–adipic mixed anhydride; AA, acetic anhydride; OSA, octenylsuccinic anhydride.

[c] T_p = peak temperature. Values are means of triplicate measurements.

[d] Conclusion temperature–onset temperature. Values are means of triplicate measurements.

[e] Native starch of inbred line treated with protease only (control).

[f] Change from the value for the control/unmodified starch.

for native Oh43 was unchanged; the values for native and acetylated Mo17 were reduced, and after reaction with AAMA, ΔH_G was unchanged for B73 and W22 starches and reduced for Mo17 and Oh43 starches).

DSC examination of retrograded pastes (Table 5) provided little additional information. Melting enthalpies (ΔH_R) for stored pastes of the two native starches with the higher RADC values were greater than ΔH_R of the other three starches and this pattern carried over to starches that had been reacted with $POCl_3$ and AA. Also, ΔH_R values increased after crosslinking with $POCl_3$ (as compared to products of crosslinking with AAMA) or substitution with OSA (as compared to products of reaction with AA). For the starches derivatized after treatment with the protease, ΔH_R for stored pastes of the two native starches with the higher RADC were still greater than those of the other three native starches, and this pattern carried over to all chemically modified starches, except those reacted with OSA (Table 6).

It was evident in analysis of the data in Tables 1 and 2 (particularly the data in Table 1) that Mo17 starch (and/or W23 starch in some cases) appeared to be somewhat of an outlier in that it's RVA parameters did not fall in the same order as its RADC. For example, if Mo17 starch is removed from the data for the native starches, there are correlations with RADC and final viscosity and pasting temperature that were not present before and, in the case of breakdown, the two starches with the lower RADC gave less breakdown than did the two with the higher RADC. New correlations were also found for the modified starches. Considering only the differences in properties resulting from modification of the native starch (Table 1), greater changes occurred in the 2 starches with the lower RADC values in peak viscosity (AAMA, OSA), final

Table 5
Melting (DSC) data for retrograded pastes[a] and differences between those of derivatized and unmodified starches for 5 inbred lines of maize with different relative average degrees of channelization (RADC).[b]

Reagent[c]	Inbred	T_p (°C)[d]	Temp. range (°C)[e]	Enthalpy (J/g)
None[f]	B73	51.67 ± 0.10	22.33 ± 0.70	8.96 ± 0.30
	W22	52.92 ± 0.20	20.77 ± 1.23	8.54 ± 0.14
	Mo17	53.49 ± 0.33	22.73 ± 0.95	8.44 ± 0.46
	W23	52.05 ± 0.42	24.87 ± 0.93	10.01 ± 0.35
	Oh43	53.10 ± 0.25	21.77 ± 0.54	9.16 ± 0.44
$POCl_3$	B73	53.33 ± 0.35	21.69 ± 0.25	8.77 ± 0.02
	Difference[g]	+1.72	−0.64	−0.19
	W22	53.84 ± 0.09	20.29 ± 0.98	8.49 ± 0.19
	Difference[g]	+0.92	−0.48	−0.05
	Mo17	53.38 ± 0.34	23.09 ± 0.24	8.94 ± 0.50
	Difference[g]	−0.11	+0.36	+0.50
	W23	53.90 ± 0.04	22.02 ± 0.20	9.44 ± 0.93
	Difference[g]	+1.85	−2.85	−0.57
	Oh43	52.20 ± 0.76	26.59 ± 0.41	9.60 ± 1.48
	Difference[g]	−0.90	+4.82	+0.44
AAMA	B73	54.34 ± 0.26	22.07 ± 0.02	5.76 ± 0.31
	Difference[g]	+2.67	−0.25	−3.20
	W22	53.95 ± 0.57	20.81 ± 0.64	5.82 ± 0.29
	Difference[g]	+1.03	+0.04	−2.72
	Mo17	54.36 ± 0.35	23.23 ± 1.39	6.44 ± 0.21
	Difference[g]	+0.87	+0.50	−2.00
	W23	54.55 ± 0.05	21.92 ± 1.04	5.52 ± 0.63
	Difference[g]	+2.50	−2.95	−4.49
	Oh43	54.51 ± 0.07	21.61 ± 1.00	6.89 ± 0.72
	Difference[g]	+1.41	+0.16	−2.27
AA	B73	51.60 ± 0.08	22.64 ± 0.47	5.04 ± 0.76
	Difference[g]	−0.07	+0.31	−3.92
	W22	53.95 ± 0.28	19.21 ± 1.27	4.58 ± 0.70
	Difference[g]	+1.03	−1.56	−3.96
	Mo17	52.12 ± 0.01	20.39 ± 0.88	4.85 ± 0.30
	Difference[g]	−1.37	−2.34	−3.59
	W23	50.49 ± 0.74	26.58 ± 2.21	5.33 ± 0.13
	Difference[g]	−1.56	+1.71	−4.68
	Oh43	51.45 ± 0.62	20.89 ± 0.25	5.12 ± 0.01
	Difference[g]	−1.65	−0.88	−4.04
OSA	B73	52.57 ± 0.14	24.45 ± 1.07	6.81 ± 0.62
	Difference[g]	+0.90	+2.12	−2.15
	W22	53.35 ± 0.18	22.66 ± 0.47	5.60 ± 0.04
	Difference[g]	+0.43	+1.89	−2.94
	Mo17	52.39 ± 0.66	26.80 ± 0.17	6.88 ± 0.19
	Difference[g]	−1.10	+4.07	−1.56
	W23	53.41 ± 0.18	23.24 ± 0.40	6.66 ± 0.25
	Difference[g]	+1.36	−1.63	−3.35
	Oh43	53.73 ± 0.36	22.95 ± 1.05	7.07 ± 0.21
	Difference[g]	+0.63	+1.18	−2.09

[a] Starch that was gelatinized in DSC pan was held at 4 °C for 7 days.
[b] Ordered from the lowest RADC value to the highest RADC value based on actin content (Widya et al., 2010).
[c] $POCl_3$, phosphoryl chloride; AAMA, acetic–adipic mixed anhydride; AA, acetic anhydride; OSA, octenylsuccinic anhydride.
[d] T_p = peak temperature. Values are means of triplicate measurements.
[e] Conclusion temperature–onset temperature. Values are means of triplicate measurements.
[f] Native starch of inbred line treated with protease only (control).
[g] Change from the value for the control/unmodified starch.

viscosity (AA (with W22 and W23 having an equal increases), OSA), pasting temperature ($POCl_3$, AAMA, AA), and setback (OSA). This analysis might indicate that, with the exception of Mo17 starch, the fewer the number of channels, the greater the effect of modification, but we did not believe that. So, as another control, the starches were also reacted with propylene oxide (PO), a reagent which is known to take at least 25 times longer to react with starch than $POCl_3$ (with the difference probably being even greater), i.e., to be a slowly reacting reagent, and which had previously been found to react throughout granules rather than only at granule surfaces (Gray & BeMiller, 2005; Huber & BeMiller, 2001), on the supposition that its reaction with starch granules would be independent of the number of channels. However, modification with PO produced more correlations than did reactions with presumably more rapidly reacting reagents. After hydroxypropylation, a steady decrease in the difference between final viscosity values for the modified starches as compared to the same values for the native starches was found, going from the starch with the lowest RADC (B73) to the starch with the highest RADC (Oh43) (when Mo17 starch was removed from the analysis), and the differences for the two starches with the higher RADC (W23 and Oh43) were less than those for the two starches with the lower RADC (B73 and W22) for peak and final viscosities and breakdown. Finally, considering the actual RVA viscosity parameters, peak and final viscosities and setback steadily

Table 6

Melting (DSC) data for retrograded pastes[a] of starches that had been treated with a protease to remove surface protein and differences between values for derivatized and unmodified starches for 5 inbred lines of maize with different relative average degrees of channelization.[b]

Reagent[c]	Inbred	T_p (°C)[d]	Temp. range (°C)[e]	Enthalpy (J/g)
None[f]	B73	55.61 ± 0.57	21.62 ± 1.31	8.67 ± 0.05
	W22	53.82 ± 0.15	19.76 ± 1.92	8.84 ± 0.29
	Mo17	51.49 ± 0.50	25.65 ± 1.17	10.07 ± 1.00
	W23	54.69 ± 0.04	21.60 ± 1.14	10.41 ± 0.14
	Oh43	53.61 ± 0.01	20.26 ± 2.04	10.10 ± 0.56
$POCl_3$	B73	53.39 ± 0.01	23.69 ± 0.71	8.55 ± 0.14
	Difference[g]	−2.22	+2.07	−0.12
	W22	52.25 ± 0.91	24.61 ± 0.17	8.29 ± 0.64
	Difference[g]	−1.57	+4.85	−0.55
	Mo17	53.21 ± 0.11	25.60 ± 0.47	11.26 ± 0.01
	Difference[g]	+1.72	−0.05	+1.19
	W23	52.03 ± 0.74	24.01 ± 0.36	9.17 ± 0.18
	Difference[g]	−2.66	+2.41	−1.24
	Oh43	52.21 ± 0.50	25.40 ± 0.50	9.24 ± 0.10
	Difference[g]	−1.40	+5.14	−0.86
AAMA	B73	54.41 ± 0.85	20.04 ± 0.48	6.04 ± 0.22
	Difference[g]	−1.20	−1.58	−2.63
	W22	53.80 ± 0.34	20.14 ± 0.14	5.90 ± 0.11
	Difference[g]	−0.02	+0.38	−2.94
	Mo17	55.26 ± 0.64	21.14 ± 0.57	6.70 ± 0.73
	Difference[g]	+3.77	−4.51	−3.37
	W23	54.31 ± 0.10	21.32 ± 0.18	6.92 ± 0.58
	Difference	−0.14	−0.28	−3.49
	Oh43	55.25 ± 0.01	21.09 ± 0.18	7.04 ± 0.37
	Difference[g]	+1.64	+0.83	−3.06
AA	B73	52.64 ± 1.04	20.03 ± 1.56	3.42 ± 0.18
	Difference[g]	−2.97	−1.59	−5.25
	W22	54.09 ± 0.51	18.22 ± 0.52	3.59 ± 0.06
	Difference[g]	+0.27	−1.54	−5.25
	Mo17	53.93 ± 0.00	19.47 ± 0.02	3.56 ± 0.46
	Difference[g]	+2.44	−6.18	−6.51
	W23	53.62 ± 0.05	20.14 ± 1.29	4.14 ± 0.06
	Difference[g]	−1.07	−1.46	−6.27
	Oh43	53.40 ± 0.34	19.86 ± 1.49	5.22 ± 0.06
	Difference	+0.21	−0.40	−4.88
OSA	B73	53.11 ± 0.45	23.57 ± 1.48	6.64 ± 0.34
	Difference[g]	−2.50	+1.95	−2.03
	W22	54.79 ± 0.52	25.17 ± 5.42	7.33 ± 1.90
	Difference[g]	+0.97	+5.41	−1.51
	Mo17	54.09 ± 0.23	21.43 ± 0.48	7.03 ± 0.21
	Difference	+2.60	−4.22	−3.04
	W23	52.63 ± 0.08	23.11 ± 0.70	6.90 ± 0.68
	Difference[g]	−2.06	+1.51	−3.51
	Oh43	52.09 ± 1.05	25.46 ± 1.58	6.67 ± 0.25
	Difference	−1.52	+5.20	−3.43

[a] Ordered from the lowest RADC value to the highest RADC value based on actin content (Widya et al., 2010).

[b] Starch that was gelatinized in DSC pan was held at 4 °C for 7 days.

[c] $POCl_3$, phosphoryl chloride; AAMA, acetic–adipic mixed anhydride; AA, acetic anhydride; OSA, octenylsuccinic anhydride.

[d] T_p = peak temperature. Values are means of triplicate measurements.

[e] Conclusion temperature–onset temperature. Values are means of triplicate measurements.

[f] Native starch of inbred line treated with protease only (control).

[g] Change from the value for the control/unmodified starch.

decreased going from B73 starch to Oh43 starch. We believe that the only way that RADC could affect reaction with PO would be via an enhancement of reagent solution penetration, which had been shown to proceed from the inside out in maize starch granules (Huber & BeMiller, 2000), but that occurs rapidly (Huber & BeMiller, 2000) and the results of this research indicate smaller changes for the granules with the higher RADC, so we believe that our results support the conclusion that any correlation between the RADC and the effects of modification with any reagents is coincidental.

Removing the Mo17 starch from analysis of the RVA data of products made after removal of channel and exterior surface protein (Table 2) also produced new correlations. Again considering only the differences in properties resulting from modification, greater changes occurred in peak and final viscosities and setback (OSA) and pasting temperature ($POCl_3$, AAMA, AA, OSA) of the products of 2 starches with the lower RADC values.

The ultimate comparison would be in DS values (Table 7). Three modified starch products were analyzed. For the isolated starches reacted with AA, the DS value for the modified B73 starch was greater than that for the modified Oh43 starch, whether or not protein was removed, indicating a more efficient reaction when fewer channels were present, the reverse of what was hypothesized. (However, it should be remembered that any AA that reacted

Table 7
DS values for the chemically modified starches from 5 maize inbred lines with different average degrees of channelization (RADC) before and after protease treatment.

Inbred[a]	Reagent	Native starch[b]	PT starch[c]
B73	AA[d]	0.237(0.003)	0.245(0.001)
W22	AA	0.223(0.002)	0.228(0.001)
Mo17	AA	0.231(0.003)	0.241(0.014)
W23	AA	0.206(0.002)	0.257(0.003)
Oh43	AA	0.205(0.000)	0.236(0.001)
B73	OSA[e]	0.022(0.001)	0.021(0.001)
W22	OSA	0.022(0.001)	0.024(0.002)
Mo17	OSA	0.022(0.001)	0.034(0.001)
W23	OSA	0.024(0.000)	0.023(0.001)
Oh43	OSA	0.025(0.001)	0.023(0.001)
B73	$POCl_3$[f]	21.5(0.5)	11.5(0.5)
Oh43	$POCl_3$[f]	11.5(0.5)	6.0

[a] Ordered from the lowest RADC value to the highest RADC value based on actin content (Widya et al., 2010).

[b] Isolated starch without protease (thermolysin) treatment (values are means of triplicate analyses).

[c] Protease (thermolysin)-treated starch (values are means of triplicate analyses).

[d] Acetic anhydride.

[e] Octenylsuccinic anhydride.

[f] Results (in μg P/g starch) are means of duplicate analyses.

with $-NH_2$ or $=NH$ groups of surface protein molecules would not have been detected, which is consistent with our results in that, when protease-treated starches were used, in every case, the DS values for the acetylated PT starches were greater than those of the acetylated native starches, which is consistent with some reagent reacting with protein.) For the starches reacted with OSA, the DS value for the modified Oh43 starch was greater than that for the modified B73 starch, but the difference was small and deproteinization had little effect on the pattern. Neither did deproteinization increase DS values. These results are consistent with a different process of reaction with OSA as compared to the other three reagents (to be presented in another paper). For the starches crosslinked with $POCl_3$, B73 starch had almost twice as much incorporated phosphorus as did Oh43 starch for both the modified native starch and the protease-treated starch. Again, this is the reverse of what was expected, especially so since, in this case, even the phosphorus incorporated into protein molecules should have been measured, so the fact that the PT starches had less incorporation of phosphorus as compared to the native starches might indicate a more efficient reaction when more reactive protein molecules are present.

4. Conclusions

There are differences in the granular structures of starches isolated from the different inbred lines of maize (that are undoubtedly related to differences in structures of the component polymer molecules) that determine differences in their pasting and paste properties and changes in them following reaction with both presumed to be rapidly reacting reagents ($POCl_3$, AAAMA, AA, OSA) and a slowly reacting reagent (PO). However, data indicate that RADC is not the most important factor in either case. Most, but not all, results also indicate that, when surface protein was present, some of the reagent reacted with it, leaving less to react with the starch polymers.

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