

Structural Changes of Debranched Corn Starch by Aqueous Heating and Stirring

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ABSTRACT

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Aqueous dispersions (2 mg/mL) of debranched corn starches of different amylose contents (waxy, normal, and high-amylose) were subjected to extensive autoclaving and boiling-stirring, and then the changes in starch chain profile were examined using medium-pressure, aqueous, size-exclusion column chromatography. As autoclaving time increased from 15 to 60 min, weight-average chain length (CL_w) of waxy, normal, and high-amylose corn starches determined using pullulan standards decreased from 46 to 41.2, from 122.1 to 96.3, and from 207.3 to 151.8, respectively. Number-average chain length (CL_n) measured by the Nelson-Somogyi method also decreased from 23.0 to 18.4, from 26.4 to 21.8, and from 66.5 to 41.5, respectively, indicating that thermal degradation of starch chains occurred. The CL_w/CL_n ratio for normal

corn starch was higher than that for waxy corn starch, indicating an increase in polydispersity of the amylose fraction. Thermal degradation was also observed when the debranched starch was subjected to the boiling-stirring treatment (0–96 hr). During 96 hr, the CL_w and relative proportion of $B \geq 2$ chains of amylopectin released by debranching waxy corn starch increased, whereas those of B1 chains decreased. This change may indicate physical aggregation of B1 chains. But branches from normal and high-amylose corn starches showed increases in CL_w and the proportion of both B1 and $B \geq 2$ chains, along with substantial decreases in those of amylose chains. Therefore, thermal degradation of amylose was greater than that of amylopectin.

Starch consists mainly of two α -glucans, amylose and amylopectin, that differ in molecular size and degree of branching. There is more amylopectin than amylose in normal starch granules. Chain length and branching characteristics are major parameters in determining functional and physical properties of amylopectin (Adkins and Greenwood 1969). Many researchers have isolated the amylopectins from starches of different botanical sources and investigated their structural characteristics (Kalichevsky et al 1990, Jane and Chen 1992, Takeda et al 1993, Yuan et al 1993). Amylose composed of linear and mildly branched chains (Takeda and Hizukuri 1987; Takeda et al 1988) plays a critical role in the physical properties of starch pastes and gels. The chain length of amylose is related to its retrogradation tendency (Suzuki et al 1981; Pfannmüller 1987). Complete isolation of either starch molecule from starch granules is difficult because of intermolecular interactions, the presence of intermediate chains, and the relatively high polydispersity.

In general, chain length and distribution of amylopectin and amylose are usually measured by high-performance, size-exclusion chromatography (HPSEC) (Yuan et al 1993; Ong et al 1994) or high-performance, anion-exchange chromatography (HPAEC) (Jane et al 1999) after complete debranching with a specific enzyme such as isoamylase.

Size-exclusion chromatography (SEC) separates the polymeric molecules on the basis of effective diameter and molecular weight (Jackson et al 1988). When a Sephadex column was used to characterize the chain profile of debranched waxy corn starch, two major peaks of amylopectin chains, representing $DP < 20$ and $DP \approx 40$, were observed (Lee et al 1968). MacGregor and Morgan (1984) used a Biogel column and obtained a trimodal distribution of debranched amylopectin in normal and waxy barley starch granules. Hizukuri (1985) examined profiles of chains released by debranching amylopectins from 20 starch species (11 A, 6 B, 3 C) using SEC, and reported that most of the amylopectins showed bimodal distributions of long and short chains but three species gave trimodal distributions. Hizukuri reported HPSEC chromatograms using im-

proved columns and techniques showing tetramodal distribution profiles for potato, tapioca, and kuzu amylopectins (Hizukuri 1986). More recently, Yuan et al (1993) used a high-pressure column (Zorbax PSM) and obtained a trimodal distribution from waxy corn starch.

Profiles obtained are a function of the column and method of starch dissolution (Jackson et al 1988). Because starch molecules occupy large volumes in solution and readily aggregate, it is difficult to measure their structural characteristics in neutral aqueous solution (Cheetham and Tao 1997). Nevertheless, characterization of starch in aqueous media is important because water is the solvent most frequently used for food and other industrial purposes. For chromatographic analysis, molecules must be completely dissolved, i.e., a molecular dispersion must be present. However, boiling in an aqueous solution was insufficient for complete dissolution (Aberle et al 1994). Delgado et al (1991), and Fishman and Hoagland (1994) used microwave heating in a pressure vessel to dissolve corn starch in water. Aberle et al (1994), and You and Lim (2000) used autoclaving to dissolve corn starch. However, no previous study has been done on the effect of heat treatment on chain profiles of debranched starches.

In the present study, branch chains of corn starches were analyzed using a medium-pressure-size exclusion column in an aqueous system. Differences in effects of autoclaving and boiling-stirring on SEC chain profiles were examined.

MATERIALS AND METHODS

Waxy and normal corn starches were provided by Samyang Genex Co. (Seoul, Korea); high-amylose corn starch (Hylon 7) was obtained from Cerestar USA, Inc. (Hammond, IN). Pullulan and dextran standards were purchased from Sigma Chemical Company (St. Louis, MO). *Pseudomonas* isoamylase was purchased in a crystalline form from Hayashibara Biochemical Laboratory (Okayama, Japan).

Starch Debranching

All starches were purified using 90% DMSO and ethyl alcohol (Klucinec and Thompson 1998). The purified starch was dried in a vacuum oven overnight. Purified starch (10 mg) was dispersed in 50 mL of water and then autoclaved at 121°C for 15 min for complete dissolution. Acetate buffer (0.1M, pH 3.5, 400 μ L) and isoamylase (2 μ L, 2,900 U/mL) were added to the starch solution, and the mixture was incubated at 45°C for 24 hr. The enzyme was inactivated by boiling the solution for 15 min, and filtered off

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through a glass filter. The debranched starch solution was freeze-dried.

Heat Treatments

Debranched starch sample was dispersed in aqueous sodium azide (NaN_3 , 0.02%) solution (2 mg/mL). The debranched starch dispersion was autoclaved at 121°C for 15–60 min or stirred using a magnetic stirrer up to 96 hr in a boiling water bath after autoclaving for 15 min.

Chain Profile Analysis

The number-average degree of polymerization (DP_n) of the debranched starch samples was determined by the Somogyi-Nelson method (Nelson 1944).

The SEC system consisted of a pump (P2000, Spectra System, San Jose, CA), an injector valve with a 0.1 mL loop (Rheodyne 7072, Cotati, CA), and differential refractive index detector (Optilab 903, Wyatt Technology, Santa Barbara, CA). The eluent was deionized and distilled water containing 0.02% NaN_3 that had been filtered through a 0.1- μm cellulose acetate filter (Whatman, UK) and degassed. The column used was a medium-pressure Superdex 75HR (Amersham Pharmacia Biotech, Uppsala, Sweden). Flow rate was 0.4 mL/min.

The starch sample solution was filtered through a 5.0- μm acrylic copolymer syringe filter (Pall Gelman Science, Ann Arbor, MI) before SEC analysis. The void and total volumes of the chromatographic system were determined by using dextran (T2000) and glucose, respectively. Pullulan standards of different molecular weights (70,000, 47,300, 22,800, 11,800, 5,900, and 738) were used for calculating relative molecular weights of the debranched starch chains. A semilogarithmic plot of the standard molecular weights versus K_{av} at the maximum refractive index value for pullulan standards was used. The regression coefficient of the relationship between $\log M_w$ and K_{av} was 0.995.

RESULTS AND DISCUSSION

Autoclaving Effects

Debranched starch samples were analyzed by $^1\text{H-NMR}$ spectroscopy to confirm the absence of α -1,6-anomeric protons (branching) which had been assigned 5.0 ppm in the NMR spectrum (Gidley 1985). No signal for branching was observed (spectrum not shown).

When debranched starch samples were boiled for 30 min, recovery after filtration (5.0 μm) was $\approx 94\%$. However, after autoclaving 15 min, complete dissolution was obtained. Relative contents of the

long chains (amylose and $B_{\geq 2}$) in chromatogram (not shown) were higher for the starch sample autoclaved for 15 min compared with that boiled for 30 min, indicating that the long chains were not readily dissolved by boiling.

Table I summarizes the changes in the weight- and number-average chain lengths (CL_w and CL_n) of the total released branches at different autoclaving times. Both chain length values measured decreased with autoclaving time, indicating that there was structural degradation by the heat treatment. For all samples, the CL_w was two- to fourfold larger than the CL_n . The difference was greater for normal and high-amylose corn starches, suggesting that the polydispersity of starch chains was increased by the presence of amylose. According to Takeda et al (1988), amylose in amylo-maize (high-amylose corn) and normal corn starch had relatively broad molecular weight distributions, implying that the polydispersity of amylose was higher than that of amylopectin. Ong et al (1994) also reported high polydispersity values (2.6 \approx 6.9) of amyloses in various starches.

Figure 1 exhibits how the starch chain profile in SEC chromatograms changed as autoclaving time increased up to 60 min. Debranched waxy corn starch chains showed no amylose peak (near the void volume of size-exclusion column), confirming the absence of long amylose chains. The amylopectin chains consisted of four major peaks: $B_{\geq 2}$, B1, and two A chains (Aa and Ab). It was noteworthy that two A chains appeared in the chromatogram.

According to Hizukuri (1986), short chains in starch (tapioca, kuzu, waxy rice) were divided into two groups: CL 11–13 and 18–19, referred to as A and short B-chains (B1 chains), respectively. Ong et al (1994) measured wheat and potato starch and obtained two A chains with CL_w 14–15 and 10, referred to as A1 and A2 chains, respectively. In this experiment, the short A chains of waxy corn starch were distinctly divided into two groups, Aa and Ab, in the SEC chromatogram with CL_w values of 18.3 and 9.2, respectively. Separate peaks for A chains might result from the capability of the column in resolving small chains.

Debranched normal corn starch showed an amylose peak but little Ab chain. Only a shoulder behind the Aa chain appeared. In high-amylose corn starch, an amylose peak and four amylopectin peaks were shown clearly as in the waxy corn starch sample. For all the three corn starches, differences in chain profiles were observed in the chromatograms from different autoclaving times.

Table II summarizes the CL_w and proportion of each chain. In the waxy corn starch sample, long amylopectin chains, $B_{\geq 2}$ and B1, showed substantial decreases in size, from 144 to 115 and from 40.0 to 31.4, respectively, as the autoclaving time increased

TABLE I
Total Weight and Number Average Chain Lengths (CL_w and CL_n) of Debranched Corn Starches After Different Autoclaving Times^a

Autoclaving Time (min)	CL_w			CL_n		
	Waxy	Normal	High Amylose	Waxy	Normal	High Amylose
15	46.0	122.1	207.3	23.0	26.4	66.5
30	43.9	117.4	180.3	22.1	24.7	49.5
60	41.2	96.3	151.8	18.4	21.8	41.6

^a Values are average of three replicates of each sample.

TABLE II
Changes in CL_w and Chain Proportion (%) of Chain Fractions of Debranched Corn Starches at Different Autoclaving Times^a

Autoclaving (min)	Waxy				Normal				High Amylose				
	$B_{\geq 2}$	B1	Aa	Ab	Amylose	$B_{\geq 2}$	B1	A	Amylose	$B_{\geq 2}$	B1	Aa	Ab
15	144 (18.8)	40.0 (14.3)	18.5 (50.6)	9.2 (16.3)	1,619 (20.3)	252 (5.8)	41.7 (23.2)	18.7 (50.7)	1,551 (35.9)	261 (12.1)	49.7 (28.5)	25.5 (15.6)	12.3 (7.9)
30	135 (20.7)	32.0 (14.2)	18.3 (45.6)	8.6 (19.5)	1,497 (19.1)	245 (5.6)	39.6 (21.3)	18.6 (54.0)	1,439 (34.5)	252 (11.8)	41.4 (27.8)	22.2 (14.8)	11.4 (11.1)
60	115 (22.5)	31.4 (14.3)	18.0 (44.3)	8.4 (18.9)	1,394 (18.2)	234 (6.3)	35.8 (19.8)	17.1 (55.7)	1,225 (31.3)	247 (11.7)	37.2 (28.0)	22.3 (16.6)	10.7 (12.4)

^a Values are average of three replicates of each sample.

from 15 to 60 min. In addition, there were slight decreases in the sizes of the short A chains (Aa and Ab) from 18.5 to 18.0, and from 9.2 to 8.4, respectively. This indicates that the longer chains were more susceptible to heating. Likewise, amylose chains present in normal and high-amylose corn starches displayed greater CL_w reduction than did amylopectin chains.

Takeda et al (1988) reported that the CL_w of normal corn amylose was 2,270–2,550 and that it contained nearly equal numbers of branched and unbranched molecules. Takeda et al (1989) determined that the amylose CL_w of Hylon 7 (high-amylose corn starch of $\approx 70\%$ amylose) had a lower value. In concurrence, the amylose of the high-amylose corn starch used in this study had a lower CL_w value than that of normal corn starch (Table II). But both values were smaller than those reported by Takeda et al (1989).

The CL_w for $B \geq 2$ and B1 chains increased proportionally with the amylose content. In agreement with the data in Table II, Cheetham and Tao (1997) reported that the amylose content had little effect either on the CL_w or the proportion of short chains, but only on the proportion amylopectin long chains.

In waxy corn starch, the relative proportion of $B \geq 2$ chains was 18.8% in the sample autoclaved for 15 min, but it increased to 22.5% as autoclaving time increased to 60 min. The increased proportion of B1 chains, however, was unchanged by autoclaving (Table II). The proportion increase of $B \geq 2$ chains could imply that there was chain aggregation. Comparing the relative proportions of each chain fraction, the increase in the amount of $B \geq 2$ chains seemed to come mainly from Aa chains, the proportion of which decreased by $>5\%$. The minor increase in the proportion of Ab chains might have also originated from the Aa chains, but by degradation in this case.

In normal corn starch, the proportions of amylose and B1 chains decreased by increasing autoclaving time from 20.3 to 18.2% and from 23.2 to 19.8%, respectively, whereas those of $B \geq 2$ and A chains increased. Particularly, the increase in the proportion of A chains was significant; this trend was opposite to that in waxy corn starch. Thermal degradation of amylose and B1 chains resulted in increases in the proportions of $B \geq 2$ and A chains, respectively. Considering the relatively high increase in the amount of Aa chains, some amylose chain degradation products must have eluted in the A chain fraction.

The different trends in chain proportion changes during autoclaving between waxy and normal corn starch are not clearly understood. Because the CL_w of $B \geq 2$ was higher in normal starch than in waxy corn starch, the $B \geq 2$ chains in normal starch appear to be more susceptible to thermal treatment, and thus the proportionate increase was not as significant as in waxy corn starch. The greatest change was found in the Aa chain fraction. A continuous increase in the proportion of A chains in normal corn starch probably arose from continuous short chain production by degradation of B1 and amylose chains. But, based on the waxy corn starch results, some aggregation must have occurred simultaneously, although this is not indicated in the data for normal corn starch.

The CL_w of all chains in high-amylose corn starch was decreased by autoclaving, but the change in chain proportion was somewhat different from those of waxy and normal corn starches. The proportions of amylose and $B \geq 2$ chains decreased, whereas those of Ab chains increased, indicating degradation of the long chains (mainly amylose chains) resulting in an increase in the proportion of short A chains. High-amylose corn starch chains were more susceptible to thermal degradation than were normal or waxy corn starch chains because of the greater amount of long chains.

If the first peak on the chromatogram (amylose in Table II) represents the whole amylose fraction, the relative content of amylose chains in normal and high-amylose corn starches is measured as 18.2–20.3 and 31.3–35.9%, respectively. These values are much lower than those reported in the literature ($\approx 25\%$ and 70%).

Cheetham and Tao (1997) compared various methods used for determination of amylose content. They found that the amylose

content of high-amylose maize starch (amylose content 84%, in the literature) could vary from 62 to 84%, depending on the method used for analysis, and they found that the higher amylose starch gave the larger deviation. Banks et al (1974) proposed that amylo maize starch contained an unusually high proportion of intermediate material that fits neither the definition of amylose nor amylopectin. Baba et al (1982) claimed that the true amylose content in amylo maize starch was significantly less than that estimated by the iodine method. Kweon et al (2000) also reported that high-amylose corn starch (*ae 7Wx*) containing $\approx 70\%$ amylose was composed of normal linear amylose (33%) and long linear branches of anomalous amylopectin (38%). They reported that amylo maize starch with 50% amylose was actually composed of $\approx 30\%$ amylose, 40% amylo maize-specific amylopectin, and 30% intermediate material, not fitting the definition of either amylose or amylopectin. Jane et al (1999) reported that B-type starches might give greater overestimation of amylose content than A- or C-type starches, because the former have a greater proportion of long amylopectin branch chains. Thus, conventional iodine binding analysis could overestimate the amylose content of high-amylose corn starch that has the B-type X-ray pattern.

We hypothesize that some of the amylose chains, especially in the branched amylose molecules, have relatively low CL_w values,

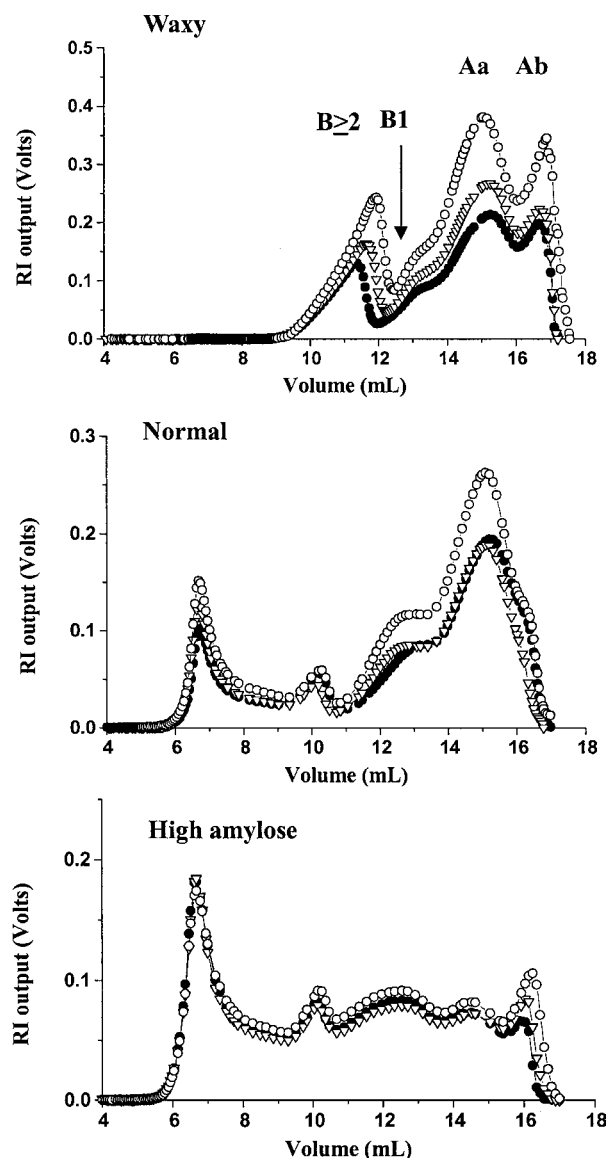


Fig. 1. Chain profiles of three corn starches with increasing autoclaving time: ●, 15 min; ▽, 30 min; and ○, 60 min.

and that, after debranching, these chains eluted together with amylopectin B chains. Therefore, the amylose content estimation based on debranching starch could lead to a lower estimation than the actual content.

According to Jane and Chen (1992), A-type starches have a larger proportion (16–27%) of short chains (DP 6–12) and smaller proportions (7–27%) of long chains (DP >37) than B-type starches. Our data are supportive of this finding. We observed that A-type corn starches (waxy and normal) had smaller proportions (33.1 and 29%, respectively, at 15 min of autoclaving) of B chains (B_{≥2} and B1) than that of the B-type high-amylose corn starch (40.6%). Regarding the effect of starch dissolution by autoclaving, You and Lim (2000) claimed that autoclaving (at 121°C) for 20 min was necessary to dissolve dry normal corn starch in an aqueous medium. Wang et al (2001) examined the effect of autoclaving (15 min) on xyloglucan, dextran and oat β-glucan and reported that autoclaving caused disruption of aggregates rather than chain degradation for xyloglucan and dextran, leading to excellent water solubility. However, they found molecular degradation with oat β-glucan under the same treatment. Therefore, thermal degradation of polysaccharide chains is possible when a heat-treatment is applied for dissolution in aqueous medium, and the level of degradation depends on the polysaccharide structure. In debranched starches, the effect of autoclaving probably depends on starch chain length. In addition, it was noteworthy that autoclaving appeared to result in some aggregation with waxy corn starch.

Boiling-Stirring Effects

Table III shows average chain lengths (CL_w and CL_n) of debranched starches at different periods of boiling and stirring after autoclaving for 15 min. For waxy corn starch chains, CL_w decreased from 46.0 to 43.7 during 1 hr of boiling but did not change significantly afterwards. The number average chain length (CL_n) of waxy corn starch was not changed in the first 1 hr, indicating that there was no degradation. However, CL_n decreased continuously from 23.2 to 17.6 as the boiling continued up to 96

hr. This indicates that thermal degradation continued during this period, although CL_w value was not much changed. The relatively constant CL_w despite the continuous decrease in CL_n indicates partial aggregation of molecules.

Unlike waxy starch, normal and high-amylose corn starches showed decreases in both CL_w and CL_n during the boiling period (≤96 hrs). Comparing the change in CL_w of waxy starch chains (from 46.0 to 43.5), normal and high-amylose starches exhibited more significant decreases (from 122.1 to 50.6 and from 207.3 to 86.3, respectively). A similar result was already observed with autoclaving (Table I).

Table IV shows the changes in CL_w and percent proportion of each starch chain fraction of debranched corn starches as the boiling-stirring time increased. In waxy corn starch, the CL_w of B_{≥2} chains decreased rapidly from 144 to 138 within 1 hr of boiling-stirring, but slightly increased afterwards, whereas the CL_w of shorter chains (B1 and A chains) tended to decrease during the heat treatment. Increases in CL_w and proportion of B_{≥2} chains during the extended treatment were probably due to association or aggregation of the small chains, perhaps facilitated by stirring.

In normal corn starch, the amylose chains displayed more substantial changes than did amylopectin chains, in congruence with the results from autoclaving. The CL_w of amylose decreased substantially from 1,619 to 841 by the boiling-stirring up to 96 hr, indicating thermal degradation of amylose chains. But B_{≥2} and B1 chains in amylopectin showed increases in CL_w and relative percentages. Especially, the proportion of B_{≥2} chain was doubled at 48 hr (Table IV) from 5.8 to 11.6%. This indicates that the degraded fraction from amylose chains appeared as B chains (B_{≥2} and B1). The CL_w of B1 and A chains at the beginning were 41.7 and 18.7, respectively, consistent with data reported by Takeda et al (46–47 and 17–18, respectively) (1993).

For high-amylose corn starch also, CL_w of amylose decreased continuously from 1,551 to 794 as the boiling-stirring time increased from 0 to 96 hr. The decreases in amylose CL_w by the boiling-stirring treatment in normal and high-amylose corn starch chains were similar. As in debranched normal corn starch, amylose

TABLE III
Changes in Total Weight and Number Average Chain Lengths (CL_w and CL_n) of Debranched Corn Starches at Different Boiling-Stirring Times^a

Boiling-stirring (hr)	CL _w			CL _n		
	Waxy	Normal	High Amylose	Waxy	Normal	High Amylose
0	46.0	122.1	207.3	23.0	26.4	66.5
1	43.7	112.5	198.7	23.2	25.7	60.8
24	44.9	80.9	135.2	22.7	21.7	50.7
48	43.9	73.3	104.2	21.9	20.8	48.1
72	42.6	58.8	94.5	18.4	19.5	40.9
96	43.5	50.6	86.3	17.6	19.0	38.5

^a Values are average of three replicates of each sample.

TABLE IV
Changes in CL_w and Chain Proportion (%) of the Chain Fractions of Debranched Corn Starches at Different Boiling-Stirring Times^a

Boiling-Stirring (hr)	Waxy				Normal				High Amylose				
	B _{≥2}	B1	Aa	Ab	Amylose	B _{≥2}	B1	A	Amylose	B _{≥2}	B1	Aa	Ab
0	144 (18.8)	40.0 (14.3)	18.5 (50.6)	9.2 (16.3)	1,619 (20.3)	252 (5.8)	41.7 (23.2)	18.7 (50.7)	1,551 (35.9)	261 (12.1)	49.7 (28.5)	25.5 (15.6)	12.3 (7.9)
1	138 (18.6)	47.4 (14.1)	17.5 (49.6)	8.8 (17.7)	1,571 (19.8)	240 (5.8)	40.2 (21.4)	18.6 (53.0)	1,540 (35.1)	256 (11.6)	48.2 (29.6)	23.0 (15.4)	12.0 (8.3)
24	140 (21.5)	42.8 (14.1)	15.4 (39.4)	8.6 (25.1)	1,384 (15.2)	289 (8.2)	42.4 (23.6)	18.7 (53.0)	1,261 (26.6)	263 (16.5)	49.3 (31.8)	22.5 (14.3)	12.6 (10.8)
48	146 (22.5)	40.0 (13.1)	12.8 (39.4)	8.4 (25.0)	1,153 (8.3)	280 (11.7)	48.9 (24.7)	17.3 (55.3)	1,079 (22.5)	281 (18.0)	58.9 (34.0)	22.6 (12.8)	12.1 (12.7)
72	145 (22.8)	34.5 (15.2)	10.8 (34.5)	8.5 (27.5)	918 (7.5)	282 (11.3)	59.4 (27.8)	16.6 (53.4)	917 (18.9)	286 (19.9)	65.6 (34.0)	18.1 (13.8)	13.7 (13.4)
96	150 (24.5)	28.6 (13.8)	12.9 (36.0)	9.1 (25.7)	841 (6.4)	280 (11.6)	68.4 (28.6)	15.4 (53.4)	794 (15.2)	288 (21.5)	73.4 (36.7)	16.6 (10.4)	14.4 (16.2)

^a Values are average of three replicates of each sample.

degradation in high-amylose corn starch led to increases in the CL_w and percent portion of B chains. The CL_w of $B_{\geq 2}$ and B1 chains increased from 261 to 288 and from 49.7 to 73.4, respectively. Also, the proportions of $B_{\geq 2}$ and B1 chains increased from 12.1 to 21.5% and from 28.5 to 36.7%, respectively. The CL_w and relative percentage of Aa decreased and those of Ab increased, indicating that Aa chains were transformed to Ab chains by the heat treatment. This trend was the same as that found with waxy corn starch.

Our data revealed that the chain lengths of the amylopectin of amylo maize starch were higher than those in normal or waxy corn starch. Boyer and Preiss (1978) reported that amylo maize kernels were deficient in one of three forms of branching enzyme found in normal maize, which results in fewer branch points in amylopectin. The proportion of A chain (Aa plus Ab) was smallest for high-amylose starch.

The starch chain profile in SEC chromatograms changed as boiling-stirring time increased up to 96 hr (Fig. 2). In waxy corn starch, as the Aa chain fraction decreased gradually, the Ab chain fraction increased. By boiling for 24 hr, a new but small B chain peak appeared. The newly formed B chains might be the degradation products of B1 chain. As the boiling time increased, the relative percent of $B_{\geq 2}$ chains increased, which was the same tendency as found with autoclaving. This is another indication that chain aggregation transferred some of the chains from shorter-chain peaks to longer-chain peaks (Table IV).

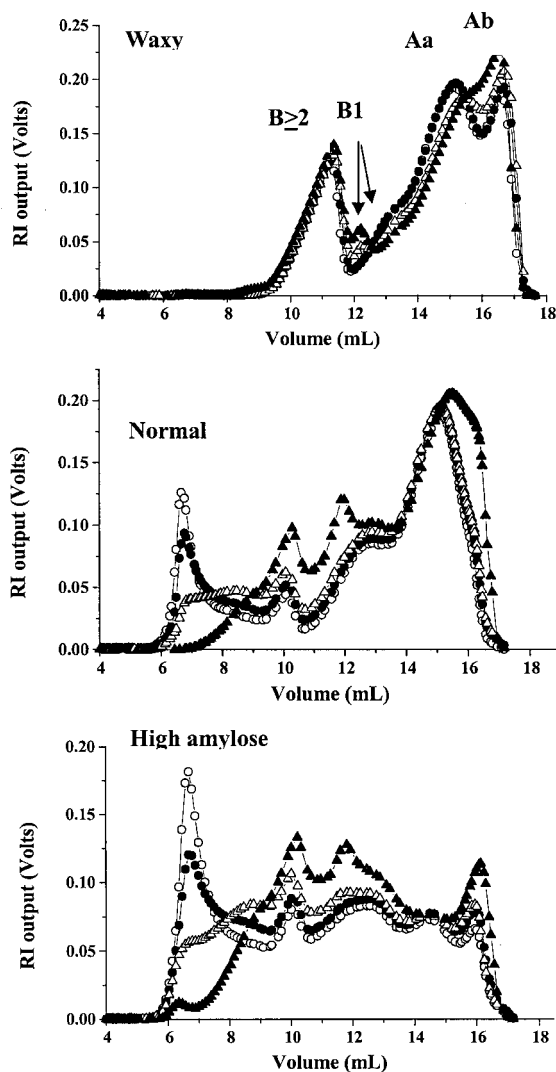


Fig. 2. Chain profiles of three corn starches with increasing boiling-stirring time: ○, 0 hr; ●, 24 hr; △, 48 hr; and ▲, 96 hr.

In normal and high-amylose corn starches, as boiling time increased, the amylose fraction in chromatograms changed most significantly. After 24 hr, there was an increase in $B_{\geq 2}$ and B1 chain fractions, especially $B_{\geq 2}$, concurrent with the loss in amylose. The increased portion of Ab chains indicates that short chains were produced by thermal degradation. Basically both autoclaving and boiling-stirring decreased the percent of the amylose. This indicates that longer chains are more susceptible to the physical treatment, especially shear force by stirring, resulting in more degradation. On the contrary, shearing could also accelerate chain aggregation and this phenomenon appeared more with the short chains, possibly due to high chain mobilities. Based on the chromatograms, 96 hr of boiling with mild stirring was a more severe treatment than was 60 min of autoclaving.

CONCLUSIONS

After dehydration, debranched starch samples need to be subjected to autoclaving (121°C, 15 min) to achieve complete dissolution in aqueous media. But autoclaving for more than 30 min or additional boiling-stirring causes thermal degradation of starch chains. Amylose chains are more susceptible to thermal degradation than are short amylopectin chains. Moreover, branch chains may aggregate during either static autoclaving or boiling-stirring. So aggregation appeared to be more significant in debranched waxy corn starch than in debranched normal or high-amylose corn starch.

In a SEC system employing water, any heat treatment for starch dissolution should be carefully monitored to minimize thermal degradation and to achieve starch dissolution for accurate analysis of chain length and proportion.

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