

Structure of Amylopectins from *ae*-Containing Maize Starches

Jeffrey D. Klucinec¹ and Donald B. Thompson^{1,2}

Cereal Chem. 79(1):19–23

Recently, Klucinec and Thompson (1998) fractionated starch from commercial normal maize endosperm and from commercial *ae*-containing endosperm into amylose (AM), amylopectin (AP), and intermediate material (IM) based on their differential abilities to precipitate with a mixture of isoamyl alcohol and 1-butanol. Amylopectin (AP) was defined as the material that did not precipitate in either case. Chain length profiles were obtained for the AP and IM fractions from *ae*-containing starch, and the profiles of these fractions were used to provide insight into the basis of the physical behavior of unfractionated starch materials. Subsequent research (Klucinec and Thompson 2002) suggested that the nature of the branching pattern might be more important than the chain length profile for an understanding of the behavior of gels of mixtures of nongranular AP and AM.

Determination of the fine structure of AP has been a goal of the starch chemist ever since it was first understood that normal starches have two main components, amylose and amylopectin. The distribution of linear chain lengths has been determined by chromatography of enzymatically debranched AP. While this approach provides useful information about chain lengths, far more detail about the fine structure can be obtained by producing and analyzing the β -limit dextrin of these molecules (Manners 1989). From the extent of hydrolysis by β -amylase, exterior chain lengths (ECL) and interior chain lengths (ICL) may be calculated (Manners 1989). The distribution of the residual chains in the debranched β -limit dextrin allows calculation of the A:B chain ratio (Fuwa et al 1987; Yuan et al 1993). The distribution of the shortest of the residual B chains also gives insight into the relationship among branch points, as these short residual B chains represent the population of the shortest interior chain lengths, which correspond to the frequency of closely associated branch points (Thompson 2000). This frequency is quite different for AP from *wx*, *ae wx*, and *du wx* starches (Yuan et al 1993).

Quantification of A chains by chromatography of debranched amylopectins of *wx*-type starches is problematic because the population of A chains and the population of short B chains overlap considerably in size-exclusion chromatograms (Akai et al 1971; Hizukuri 1985, 1986; Yuan et al 1993; Klucinec and Thompson 1998). However, for debranched β -limit dextrans, the residual A chains and residual B chains may be nearly completely separated (Baba and Arai 1984; Fuwa et al 1987; Inouchi et al 1987; Yuan et al 1993), allowing calculation of the A:B chain ratio. Baba and Arai (1984), Fuwa et al (1987), and Yun and Matheson (1993) have examined the isolated amylopectin from *ae* starches in a non-*wx* background by analyzing debranched β -limit dextrans. These three groups independently investigated the structure of amylopectin from three different *ae* starches, but none of these three studies compared the AP structures of different *ae*-containing starches. Moreover, analysis of the debranched β -limit dextrin of AP from the double mutant *ae du* has not been reported.

The purpose of the present study was to investigate the β -limit dextrans of AP fractions from several *ae*-containing starches (Klucinec

and Thompson 1998, 2002) to explore differences in the branching pattern among these AP.

MATERIALS AND METHODS

Native Starches

Two commercial starches from *ae* maize (Hylon V [*aeV*] and Hylon VII [*aeVII*], nominal amylose content of 50 and 70%, respectively, according to the manufacturer) were gifts from National Starch and Chemical Company (Bridgewater, NJ). A starch from *ae wx* maize was a gift from Cerestar USA, Inc. (Hammond, IN). A commercial starch from *ae du* maize was a gift from Cerestar USA, Inc. (Hammond, IN). Commercial starches from normal maize (*n*) (common corn starch, Melojel) and *wx* maize (waxy maize, Amioca) were gifts from National Starch and Chemical Co.

Nongranular starches were prepared from starting materials as described previously (Klucinec and Thompson 1998). The AP fractions from the *n*, *aeV*, *aeVII*, and *ae du* commercial starches were separated from IM and AM by differential alcohol precipitation, also as described previously.

Preparation of Debranched Starches with Intact External Chains

Nongranular starches with intact external chains were debranched using the method of Klucinec and Thompson (1998). The average chain lengths (CL) were determined by calculating the ratio of the total carbohydrate concentration (Dubois et al 1956) to the concentration of reducing ends (Hizukuri et al 1981; Jane and Chen 1992).

Preparation of Debranched β -Limit Dextrans

The β -limit dextrans were prepared using the method described by Yuan et al (1993) with modifications. Nongranular starches and starch fractions (12 mg) were dispersed in 120 μ L of 90% dimethyl sulfoxide (DMSO) by heating in a boiling water bath for 10 min. Warm sodium acetate buffer (880 μ L, 50°C; 0.02*N*, pH 6.0) was mixed with the DMSO-dispersed starches. The mixtures were then heated for 10 min in a boiling water bath and cooled to 50°C in a shaking water bath. A 50- μ L aliquot of a barley β -amylase (Megazyme International Ireland, Ltd., County Wicklow, Ireland) solution (250 U/mL; 0.02*N* sodium acetate, pH 6.0) was added, and the samples were held for 24 hr at 50°C with constant agitation. The samples were then heated in a boiling water bath for 10 min and returned to the 50°C shaking water bath, at which time an additional 50 μ L of the barley β -amylase solution was added. The samples were held an additional 24 hr at 50°C before they were removed from the shaking water bath, heated in a boiling water bath for 10 min, and cooled to room temperature (\approx 22°C). A 0.2-mL aliquot was reserved for β -amylolysis limit calculations based on the total carbohydrate (Dubois et al 1956) and the reducing capacity (Robyt and Whelan 1968) of the solutions. The β -amylolysis was considered complete because preliminary work with additional β -amylase did not increase the reducing capacity of the digest.

A 0.5-mL aliquot of each digestion mixture was mixed with 1.5 mL of ethanol, held at 4°C for 30 min, and then centrifuged at 250 \times g at room temperature for 10 min in a microcentrifuge. The precipitated β -limit dextrans were washed at room temperature twice with 0.5 mL of ethanol and once with 0.5 mL of acetone. Samples were cen-

¹ Department of Food Science, Penn State University, University Park, PA 16802.

² Corresponding author. Phone: 814-863-2950. Fax: 814-863-6132. E-mail: dbt1@psu.edu

trifuged at $1,000 \times g$ at room temperature for 10 min after each wash of ethanol or acetone. The recovered β -limit dextrans were dried in a forced-air oven at 50°C for 30 min and allowed to cool to room temperature.

The β -limit dextrans (≈ 3 mg) were mixed with 50 μL of 90% DMSO and heated in a boiling water bath for 10 min. While the dispersed β -limit dextrans were still hot, sodium acetate buffer (350 μL , 50°C ; 0.02N, pH 4.75) was added. The mixtures were then heated in a boiling water bath for 10 min and cooled to 37°C in a shaking water bath. To the cooled mixtures, 40 μL of an iso-amylase solution (Megazyme, 0.5 U/mL, 0.02N sodium acetate, pH 4.75) was added. The samples were held for 24 hr at 37°C with constant agitation, placed in a boiling water bath for 10 min, and then returned to the 37°C shaking water bath. To these solutions of partially debranched β -limit dextrin, 40 μL of a pullulanase solution (Megazyme, 0.43 U/mL, 0.02N sodium acetate, pH 4.75) was added. The samples were then held for an additional 24 hr at 37°C , boiled for 10 min, allowed to cool to room temperature, and then centrifuged for 10 min at $5,000 \times g$. A 100- μL aliquot of each completely

debranched β -limit dextrin solution was added to 900 μL of DMSO and reserved for analysis by high-performance size-exclusion chromatography (HPSEC). A 0.2-mL aliquot of each mixture was reserved for β -amylolysis limit calculations based on the total carbohydrate (Dubois et al 1956) and the reducing capacity (Robyt and Whelan 1968) of the solutions. Debranching was considered complete because preliminary HPSEC of digests with additional aliquots of pullulanase did increase either the proportion of the chromatogram area attributable to chains with DP 2 relative to those with DP 3, or the proportion of the chromatogram area attributable to chains with DP 2+3.

Chromatography of Debranched β -Limit Dextrans

Samples of debranched β -limit dextrin in 90% DMSO were prepared for chromatography by heating in a boiling water bath for 10 min, cooled to room temperature, and then centrifuged at $5,000 \times g$ at room temperature in a microcentrifuge. The HPSEC system and the conditions of the separation were the same as used previously (Klucinec and Thompson 1998). In addition to the standards for

TABLE I
Chain Length Distributions of Debranched β -Limit Dextrans

Sample ^a	Chromatographic Region ^b						
	B _L Chains			B _S Chains		A Chains	
	I	II	III	IV	V	VI	VII
<i>wx</i>							
wt%	1.9	12.3	30.9	30.6	7.9	9.7	6.7
	(2.38, 1.35)	(12.85, 11.82)	(31.54, 30.29)	(29.62, 31.62)	(7.74, 8.06)	(9.42, 9.90)	(6.45, 6.96)
mol%	0.1	1.1	9.6	24.4	11.7	25.3	27.8
<i>ae wx</i>							
wt%	2.1	28.7	35.4	16.8	4.4	7.2	5.6
	(2.03, 2.09)	(29.37, 27.94)	(35.92, 34.83)	(16.00, 17.58)	(4.19, 4.51)	(7.02, 7.29)	(5.46, 5.75)
mol%	0.1	4.4	14.1	17.6	8.5	24.9	30.6
<i>n AP</i>							
wt%	3.2	11.3	30.5	30.0	8.5	9.5	7.0
	(3.75, 2.80)	(11.62, 10.88)	(30.80, 30.19)	(28.86, 31.20)	(8.67, 8.29)	(9.51, 9.47)	(6.79, 7.18)
mol%	0.1	1.0	9.2	23.6	12.3	24.9	28.8
<i>ae du AP</i>							
wt%	7.2	19.4	30.4	23.9	5.0	8.1	6.0
	(7.72, 6.75)	(19.02, 19.78)	(30.60, 30.27)	(23.37, 24.40)	(5.18, 4.90)	(8.11, 8.14)	(6.10, 5.81)
mol%	0.8	4.0	12.4	19.0	7.6	27.3	29.0
<i>aeV AP</i>							
wt%	9.7	24.5	34.2	14.7	3.4	6.7	4.7
	(9.74, 9.62)	(24.75, 24.27)	(33.69, 34.71)	(14.82, 14.54)	(3.44, 3.39)	(6.81, 6.54)	(4.78, 4.64)
mol%	0.8	4.1	14.4	17.5	7.5	27.1	28.6
<i>aeVII AP</i>							
wt%	6.8	36.7	32.2	11.5	2.8	5.5	4.6
	(7.09, 6.40)	(37.07, 36.40)	(32.51, 31.92)	(10.39, 12.62)	(2.71, 2.83)	(5.67, 5.27)	(4.56, 4.56)
mol%	0.5	6.9	15.7	15.3	7.1	24.9	30.1

^a Values in parentheses are means of two independent β -limit dextrin preparations and digestions.

^b Divisions between regions I and II and III and IV were based on the minima observed for *wx*; division between regions II and III was based on the minima observed for *ae wx*; division between region IV and V was based on the inflection observed in the molar response of *wx*; divisions between regions V, VI, and VII were based on the minima observed for each chromatogram.

TABLE II
 β -Amylolysis Limits, Chain Lengths, and Molar Chain Ratios^{a,b}

Sample	β -Amylolysis Limit	CL	ECL	ICL	CL _{β}	CCL	A:B	A:B _S :B _L	B _S :B _L
<i>wx</i>	54.2 \pm 0.4	23.3 \pm 3.6	14.6	7.7	8.7 \pm 0.0	13.3	1.1	53:36:11	3.3
<i>ae wx</i>	55.4 \pm 0.6	32.8 \pm 0.3	20.2	11.6	11.6 \pm 0.0	20.5	1.3	55:26:18	1.4
<i>n AP</i>	57.3 \pm 1.5	26.5 \pm 2.8	17.2	8.3	8.5 \pm 0.2	12.9	1.2	54:36:10	3.6
<i>ae du AP</i>	62.6 \pm 0.2	30.6 \pm 1.0	21.4	8.2	10.1 \pm 0.1	17.0	1.3	56:32:13	2.5
<i>aeV AP</i>	62.6 \pm 4.4	40.8 \pm 0.3	27.5	12.3	12.7 \pm 0.1	23.0	1.3	56:25:19	1.3
<i>aeVII AP</i>	60.6 \pm 1.5	48.5 \pm 0.7	31.4	16.1	14.5 \pm 0.4	26.6	1.3	55:22:23	1.0

^a AP = amylopectin, CL = average chain length of AP, ECL = average external chain length of AP, ICL = average internal chain length of AP (includes residual A chains of the β -limit dextrin), CL _{β} = average chain length of the AP β -limit dextrin (includes residual A chains of the β -limit dextrin), CCL = average core chain length of AP (average chain length of residual B chains of the β -limit dextrin), A:B = molar ratio of A chains (regions VI and VII) to B chains (regions I through V), A:B_S:B_L = molar ratio of A chains (regions VI and VII) to short B chains (regions IV and V) to long B chains (regions I, II, and III), B_S:B_L = molar ratio of short B chains to long B chains.

^b Values for limit β -amylolysis, CL, and CL _{β} reported as mean \pm standard deviation of triplicate analyses of total carbohydrate (Dubois et al 1956) and reducing capacity (Hizukuri et al 1981; Jane and Chen 1992) from two independently debranched samples of an AP. ECL, ICL, and CCL calculated according to Yun and Matheson (1993). Molar chain ratios are means from HPSEC chromatograms of two independently debranched samples of AP.

calibration in that work, glucose and maltose were also used to generate the calibration curve. Injections of debranched β -limit dextrin solution were 50 μ L.

The differential refractive index response at each time was divided by the molecular weight of the carbohydrate calculated to elute at that time to obtain a relative molar response (Yuan et al 1993). Chromatograms of debranched β -limit dextrans (Fig. 1) were divided into seven regions for comparison. The divisions between regions I and II and between regions III and IV were based on the minima observed for *wx* starch, and the division between regions II and III was based on the minima observed for *ae wx* starch. The division between region IV and V was based on the inflection observed in the molar response of *wx* starch. Finally, the divisions between regions V, VI, and VII were based on the minima observed for each chromatogram.

The A:B chain ratio was calculated according to Yuan et al (1993). The average β -limit dextrin chain length (CL_{β}), the average external chain length (ECL), the average internal chain length (ICL), and the average core chain length (CCL) were calculated according to Yun and Matheson (1993). Regions VI and VII (DP 3 and DP 2, respectively) were considered the residues of A chains (Manners 1989). Regions I, II, and III were considered long B chain residues (B_L), and regions IV and V were considered short B chain residues (B_S).

RESULTS

The β -limit dextrans of the *wx* starch had a chain distribution that closely matched the distribution of chains of the *n* AP (Fig. 1, Table I). These starches had a similar molar fraction of chains in combined regions I, II, and III: 10.8% for *wx* and 10.3% for *n*. The molar fraction of chains in combined regions I, II, and III for *ae*-containing starches was much higher: 18.4% for *ae wx*, 17.3% for *ae du*, 19.4% for *aeV*, and 22.6% for *aeVII*.

The *wx* and *n* AP had the lowest CL (Table II). The AP from the *ae*-containing endosperm had the highest CL (30.6 for *ae du* AP to 48.5 for *aeVII* AP). The present work allowed calculation of the ECL, ICL, and CCL of the starches, which followed a pattern similar to that of the CL, with the *wx* and *n* AP having similar and lowest values, and the starches from the *ae*-containing endosperm having highest values but varying greatly.

The A:B molar ratios for all the starches and starch fractions were similar (1.1 to 1.3) (Table II). The $B_S:B_L$ molar ratios were highest for the *wx* (3.3) and *n* AP (3.6). These values were at least twice the $B_S:B_L$ of all but one of the starches from the *ae*-containing endosperm. The exception was *ae du* AP ($B_S:B_L$ 2.5).

DISCUSSION

Even though the material in region I eluted within the same time range as amylose (data not shown), we could not prove that this material was not long chains from AP. Consequently, calculation of the molar proportion of BL chains included region I for all starches. The inclusion of this population in calculations had a negligible effect on the A:B, A: $B_S:B_L$, and $B_S:B_L$ molar ratios.

The chromatograms in Fig. 1 agree well with previously reported chromatograms of the β -limit dextrans of *wx*-type starches (Yuan et al 1993) and of the AP of *ae* starch from the Oh43 inbred line (Inouchi et al 1987). The β -amylolysis limit values (Table II) are in reasonable agreement with previous reports of β -amylolysis limits of *wx*-containing starches (52–54%) (Yun and Matheson 1993) and the AP of *ae* starches (60–62%) (Takeda et al 1993; Yun and Matheson 1993). The similar β -amylolysis limits for the *n* AP, *wx* starch, and *ae wx* starch are consistent with the idea that both the external chains and the internal chains are proportionately longer for *ae wx* starch than for *wx* starch or *n* AP. The somewhat higher β -amylolysis limit for the three non-*wx*-containing *ae*-type starches suggests that the ratio of ECL to ICL is proportionately greater for these AP than for *wx* starch, *ae wx* starch, and *n* AP. The CL, CL_{β} ,

ECL, ICL, and CCL for AP from *n* AP, *wx* starch, *ae wx* starch, and *ae* AP are in general agreement with previously reported values for starches from the same genotypes in undisclosed backgrounds (Yun and Matheson 1993).

The A:B chain ratios for the *n* AP, the *wx*-type starches, the *aeV* AP, and the *aeVII* AP determined from the chromatograms (Table II) are consistent with previously reported A:B chain ratios (1.1 to 1.5) for *n*, *wx*, and *ae* starches (Fuwa et al 1987; Inouchi et al 1987; Yun and Matheson 1993; Yuan et al 1993). The A:B chain ratios for *aeV* AP and *aeVII* AP are lower than the A:B ratio (1.7) for *ae* starch calculated by Inouchi et al (1987) from unfractionated *ae* starch. But their calculation was based on a chromatogram with a large amylose peak that overlapped the long B chain peak. Inouchi et al (1987) noted that the high amylose content of the *ae* starch may have affected the A:B chain ratio. The A:B chain ratios for the *aeV* AP and *aeVII* AP (Table II) are consistent with the A:B chain ratio (1.2) calculated by Yun and Matheson (1993) for amylopectin fractionated by concanavalin A precipitation from *ae* starch. The A: $B_S:B_L$ chain ratios for the *wx* starches are in general agreement with chain ratios previously reported as A: $B_1:(B_2+longer)$ for *wx* and *ae wx* starch from the Ia5125A inbred line (*wx* 52:41:7, *ae wx* 52:33:15), and the W64A inbred line (*wx* 52:42:6, *ae wx* 60:20:20) (Yuan et al 1993). The $B_S:B_L$ chain ratios for the *n* AP and the *wx* starches are consistent with previously reported $III_{\beta}:II_{\beta}$ chain ratios for *ae* (1.1), *ae wx* (1.1), *wx* (3.2), and *n* (2.9) (Fuwa et al 1987; Inouchi et al 1987). These ratios correspond to the weight ratios in the present report for *wx* (48:45) and *ae wx* (21:65) (Table I).

The association between longer CL in *ae*-containing starches and a diminished activity of specific isoforms of starch branching enzymes (SBE) has been described previously (Boyer et al 1976a; Boyer and Preiss 1978). However, it is unclear why there are two populations of B chains (as observed after debranching the β -limit dextrin),

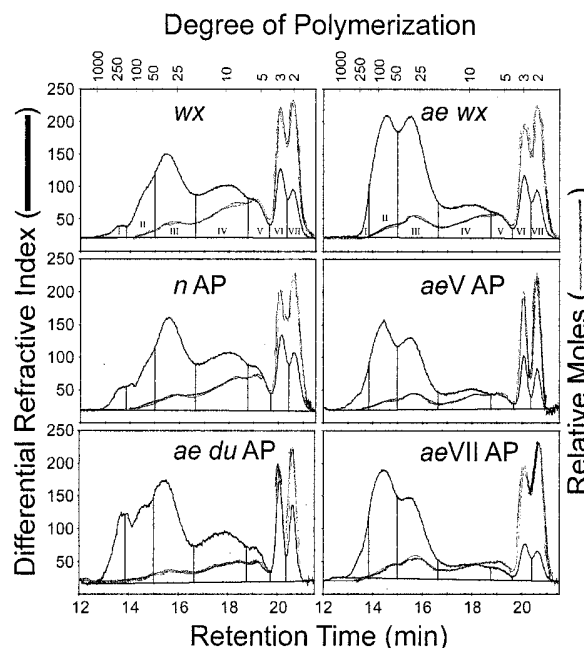


Fig. 1. Mass-basis and molar-basis chromatograms of debranched β -limit dextrans (*wx* = *wx* maize starch, *ae wx* = *ae wx* maize starch, *n* = normal maize starch, *aeVII* = *aeVII* maize, *aeV* = *aeV* maize, AP = amylopectin from differential alcohol precipitation) (Klucinec and Thompson 1998). Divisions between regions I and II and III and IV were based on the minima observed for *wx*; division between regions II and III was based on the minima observed for *ae wx*; division between region IV and V was based on the inflection observed in the molar response of *wx*; divisions between regions V, VI, and VII were based on the minima observed for each chromatogram.

and why the B_L chains tend to be favored over B_S chains in AP from *ae*-containing starches. While the ICL does increase in AP from *ae* starch, indicating that the average distance between branches increases, this average value is not necessarily representative of the difference between branch points in a cluster. The population of longer B chains is favored over the population of shorter B chains in the AP from *ae*-containing starches. If these long B chains link clusters (Thompson 2000), then the number of chains per cluster would decrease, which is another way of saying that fewer branch points would be found in periodic clusters of branch points. This outcome might be simply attributed to a lower starch branching activity relative to starch synthase activity for the *ae*-containing starches compared with this ratio for *wx* and *n* starches. The *ae du* AP had a chromatographic profile (Table I) and chain length parameters (Table II) that were more similar to those for *wx* starch and *n* AP than for the other *ae*-containing starches. Examination of the β -limit dextrins of *du* (Inouchi et al 1987) and *du wx* (Fuwa et al 1987) showed a higher ratio of short B chains to long B chains (Fr. III β :Fr. II β 4.0 for *du* and 6.1 *du wx*) than for normal starch (Fr. III β :Fr. II β 2.9). The observation that the B_S : B_L ratios for *du* and *du wx* were higher than for *wx* and *n* starch could be attributed to a higher starch branching enzyme activity relative to starch synthase activity in *wx* and *n* starch. Gao et al (1998) suggested that *Du* was the structural gene coding for starch synthase II (SSII), with the reduction in SSII activity being a direct result of the mutation. With both starch synthase activity and branching enzyme activity reduced for *ae du* as compared with *n*, *ae du* could have a balance of synthase and branching activities closely resembling of the ratio in the *wx* and *n* starch.

Studies have demonstrated that the isoamylase-debranched AP from *ae*-containing starches from different sources have somewhat different chain length profiles (Takeda et al 1993; Klucinec and Thompson 1998; Shi et al 1998) and that the background of the starch may influence this profile (Boyer et al 1976b; Yuan et al 1993; Shi et al 1998; Sidebottom et al 1998). Yuan et al (1993) showed that the debranched β -limit dextrins of *wx* and *ae wx* starches in the W64A dent corn inbred line were different from the debranched β -limit dextrins of genotypes in the Ia5125 sweet corn inbred line. Klucinec and Thompson (1998) showed that the debranched *aeV* AP and *aeVII* AP had different chain length distributions: *aeVII* AP had more long chains than *aeV* AP. In a related study, Shi et al (1998) observed that the isoamylase-debranched high-amylose starch with the lower SBEII activity had a lower proportion of short chains. These differences in the chain length profiles of these high-amylose starches have been discussed within the context of their genetic background (Sidebottom et al 1998; Shi et al 1998). In the present work, the background of the starch also influences the chain length distribution of the residual B chains of the β -limit dextrins of starches containing the *ae*-mutation, indicating that the pattern of branching has also been affected.

Numerous investigations have related the chain length distribution of isoamylase-debranched starches to the retrogradation behavior of the starch. Klucinec and Thompson (1999) suggested that the differing retrogradation behavior for a series of high-amylose maize starches could be associated with the chain length distributions of the AP fractions. Other aspects of the fine structure may be equally important with respect to the rheological behavior of branched molecules. If the formation of double helices by the external chains of amylopectin is responsible for enthalpy as observed by differential scanning calorimetry (Cooke and Gidley 1992), the ECL may also be the basis for physical junction zones in starch gels. The ECL may also be responsible for the extent and nature of any interaction between amylose and amylopectin, as suggested by Klucinec and Thompson (1999, 2002). A longer ICL (or CCL) may also have an influence on the retrogradation behavior of amylopectin molecules as suggested for *wx* starches (Shi and Seib 1995; Thompson and Blanshard 1995; Liu and Thompson 1998). The chain length distribution provides rudimentary information related

to the fine structure of AP; analysis of β -limit dextrins of AP allows a more indepth understanding of how the AP structures contribute to the unusual properties of high-amylose maize starches. It is clear that in addition to the high amylose content, the nature of the AP in high-amylose starch can contribute to the physical behavior (Klucinec and Thompson 1999, 2002). We suggest that the AP branching pattern of *ae*-containing starches and the CL profile of the AP may both contribute to the contribution of AP to the different physical behaviors of high-amylose starches.

LITERATURE CITED

- Akai, H., Yokobayashi, K., Misaki, A., and Harada, T. 1971. Structural analysis of amylopectin using pseudomonas isoamylase. *Biochim. Biophys. Acta* 252:427-431.
- Baba, T., and Arai, Y. 1984. Structural characterization of amylopectin and intermediate material in amylo maize starch granules. *Agric. Biol. Chem.* 48:1763-1775.
- Boyer, C. D., and Preiss, J. 1978. Multiple forms of starch branching enzyme of maize: Evidence for independent genetic control. *Biochem. Biophys. Res. Comm.* 80:169-175.
- Boyer, C. D., Garwood, D. L., and Shannon, J. C. 1976a. Interaction of the amylose-extender and waxy mutants of maize. *J. Heredity* 67:209-214.
- Boyer, C. D., Garwood, D. L., and Shannon, J. C. 1976b. The interaction of the amylose-extender and waxy mutants of maize (*Zea mays* L.). Fine structure of amylose-extender waxy starch. *Starch* 28:405-410.
- Cooke, D., and Gidley, M. J. 1992. Loss of crystalline and molecular order during starch gelatinization: Origin of the enthalpic transition. *Carbohydr. Res.* 227:103-112.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350-356.
- Fuwa, H., Glover, D. V., Miyaura, K., Inouchi, N., Konishi, Y., and Sugimoto, Y. 1987. Chain length distribution of amylopectins of double- and triple-mutants containing the waxy gene in the inbred Oh43 maize background. *Starch* 39:295-298.
- Gao, M., Wanat, J., Stinard, P. S., James, M. G., and Myers, A. M. 1998. Characterization of *dull1*, a maize gene coding for a novel starch synthase. *Plant Cell* 10:399-412.
- Hizukuri, S. 1985. Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydr. Res.* 141:295-306.
- Hizukuri, S. 1986. Polymodal distribution of the chain length of amylopectins, and its significance. *Carbohydr. Res.* 147:342-347.
- Hizukuri, S., Takeda, Y., Yasuda, M., and Suzuki, A. 1981. Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydr. Res.* 94:205-213.
- Inouchi, N., Glover, D. V., and Fuwa, H. 1987. Chain length distribution of amylopectins of several single mutants and the normal counterpart, and sugary-1 phytoglycogen in maize (*Zea mays* L.). *Starch* 39:259-266.
- Jane, J.-L., and Chen, J.-F. 1992. Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.* 69:60-65.
- Klucinec, J. D., and Thompson, D. B. 1998. Fractionation of high-amylose maize starches by differential alcohol precipitation and chromatography of the fractions. *Cereal Chem.* 75:887-896.
- Klucinec, J. D., and Thompson, D. B. 1999. Amylose and amylopectin interact in retrogradation of dispersed high-amylose starches. *Cereal Chem.* 76:282-291.
- Klucinec, J. D., and Thompson, D. B. 2002. Amylopectin nature and amylose-to-amylopectin ratio as influences on the behavior of gels of dispersed starch. *Cereal Chem.* 79:24-35.
- Liu, Q., and Thompson, D. B. 1998. Effects of moisture content and different gelatinization heating temperatures on retrogradation of waxy-type maize starches. *Carbohydr. Res.* 314:221-235.
- Manners, D. J. 1989. Recent developments in our understanding of amylopectin structure. *Carbohydr. Polym.* 11:87-112.
- Robyt, J. F., and Whelan, W. J. 1968. The α -amylases. Pages 430-476 in: *Starch and Its Derivatives*. J. F. Robyt and W. J. Whelan, eds. Chapman and Hall: London.
- Shi, Y.-C., and Seib, P. A. 1995. Fine structure of maize starches from four *wx*-containing genotypes of the W64A inbred line in relation to gelatinization and retrogradation. *Carbohydr. Polym.* 26:141-147.
- Shi, Y.-C., Capitani, T., Trzasko, P., and Jeffcoat, R. 1998. Molecular

- structure of a low-amylopectin starch and other high-amylose maize starches. *J. Cereal Sci.* 27:289-299.
- Sidebottom, C., Kirkland, M., Strongitharm, B., and Jeffcoat, R. 1998. Characterization of the difference of starch branching enzyme activities in normal and low-amylopectin maize during kernel development. *J. Cereal Sci.* 27:279-287.
- Takeda, C., Takeda, Y., and Hizukuri, S. 1993. Structure of the amylopectin fraction of amylo maize. *Carbohydr. Res.* 246:273-281.
- Thompson, D. B. 2000. On the non-random nature of amylopectin branching. *Carbohydr. Polym.* 40:223-239.
- Thompson, D. B., and Blanshard, J. M. V. 1995. Retrogradation of selected *wx*-containing maize starches. *Cereal Foods World* 40:670.
- Wang, Y.-J., White, P., Pollak, L., and Jane, J. 1993. Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background. *Cereal Chem.* 70:171-179.
- Yuan, R. C., Thompson, D. B., and Boyer, C. D. 1993. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three *wx*-containing genotypes in two inbred lines. *Cereal Chem.* 70:81-89.
- Yun, S.-H., and Matheson, N. K. 1993. Structures of the amylopectins of waxy, normal, amylose-extender, and *wx:ae* genotypes and of the phyto-glycogen of maize. *Carbohydr. Res.* 243:307-321.

[Received December 6, 2000. Accepted July 23, 2001.]