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Fine structure of maize starches from four *wx*-containing genotypes of the W64A inbred line in relation to gelatinization and retrogradation

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Maize starches from four *wx*-containing genotypes (*wx*, *du wx*, *ae wx*, and *ae du wx*) of the W64A inbred line were examined to determine their gelatinization and retrogradation properties and the fine structure of their amylopectins (AP). Chain-length distribution profiles of the APs showed distinct patterns according to genotype. The *wx* and *du wx* starches had a large proportion of short chains and showed A-type X-ray patterns, whereas the *ae wx* and *ae du wx* starches had a large proportion of long chains and showed B-type X-ray patterns. The *ae wx* starch had the highest values of onset melting temperature (T_o) and heat uptake (ΔH), both for gelatinization and after retrogradation, which were attributed to a greater proportion of long chains (degree of polymerization > 16) in the AP. Debranching of β-limit dextrins also revealed that the interior chain-length distribution of AP from the *ae wx* genotype constituted a large proportion of long chains. The extent of retrogradation increased in the order *wx*, *du wx*, *ae wx* and *ae du wx* starches and appeared to be proportional to the level of unit chains with DP 16–30 and inversely proportional to the level of short chains with DP 6–11.

INTRODUCTION

Starch is used widely in foods as a thickening, texturizing, binding, or stabilizing agent. In the United States, maize is the most prominent commercial source of starch. The yield of normal maize crops is high, and many varieties of maize are available. Waxy maize (from the *wx* genotype) is cultivated to obtain its special starch, which contains essentially 100% amylopectin (AP).

Other *wx*-containing genotypes (*ae wx*, *du wx* and *ae du wx*) contain AP of altered fine structure, which affects the gelatinization, swelling, and retrogradation properties of their starches. Starches from those double- or triple-mutant genotypes have the potential to be used in foods without chemical modification or to serve as raw materials to make modified starches with unique properties (De Boer, 1991).

A number of investigators (Baba & Arai, 1984; Boyer & Liu, 1985; Fuwa *et al.*, 1987; Inouchi *et al.*, 1987, 1991*a*, *b*; Sanders *et al.*, 1990; Takeda *et al.*, 1993; Yuan *et al.*, 1993; Wang *et al.*, 1992, 1993*a*, *b*) have examined the gelatinization and retrogradation of maize starches by differential scanning calorimetry (DSC) and the chain-length distribution of APs by high-performance size-exclusion chromatography (HPSEC). In recent years, high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), has been used to separate individual unit chains of linear dextrins up to degree of polymerization (DP) 70 (Koizumi *et al.*, 1989). Using HPAEC-PAD, Koizumi *et al.* (1991) demonstrated differences in chain-length distributions among APs from seven starches. In a previous study (Shi & Seib, 1992), we examined four waxy cereal starches by HPAEC-PAD and found that the extent of retrogradation of the four starches was directly proportional to the mole fraction of unit chains with DP 14–24 and inversely proportional to the mole fraction of unit chains with DP 6–9.

The aims of this study were to characterize maize starches from four *wx*-containing genotypes (*wx*, *du wx*, *ae wx*, and *ae du wx*) by HPAEC-PAD and to relate, if possible, the fine structure of the starches to their gelatinization and retrogradation properties.

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MATERIALS AND METHODS

Materials

Maize samples from four genotypes (*wx*, *du wx*, *ae wx*, and *ae du wx*) were provided by Dr C. D. Boyer at Pennsylvania State University (University Park, PA). The starches were isolated after soaking the kernels in 20 mM sodium acetate buffer at pH 6·5 containing 10 mM mercuric chloride (Boyer & Liu, 1985). The starch granules were purified by extractions with saline and toluene. After air-drying, the moisture levels in the starches were 10·0–11·7%.

Amylopectin (AP) was prepared by dissolving starch granules in aqueous 90% methyl sulfoxide followed by precipitation of AP with ethanol (Ring *et al.*, 1987). The precipitated material was collected by centrifugation, washed three times with ethanol and once with acetone, and vacuum-dried overnight in a desiccator containing $CaCl_2$.

Pullulanase (EC 3.2.1.41) from *Enterobacter aerogenes*, and isoamylase (EC 3.2.1.68) from *Pseudomonas amyloderamosa* were obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Crystalline sweet potato β-amylase (EC 3.2.1.2), type 1-B, was obtained from Sigma Chemical Co. (St Louis, MO).

Differential scanning calorimeter (DSC)

Gelatinization of the starches was determined (Shi & Seib, 1992) at starch-to-water ratios of 1:1, 1:2 and 1:3 (w/w). After a mixture of starch and water had been heated in a DSC pan, the starch gel was stored at 4 C for 1 week, then at room temperature (23 ± 1 C) for 4 weeks. The sample pan was rescanned in the DSC, and the extent of retrogradation of the starch gel was estimated from the enthalpy of the endotherm.

X-ray diffraction

X-ray diffraction patterns of the maize starches were recorded on a Philips X-ray diffractometer, with Cu-K_α radiation at 35 kV and 20 mA, a theta compensating slit, and a diffracted beam monochromator. Relative crystallinity was estimated from the ratio of the area of peaks to the total area of a diffractogram (Komiya & Nara, 1986).

In addition to the X-ray diffractograms recorded on the starches with ~10% moisture, the starches from the *ae wx* and *ae du wx* also were recorded at ~20% moisture. To prepare the samples, *ae wx* and *ae du wx* starches (3 g each) were added to water (15 ml) at 25°C, the mixtures centrifuged, and the supernatants discarded. The sedimented starches were air-dried at room temperature for 4 h and contained approximately 20% moisture.

Fine structure of amylopectin (AP)

AP was debranched by isoamylase, and the unit chain-length distribution was determined by HPAEC-PAD (Shi & Seib, 1992). β-Limit dextrin from AP was prepared and debranched as described by Yuan *et al.* (1993), and the unit chain-length distribution of debranched β-limit dextrin was determined by HPAEC-PAD.

RESULTS AND DISCUSSION

X-ray diffractograms

Figure 1 shows X-ray diffractograms of the four waxy starches with ~10% moisture. The starches from the *wx* and *du wx* genotypes had three strong reflections at 2θ values of 15, 17 and 23°, which are typical of the A-polymorphic form (Zobel, 1988). In comparison, the *ae wx* or *ae du wx* starch gave a peak of reduced intensity at 15° and a broad peak between 20 and 26°. In addition, peak 2 occurred at a slightly lower angle of refraction. Those characteristics indicated that the *ae wx* and *ae du wx* starches were of the B-polymorphic form (Zobel, 1993, personal communication).

Diffractograms of the *ae wx* and *ae du wx* starches containing increased moisture verified that the two starches were indeed of the B-type. Figure 2 shows that two peaks were resolved at 2θ of 24° and 26° when the

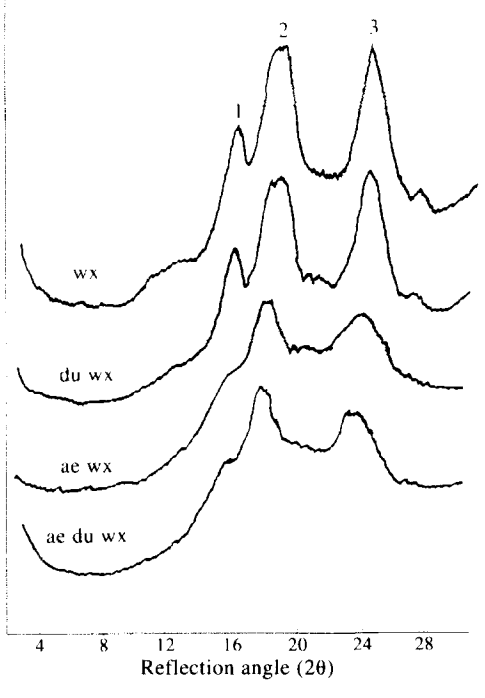

Fig. 1. X-ray diffractograms of *wx*, *du wx*, *ae wx* and *ae du wx* maize starches with ~10% moisture. Relative crystallinities were 50, 51, 35, and 33%, respectively.

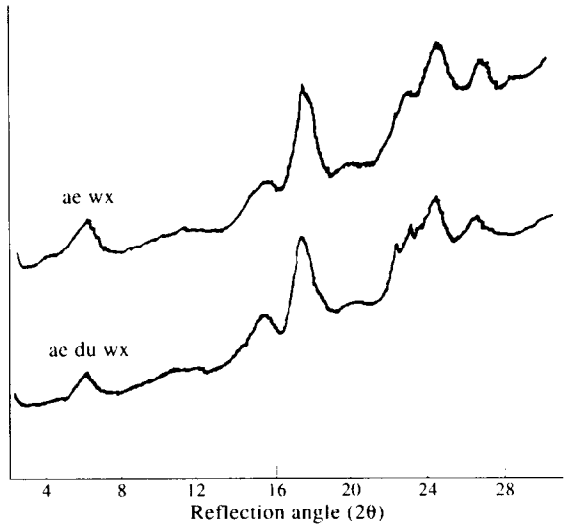

Fig. 2. X-ray diffractograms of *ae wx* and *ae du wx* maize starches with ~20% moisture.

ae wx and *ae du wx* starches contained ~20% moisture. Moreover, an additional peak was observed at a low angle of 2θ 5·6°, which is characteristic of B-type starch (Zobel, 1988). Previously, Brown *et al.* (1971) and Yuan *et al.* (1993) reported that *ae wx* starch gave a B-type X-ray pattern, but the X-ray data were not given.

Of the four starches examined, the two B-type starches were noted to have lower crystallinities as determined from the X-ray diffractograms (Fig. 1). Low X-ray crystallinity is not necessarily synonymous with poorly ordered starch molecules in the granules, but could be due to small sized crystallites (Colonna *et al.*, 1982; Colonna & Mercier, 1985). In addition to low swelling power in water, high-amylose maize starches gave high yields of acid-resistant residues when lintnerized in 2·2 N hydrochloric acid at 35°C, even though those starches showed low X-ray crystallinities (Colonna *et al.*, 1982; Colonna & Mercier, 1985).

Gelatinization and retrogradation properties

Gelatinization properties of the starches from *wx*, *du wx*, *ae wx* and *ae du wx* genotypes are summarized in Table 1. At starch to water ratios of 1:3 and 1:2, the endothermic peak for *ae wx* starch was the broadest, followed by that for *ae du wx* starch. The onset gelatinization temperatures (T_o) of the four native starches in 75% (w/w) water ranged from 51·3°C (*du wx*) to 61·8°C (*ae wx*). Those T_o values were lower than the T_os reported by Yuan *et al.* (1993) for the same inbred line, but they were in the same order among the starches. The gelatinization enthalpies (ΔH) ranged from 3·61 cal/g for the *ae du wx* starch to 4·40 cal/g for the *ae wx* starch. The ΔH values for *wx*, *du wx*, and *ae wx* starches are consistent with those reported by Yuan *et al.* (1993) from the same inbred line. Among the four genotypes, the *ae wx* had the highest gelatinization temperature as well as the highest ΔH.

Table 1. Gelatinization properties of four *wx*-containing maize starches from W64A inbred line

S:W[a]	Starch	T_o (°C)[b]	T_c (°C)[b]	T_c–T_o (°C)	ΔH (cal/g)[c]
1:3	*wx*	54·2	75·1	20·9	3·68
	du wx	51·3	75·7	24·4	3·72
	ae wx	61·8	95·4	33·6	4·40
	ae du wx	52·6	81·1	28·5	3·61
1:2	*wx*	54·8	80·0	25·2	3·71
	du wx	51·9	80·3	28·4	3·76
	ae wx	61·7	97·6	35·9	4·35
	ae du wx	51·6	82·0	30·4	3·51
1:1	*wx*	54·0	96·8	42·8	3·57
	du wx	52·2	96·2	44·1	3·59
	ae wx	61·9	105·1	43·2	4·22
	ae du wx	53·3	103·8	50·5	3·55

[a] Starch:water ratio (w/w).
[b] Standard deviation ± 0·4°C.
[c] Standard deviation ± 0·08 cal/g.

After gelatinization, the starch gels were stored at 4°C for 1 week and then at 23°C for 4 weeks. The retrograded gels of all four starches gave similar T_o values (Table 2), in contrast to the differences in gelatinization temperatures between the native starches (Table 1). At each starch concentration, the retrograded gels of the *ae wx* and *ae du wx* starches displayed broader endothermic peaks than those of the *wx* and *du wx* starches (Table 2).

For each genotype, T_o of a retrograded gel was highest at a starch:water ratio of 1:3. This agrees with the findings of Ward (1991), who reported that T_o was highest at 25% solids level when retrogradation of maize and wheat AP was examined between 25 and 45%. At low starch concentration, or high water

Table 2. DSC melting properties of crystallites formed in starch gels[a]

S:W[b]	Starch	T_o (°C)[c]	T_c (°C)[c]	T_c–T_o (°C)	ΔH (cal/g)[d]
1:3	*wx*	52·5	63·3	10·8	1·16
	du wx	51·5	61·1	10·6	2·48
	ae wx	53·5	84·8	31·3	3·86
	ae du wx	52·4	74·0	21·6	3·37
1:2	*wx*	50·4	65·2	14·8	2·31
	du wx	49·6	65·6	16·0	3·04
	ae wx	52·6	92·0	39·4	4·30
	ae du wx	49·7	72·0	22·3	3·53
1:1	*wx*	50·2	70·9	20·7	3·17
	du wx	48·8	70·0	21·2	3·44
	ae wx	50·7	93·1	42·4	4·48
	ae du wx	49·5	91·0	41·5	3·57

[a] Starch gel in DSC pan was stored at 4°C for 1 week and at 23°C for 4 weeks.
[b] Starch:water ratio (w/w).
[c] Standard deviation ±0·4°C.
[d] Standard deviation ±0·07 cal/g.

content, the starch molecules have more mobility and probably recrystallize with increased perfection.

In this study, T_0 values of the retrograded starch gels were approximately 10 C higher than those reported by Yuan *et al.* (1993) for gels from the same inbred line. These differences in T_0 values probably were due to different storage conditions for starch gels. In the work reported here, starch gels were stored at 4 C for 1 week followed by 23 C for 4 weeks. In the study by Yuan *et al.* (1993), starch gels were stored only at 4 C for 7 days, without the subsequent storage at 23 C. Nakazawa *et al.* (1985) and Zeleznak & Hoseney (1987) showed that T_0 of a retrograded starch increased with increasing storage temperature, presumably because the crystallites were annealed to increased perfection at higher storage temperatures.

The ΔH values for the retrograded gels in this investigation ranged from 1·2 to 4·5 cal/g (Table 2). At a given concentration, ΔH always increased for gels in the following order: *wx* < *du wx* < *ae du wx* < *ae wx*. Furthermore, those ΔH values closely represented the maximum recrystallization of the starch gels, because we stored the gels for an extended period of time first at a cold temperature (4 C, 1 week) for nucleation and then at a warm temperature (23 C, 4 weeks) for propagation (Zeleznak & Hoseney, 1987; Slade & Levine, 1991). We found that a gel of *wx* maize starch at 25% solids gave no retrogradation endotherm, if it was stored at 4 C for 1 day (instead of 1 week) followed by storage at 23 C for 4 weeks. The same was true for waxy rice starch at 25% solids (Shi & Seib, unpublished data). These results are different from the findings on gels from wheat and normal corn starches, which showed significant retrogradation under the same storage conditions (Ward, 1991). Apparently, the rate of nucleation at 4 C is slow for waxy starches compared with those starches that contain amylose.

For starches from the *wx* and *du wx* genotypes, the extent of retrogradation (ΔH) of their gels increased as the starch: water ratio changed from 1:3 to 1:1 (Table 2). However, the concentration of starch had much less effect on ΔH for the *ae wx* and *ae du wx* starches. In evaluating gels with starch concentrations of 10 and 30%, Yuan *et al.* (1993) reported similar findings for the *wx*, *du wx* and *ae wx* starches. In another study on wheat and maize AP retrogradation (Ward, 1991), starch solids concentration (25–45%) had a more significant effect on what AP, which retrograded to a lesser extent than maize AP. In all those studies, starch concentration seemed to have a noticeable effect on the retrogradation of a starch with low retrogradation tendencies.

Fine structure of amylopectin

Yuan *et al.* (1993) recently reported the size-exclusion high-performance liquid chromatograms of the debranched starches from *wx*, *du wx* and *ae wx* genotypes of the W64A inbred line. In the present investigation, those three starches plus *ae du wx* starch were debranched and separated by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Representative HPAEC-PAD chromatograms for the four starches are shown in Fig. 3. Those chromatograms appeared to be distinct for each genotype.

Because there is uncertainty about the molar response of the PAD detector with unit chains above DP 17 (Koizumi *et al.*, 1991, Shi & Seib, 1992), in this investigation, we plotted the area percent of each peak in a chromatogram vs the corresponding DP. Figure 4 shows the chain-length distributions compared to the *wx* sample. The starch from the *du wx* genotype was characterized by a high proportion of chains between DP 11 and 16. The *ae wx* genotype had a reduced number of short chains (DP 6–18) and an increased number of long chains (DP > 18). The *ae du wx* starch was unique in having a bimodal distribution of chains with peaks at DP 15 and 20 (shoulder).

Chromatography of the debranched β-limit dextrins produced maltose, maltotriose, and the interior chains of the respective APs as determined by HPAEC-PAD. Maltose and maltotriose were the stubs of A chains after β-amylase action, and they were not considered as part of the interior chains of the APs. To compare interior unit chain-length distributions of the APs, the percent areas of chains longer than DP 3 were calculated and plotted vs their DPs (Fig. 5). The *du wx* and *ae du wx* starches contained an increased number of short chains compared to the *wx* starch, whereas the *ae wx* starch contained an increased number of chains longer than DP 25.

Relation between fine structure and properties of the four structures

Hizukuri (1985) studied the relationship between the chain-length profiles of AP and the crystalline structure of starch granules. He found that the APs from A-type starches had a shorter average chain length and a larger proportion of short chains than the APs from B-type starches. In agreement with his findings, our work showed that the starches from *wx* and *du wx* had large proportions of short chains and gave A-type X-ray patterns, whereas the *ae wx* and *ae du wx* starches had large proportions of long chains and gave B-type X-ray patterns.

According to Hizukuri's revised cluster model of AP (Hizukuri, 1986), unit chains with DP 6–30 comprise A and B1 chains and give rise to single clusters. The sum of the A and B1 chains constitutes about 80–90% of the total chains. The remaining 10–20% of chains are involved mainly in intercluster connections. Double helices would be formed mainly from A and B1 chains in AP.

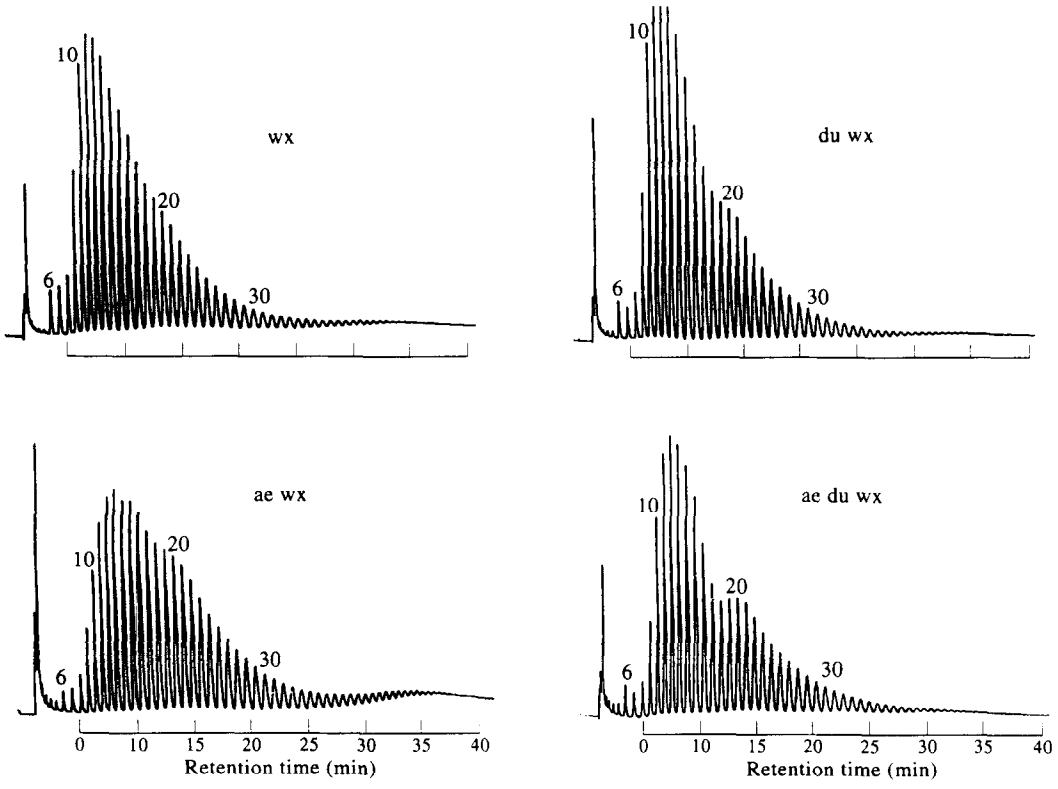

Fig. 3. Chromatograms of debranched *wx*, *du wx*, *ae wx* and *ae du wx* maize starches determined by HPAEC-PAD. Numbers above peaks are chain lengths. Peaks of DP 11–13 were off-scale in the chromatogram of *du wx*.

Among the four genotypes, the *ae wx* starch had the highest proportion of chains longer than DP 16 (Table 3), which probably explains why it gave the highest gelatinization temperature (Table 1). Yuan *et al.* (1993) suggested that the long chains in *ae wx* starch could form long double helices requiring high temperatures for dissociation. Because the *ae wx* starch showed low X-ray crystallinity (Figs 1 and 2), the double helices may not have been entirely in the crystalline registry. Indeed, the double helix content of a starch estimated by NMR spectroscopy is considerably higher than the percent crystallinity obtained from X-ray diffraction (Gidley & Bociek, 1985; Zobel, 1992). Accordingly, crystalline order would account for only part of the granular order (Zobel, 1992).

Recently, Cooke and Gidley (1992) suggested that the enthalpy of gelatinization (ΔH) primarily reflected the loss of double-helical order rather than loss of X-ray crystallinity. Their analysis may explain a low X-ray crystallinity but high ΔH such as shown by starch from the *ae wx* genotype. In this case, the high proportion of long chains in the *ae wx* starch could result in double helices varying in size and perfection. The result could then be a large but broad endothermic peak observed upon heating in the DSC. Previously, Sanders *et al.* (1990) and Yuan *et al.* (1993) attributed the broad endotherm of *ae wx* starch to crystallites varying in size and perfection.

In addition to overall chain length distribution, the interior chains of APs (Fig. 5) were affected by different genotypes. This finding suggested that amorphous regions as well as crystalline regions were altered by the mutations. For example, the *ae wx* starch contained a greater proportion of long interior chains (Fig. 5) as well as longer average chains in its AP when compared with *wx* starch, in agreement with the results of Yuan *et al.* (1993). One effect of altered amorphous regions in the *ae wx* starch could be that these regions would have low mobility due to the association between long chains. In turn, the amorphous regions would require a high temperature to incur swelling that could contribute to the disruption of the crystalline regions, i.e. co-operative melting. The effect of surrounding amorphous regions on the melting of starch crystallites was proposed and demonstrated by Donovan (1979) and Donovan and Mapes (1980). Since then, a number of researchers (Biliaderis *et al.*, 1981; Muhr *et al.*, 1984; Shi & Seib, 1992; Morrison *et al.*, 1993) have reported that the melting temperature of the crystallites in a granular starch is greatly affected by the surrounding amorphous regions.

Another explanation for an *ae* mutant genotype having low X-ray crystallinity but being resistant to swelling or melting is to consider the glass transition of the amorphous regions and its relationship to melting (Slade & Levine, 1988). For example, AP in another *ae*

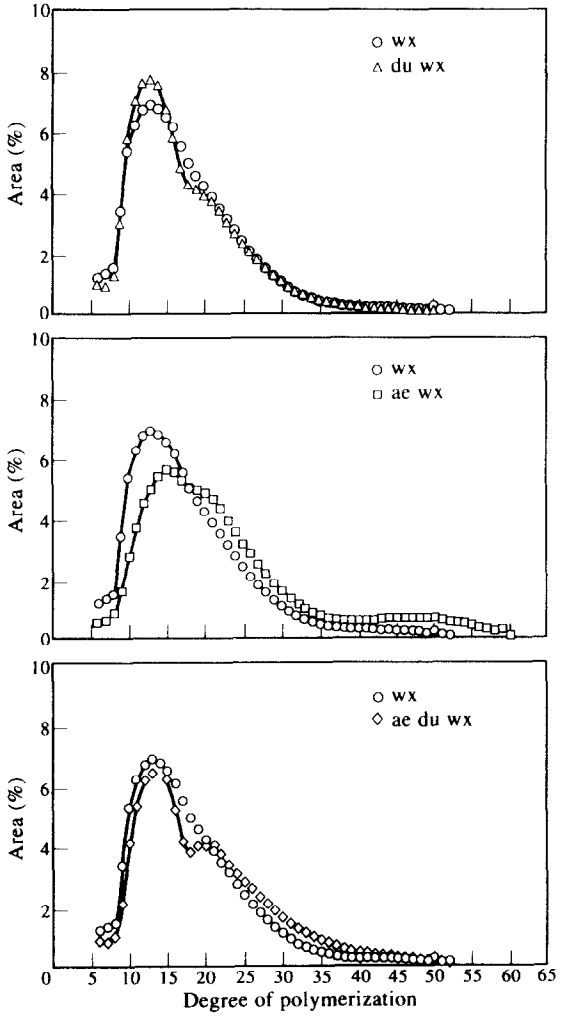

Fig. 4. Chain-length distributions of *du wx*, *ae wx* and *ae du wx* maize starches, each compared with *wx* maize starch.

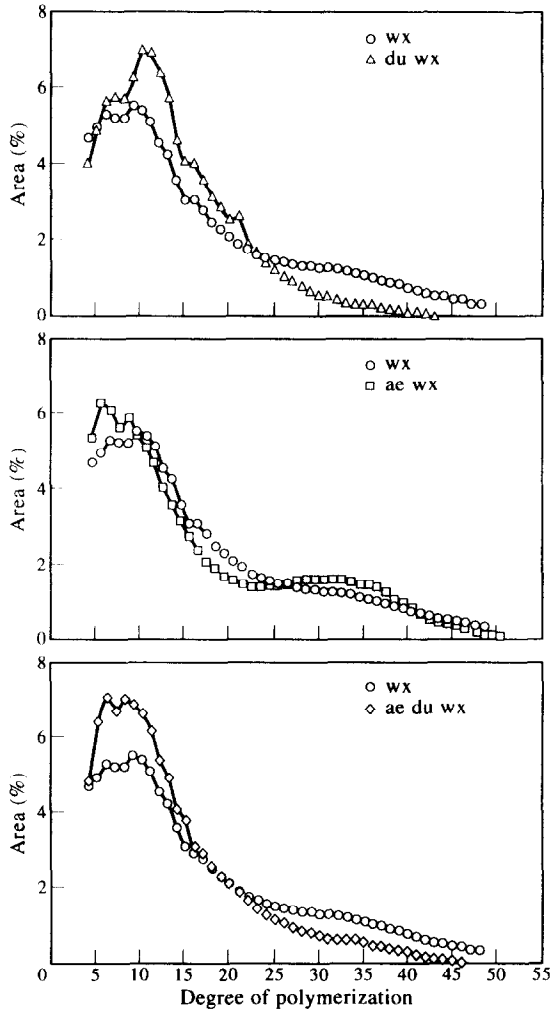

Fig. 5. Interior chain-length distributions from β-limit dextrin of *du wx*, *ae wx* and *ae du wx* maize starches, each compared with *wx* maize starch.

mutation, high amylose maize starch mutation (amylomaize) was reported as having an average interior chain length that was 9 glucose units longer than that in waxy maize AP (Baba & Arai, 1984). More recently, Takeda *et al.* (1993) showed that the APs of high amylose maize starches compared with normal maize contained a large proportion of long chains and a small proportion of short chains. Thus, an elevated glass transition was proposed due to long, unclustered, non-crystalline branches in the AP (Slade & Levine, 1988). Presumably, the glass transition occurs before the melting of the crystallites in granules and the chain mobility required for swelling and melting would be impeded accordingly.

Previously, we observed that the retrogradation of four waxy cereal starches appeared to be directly proportional to the mole fraction of unit chains with DP 14–24 and inversely proportional to the mole fraction of unit chains with DP 6–9 (Shi & Seib, 1992). In this study, the differences in retrogradation of the starches from *wx*, *du wx*, *ae wx*, and *ae du wx* genotypes (Table 2) could be explained in a similar manner by

Table 3. Areas (% of total) of groups of unit chains in maize starches from four genotypes

Starch	Degree of polymerization				
	6–11	12–16	17–20	21–30	30–60
wx	19·3	33·2	19·4	23·5	4·8
du wx	19·0	35·4	17·1	22·7	5·8
ae wx	10·4	26·0	20·1	30·1	13·1
ae du wx	14·7	31·0	16·2	27·4	10·7

their chain-length distributions (Fig. 4 and Table 3). The four starches retrograded in increasing order of *wx*, *du wx*, *ae du wx*, and *ae wx* as indicated by ΔH values (Table 2).

The starch from the *ae wx* genotype contained the highest proportion of unit chains with DP 17–30 and the lowest level of short chains with DP 6–11 (Table 3), which resulted in the highest extent of retrogradation among the four starches. The starch from *ae du wx*,

when compared to those from *wx* and *du wx*, had a lower number of chains between DP 17–20 but a much greater number of chains between DP 21–30. In addition, the *ae du wx* starch had fewer short chains (DP 6–11) than the *wx* and *du wx* starches. As a result, the *ae du wx* starch retrograded to a greater extent than did the *wx* and *du wx* starches.

When compared to the *wx* starch, the *du wx* starch had a reduced percentage of short chains particularly with DP 6–9 (Fig. 4), a higher number of unit chains with DP 12–16, and a lower number of unit chains with DP 17–30 (Table 3). The net effect was that the *du wx* starch retrograded to a greater extent than did the *wx* starch, particularly at 25% starch solids (Table 2).

CONCLUSIONS

Maize starches from the *wx*, *du wx*, *ae wx*, and *ae du wx* genotypes had distinct chain length distributions between DP 6–60. Amorphous as well as crystalline regions of the starches were altered in those genotypes. Certain groups of unit chains were related to each starches' polymorphic crystalline form, gelatinization temperature, and extent of retrogradation.

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