Fine Structure of Corn Amylose and Amylopectin Fractions with Various Molecular Weights†

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Characterization of starch molecular structure is vital to comprehending starch structure−function relationships. Four corn starches were fractionated by aqueous leaching into six amylose and five amylopectin fractions. Molecular weight (MW), branching, and chain lengths were determined by size exclusion chromatography and laser light scattering (SEC/LLS); percent crystallinity was determined by X-ray analysis. Amylose fractions had different weight-average molecular weights (M_w = 1.03−4.89E+05) and branching ratios (0.3−1.0), and branching was seemingly inversely correlated with M_w. Amylose fractions had similar polydispersities (1.3−1.9) and branch points per chain of 1000 glucose units (0.6−3.0). Degree of polymerization (DP) increased (324−1014) proportionally to M_w. For amylopectin, some fractions had different M_w values (7.08−9.88E+07) but statistically similar polydispersities (1.1−1.2) and branching ratios (1.5−4.3). The highest M_w amylopectin fraction had longer branched chains (long, intermediate, short) than those of its lower M_w counterparts. DP for short branched chains was correlated with M_w. High to low M_w amylopectin fractions with branching values >1.5, and short branched chains (DP = 15−18), showed high crystalline ratios (>28%). Similar MW and branching trends were observed in a previous study using SEC/refractive index and wet chemistry, respectively. These studies suggest that MW characterization possesses the potential for predicting some molecular structures in starch.

Keywords: *Starch; corn; amylose; amylopectin; molecular weight; branching; size exclusion chromatography; laser light scattering*

INTRODUCTION

Despite its use in a wide array of products, new uses for starch continue to be explored. A limited understanding of starch structure−function relationships has impeded development of new uses. Starch functionality has traditionally been characterized in reference to the botanical source and the ratio of amylose to amylopectin in starch granules. Nevertheless, it is now known that amylose and amylopectin from the same botanical source can have distinct molecular weight and structure characteristics (Hizukuri, 1985; Takeda et al., 1992, 1993; Ong et al., 1994).

To begin to understand the manner in which starch polymers behave under given situations, it is necessary to isolate and characterize the molecular conformation and structures of pure amylose or amylopectin subfractions. However, understanding polymer subfraction molecular structure should only be construed as a necessary first step in truly understanding polymer structures and their interaction in the native granule or in a starch polymer mixture. Starch polymer behavior depends on measurable parameters such as molecular weight (MW) distribution, the nature of chain branching, and chain lengths (Weaver et al., 1994). Starch MW is often influenced by botanical source, starch isolation procedures, amylose and amylopectin separation methods, and especially the technique used to determine polymer MW, such as ultracentrifugation, "wet" chemistry, gel permeation chromatography (GPC), or size exclusion chromatography (SEC), and the type of detector used (Ong et al., 1994).

Since its early development by Einstein, Raman, Debye, and Zimm, the light scattering detector has gained wide use in chemical physics (Wyatt, 1993). Light scattering (LS) coupled with GPC/SEC has become a powerful analytical tool in determining absolute MW (*z*, weight, and number average), size distributions, mean square radius and its various averages, molecular conformation and structure, and branching ratios (Wyatt Technology, 1992). LS does not depend on pump speed and use of standards (known MW) as does GPC/SEC, but is limited to resolving sizes down to about a 20th (10^4) of the laser's incident wavelength.

GPC/SEC separates macromolecules by hydrodynamic volume. Molecular size is determined using LS by measuring the angular variation of scattered light and it is independent of molecular weight. Concentration at each elution volume can be measured in-line by using a differential refractive index (DRI) detector, which depends on the specific refractive index (d*n*/d*c*) of the solute in solution. The specific refractive index increment represents the change in refractive index as a function of solute concentration. It is determined using the following equation (Yu and Rollings, 1987):

$$\frac{[dn/dc]_{\text{unknown polymer}}}{[dn/dc]_{\text{known polymer}}} = \frac{[\text{total mass injected/integrated DRI response}]_{\text{known}}}{[\text{total mass injected/integrated DRI response}]_{\text{unknown}}} \quad (1)$$

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THEORY

MW. MW can be determined as a function of excess Rayleigh ratio (R_ϕ). Excess R_ϕ is the difference between scattered light intensity of the solvent and that of solution. The relationship between R_ϕ and weight-average molecular weight (M_w) was given by Zimm (1948) as

$$R_\phi/(Kc) = M_w P(\phi) - 2A_2 c M_w^2 P^2(\phi) \quad (2)$$

where R is the excess Rayleigh ratio, K is an optical constant $= 4\pi^2 n_0^2 (dn/dc)^2/\lambda_0^4 N$, n_0 is the refractive index of solvent at incident radiation, λ_0 is the incident radiation wavelength, N is Avogadro's number (6.022×10^{23}), c represents solute concentration (g/mL), M_w is weight-average molecular weight, $P(\phi)$ is the form factor related to the solute's shape and size in solution and modulates scattered radiation intensity due to finite molecule size and to the polymer's deviation from sphericity, and A_2 is the second virial coefficient.

MW is calculated across the chromatogram, at constant elution volumes (M_v). The M_v values are then used to calculate z-average (M_z), weight-average (M_w), and number-average (M_n) MW values as shown below (Yu and Rollings, 1987):

$$M_z = \Sigma c_v/\Sigma(c_v/M_v) \quad (3)$$

$$M_w = \Sigma c_v M_v/\Sigma c_v \quad (4)$$

$$M_n = \Sigma c_v M_v^2/\Sigma c_v M_v \quad (5)$$

Branching Ratio. Branching ratio (g_M) is calculated by comparing the mean square radius of gyration (R^2) of branched (b) and linear (l) polymers of the same MW as given below (Zimm and Stockmayer, 1949):

$$g_M = (R^2)_b/(R^2)_l \quad (6)$$

For a given MW, the branched polymer will have a smaller radius, so g_M will be between 0 and 1 (Wyatt Technology, 1993). Before the advent of multiple-angle laser light scattering (MALLS), intrinsic viscosity data were often used to calculate g_M (Zimm and Kilb, 1959; Kawahara et al., 1984; Weaver et al., 1988; Nishinari et al., 1991).

Chain Length Distribution. The chain lengths (degree of polymerization, DP) of starch polymers can be determined by physical and chemical means based on reducing power (Dubois et al., 1956; Lindsay, 1973; Hizukuri et al., 1981; Zhang and Jackson, 1992). These methods are often laborious and prone to errors.

Separation of enzymatically hydrolyzed starch polymers by SEC/GPC has proven essential for the study of amylose and amylopectin chain degree of polymerization (Akai et al., 1971; Hizukuri, 1985; Takeda et al., 1990; Yuan et al., 1993). Isoamylase (EC 3.2.1.68) and pullulanase (EC 3.2.1.41) enzymes have been used widely for this purpose, since they hydrolyze exclusively α-D(1−6) glycosidic bonds between adjacent chains. The use of anion exchange chromatography coupled with pulsed amperometric detection (Koizumi et al., 1989; Ammeraal et al., 1991; Ward et al., 1994) or SEC/LS/DRI (Lee et al., 1968; Yu and Rollings, 1987; Ong et al., 1994) has greatly aided studies of chain length distribution.

Crystallinity. Starch granules display diffraction patterns (A, B, C, V), indicative of their polymeric order, when exposed to X-rays. The patterns differ in their interplanar spacings and reflection intensities (Nara and Komiya, 1983; Zobel, 1988). Starch crystalline order governs granule moisture sorption (Guibot et al., 1961; Nara et al., 1978), gelatinization (Cook and Gidley, 1992), and retrogradation (Zobel, 1988) properties. Generally, crystallinity, as measured by X-ray diffraction, increases with the amount of moisture adsorbed (Nara, 1978). Although moisture sorption cannot take place inside the crystalline portion of an intact granule, crystallinity reaches a maximum when a saturation moisture state is reached. Under this condition, six molecules of water are bonded to each glucose unit.

Molecular order (double-helical) and crystallinity are disrupted concurrently during gelatinization (Cook and Gidley, 1992). In granules, crystallinity is attributed to the ordered arrangements of adjacent double helix amylopectin branches (French, 1984). The fine structure of amylopectin has been reported to influence the retrogradation behavior of starch (Shi et al., 1991; Zhang and Jackson, 1992; Yuan et al., 1993). It has also been reported that the crystalline nature of amylopectin depends on its weight-average chain length (Hizukuri, 1985).

The objective of this study was to characterize the molecular weight, branching ratio, and chain length distribution of amylose and amylopectin fractions from four corn starches using high-performance size exclusion chromatography/multiangle laser light scattering (HPSEC/MALLS) and to compare these parameters to those obtained via high-performance size exclusion chromatography/differential refractive index. Another objective was to determine the pattern and percentage crystallinity of the starch fractions.

MATERIALS AND METHODS

Starch Fractionation. Six amylose and five amylopectin fractions were obtained from regular, waxy, 50% high-amylose (hAM), and 70% hAM corn starches (American Maize Products, Hammond, IN) by aqueous leaching with gentle stirring and subsequent alcohol precipitation as shown in Table 1 and outlined by Mua and Jackson (1995, 1997). The samples were then freeze-dried. Four amylopectin fractions were obtained from 50 and 70% hAM starches, but they were <80% pure; hence, they were not considered for characterization.

Determination of MW and Branching. Amylose and amylopectin fractions were solubilized in aqueous 90% (v/v) methyl sulfoxide by boiling for 30 min and holding at 52 °C for 1−4 h to prevent precipitation of solubilized material. Each solution was subsequently filtered (1.2 μm filter) before being injected (25 μL) into a HPSEC system consisting of 4 KS-series Shodex Ionpack columns (Showa Denko, Tokyo, Japan) as described by Jackson (1991). The columns were connected in series to a DAWN-SF MALLS photometer, equipped with a He−Ne laser (623.8 nm) and 18 angled detectors (Wyatt Technology Inc., Santa Barbara, CA) and subsequently in-line to a refractive index detector (Waters Model 410, Millipore Co., Milford, MA). Deionized distilled water, pumped at a rate of 1.0 mL/min, was used as the mobile phase. Absolute M_w and M_n molecular weights and polydispersities (M_w/M_n) were calculated using Astra software (version 3.04, Wyatt Technology). Branching ratio and branch frequency per 1000 glucose units (LCBF) were analyzed using EASI software (version 7.04, Wyatt Technology).

Molecular weight (M_z, M_n) and polydispersity (M_z/M_n) values from this study were compared with apparent values obtained in a previous study. In the previous study, samples were injected into the same 4 KS-series column HPSEC system in-line with a refractive index detector (Mua and Jackson, 1995, 1997). Apparent M_w, M_n, and (M_z/M_n) values were determined

Table 1. Sample Preparation Treatments and Polymer Fraction Identification

corn starch type (slurries % w/v)	heat treatments and holding times	centrifugation components (3000*g*/8 min)	solvent used for precipitation (a) or dispersion (b)	polymer fraction	sample identification
waxy (1.5)	75 °C for 30 min	residue	methanol (b)[a]	amylopectin	AP1
		supernatant	methanol (a)[b]	amylopectin	AP2
regular (4.0)	60 °C for 1 h[c]	final residue	methanol (b)[a]	amylopectin	AP3
	70 °C for 1 h[d]	final residue	methanol (b)[a]	amylopectin	AP4
		supernatants	butanol (a)[e]	amylose	AM1
	80 °C for 1 h[f]	final residue	methanol (b)[a]	amylopectin	AP5
		supernatants	butanol (a)[e]	amylose	AM2
50% amylose (3.0)	90 °C for 1 h[f]	supernatants	ethanol (a)[g]	amylose	AM3
	100 °C for 1 h[f]	supernatants	ethanol (a)[g]	amylose	AM4
70% amylose (3.0)	90 °C for 1 h[f]	supernatants	ethanol (a)[g]	amylose	AM5
	100 °C for 1 h[f]	supernatants	ethanol (a)[g]	amylose	AM6

[a] Methanol (85% v/v) was used to reslurry residue, followed by centrifugation. [b] Methanol (one-third the volume of the supernatant) was used to precipitate amylopectin from supernatant. [c] After first centrifugation, residues were reslurried, reheated, and centrifuged; procedure was repeated four times. [d] After first centrifugation, residues were reslurried, reheated, and centrifuged; procedure was repeated three times. [e] Butanol (one-third the volume of the supernatant) was used to precipitate amylose from supernatant. [f] After first centrifugation, residues were reslurried, reheated, and centrifuged; procedure was repeated two times. [g] Ethanol (half the volume of the supernatant) was used to precipitate amylose from supernatant.

using pullulan standards of known molecular weights (HPSEC/DRI) as described by Jackson (1991).

Determination of Chain Length (DP). Amylopectin and amylose (44 mg of each fraction) were solubilized in 4 mL of 90% DMSO by boiling for 15 min. Each solution was then cooled to 50 °C, and 5.25 mL of sodium acetate buffer (0.1 M, pH 3.5) was added. After 0.75 mL (161.25 units) of pullulanase enzyme (EC 3.2.1.41, Megazyme USA Inc., Boseman, MT) was added, the mixture was subsequently held at 45 °C for 18 h in a shaking water bath. A drop (0.1 M) of 3,5-dinitrosalicylic acid was added, and the solution was held for another 6 h to precipitate any proteins (protein binds to the chromatography columns). To assure protein denaturation, the mixture was boiled for 10 min. Samples were then centrifuged at 3000*g* for 10 min before being filtered (1.2 μm). One milliliter of the filtrate was mixed with 120 mg of mixed bed resin (Bio-Rad Laboratories, Hercules, CA) and shaken at ambient temperature for 30 min in a water bath to remove any ions in solution (Zhang and Jackson, 1992). Fifty microliters of deionized filtrate was then injected into the HPSEC/MALLS system.

Weight-average degree of polymerization (DP_w) was determined by fitting the M_w values calculated from polymodal chromatograms for debranched amylopectins into an equation derived from a calibration curve. Debranched amylopectin chromatograms were divided into three portions at the minima of each curve as shown in Figure 1a−e. Each portion represented a different branched chain length distribution. For AP1, AP4, and AP5, some replicate chromatograms gave bimodal distributions. Each bimodal chromatogram was divided into three regions (F-1, F-2, F-3) using the minima (retention time) derived from its replicate trimodal sample chromatograms. The calibration curve was obtained by injecting pullulan (known molecular weigh) and dextran 3000 (polymerized glucose units) standards. Due to pullulan and dextran standard calibration limitations (>30 min elution times), the highest M_w portion (<30 min elution) of each debranched amylopectin chromatogram could not be analyzed. Amylose M_n was used to calculate number-average degree of polymerization (DP_n) for amylose fractions. Values obtained from this study were compared to apparent branching ratios (BPNI) determined via "wet" chemistry in a previous study (Mua and Jackson, 1996).

X-ray Diffraction. Amylose or amylopectin fraction powders (1 g) were exposed to an X-ray beam (40 kV, 30 mA). The beam's source was Cu K_α radiation generated by a PAD V Scintag X-ray diffractometer and passing through a graphite monochromator filter. A diffraction angle of 2θ was used, and each sample was scanned from 2 to 30°. Curves were smoothed using a fast Fourier transform function (PeakFit software, version 2.0, Jandel Scientific Co., Corte Madera, CA). The areas (pixels squared) of crystalline and amorphous regions were calculated as described by Nara and Komiya (1983) using Sigma Scan Pro software (version 2.0, Jandel Scientific).

Statistical Analysis. Analysis of variance was used to analyze data collected in duplicate for HPSEC/MALLS, and in triplicate for X-ray analysis, using Statistical Analysis System software (version 6.11, SAS Institute Inc., Cary, NC). All measurements were obtained using a completely randomized block design. Mean values and least significant differences (LSD) at $P < 0.05$ were calculated for each analysis. Regression analysis was used to determine relationships between M_w and structural attribute variables.

RESULTS AND DISCUSSION

MW, Branching, and Chain Lengths. *Amylose.* MW and structural attributes of the six amylose fractions (AM1, AM2, AM3, AM4, AM5, AM6), as determined by HPSEC/MALLS, are shown in Table 2. There was a significant difference ($P < 0.05$) between some amylose fractions in M_w and M_n, branching ratios, and DP_n, while polydispersity (M_w/M_n) and longh-chain branch frequencies (LCBF) were not statistically different. Amylose fractions (AM2, AM4, AM1, AM5, AM6, AM3) ranged in order of decreasing M_w from 4.89E+05 to 1.03E+05, while branching ratios (0.3−1.1) differed (Table 2) and seemed to increase with a decrease in M_w (Table 3). Four amylose fractions (AM4, AM5, AM6, AM3, ranging from high to low M_w), showed similar branching ratios, but two of these fractions (AM3, AM4) were more branched than their high (AM2) and low (AM1) M_w counterparts. The high M_w fraction (AM2) had a higher ($P < 0.05$) DP_n than the other amylose fractions. There was a linear relationship between DP_n and M_w (Table 3).

The findings of this study generally agree with those found in the literature (Takeda et al., 1988, 1989, 1990, 1992; Takeda and Preiss, 1993) in that high-amylose starch amyloses had lower DP_n values than regular corn starch amylose. Our DP_n values and branching ratios were also similar to literature values. Regular starch amyloses in this study showed a lower (1.35−1.50) polydispersity than those (1.46−2.66) in the literature. Except for amyloses from 70% amylose starch, the numbers of branch chains per molecule for amyloses from regular and 50% amylose starches were lower for this study than those in the literature.

The reason for the difference in polydispersity may be due to the method of starch fractionation (aqueous dispersion vs aqueous leaching) and method of fraction analysis (HPSEC/DRI vs HPSEC/MALLS). Aqueous dispersion separates amylose or amylopectin fractions possessing wider polydispersities than aqueous leaching

Table 2. Molecular Weight and Structural Parameters of Corn Amylose (AM) and Amylopectin (AP) Fractions[a] Determined by HPSEC/MALLS

						chain length (DP)[f]			
sample	M_w[b]	M_n[c]	M_w/M_n[d]	branching ratio	LCBF[e]	F-1[g]	F-2[h]	F-3[i]	crystallinity[l] (%)
AM1[j]	1.36E+05	1.18E+05	1.3593	0.3350	1568	500			22
AM2	4.89E+05	3.27E+05	1.5241	0.3372	956	1014			24
AM3	1.03E+05	6.78E+04	1.5147	1.0480	743	363			18
AM4	2.47E+05	1.33E+05	1.9037	0.8456	961	864			13
AM5	1.15E+05	6.25E+04	1.8765	0.6848	401	472			18
AM6	1.06E+05	5.44E+04	1.9427	0.7339	313	324			15
LSD[k]	7.91E+04	7.67E+04	0.6210	0.4554	1779.4	540.7			12.4
AP1	9.88E+07	9.11E+07	1.0843	2.0182		139	86	37	21
AP2	7.98E+07	7.62E+07	1.0466	1.4907		113	41	15	28
AP3	8.94E+07	7.21E+07	1.2754	4.3483		86	59	12	17
AP4	7.63E+07	6.61E+07	1.1670	2.9391		118	37	16	30
AP5	7.08E+07	5.85E+07	1.2031	1.5770		173	56	18	36
LSD	1.70E+07	2.85E+07	0.3384	3.4216		74.3	23.5	8.6	18.3

[a] Values are means of two analyses. [b] Molecular weight weight-average. [c] Molecular weight number-average. [d] Polydispersity. [e] Long-chain branch frequency (numbers indicate where branch points occur per 1000 polymerized glucose units). [f] Number-average degree and weight-average degree of polymerization for amylose and amylopectin, respectively. [g,h,i] Represent long, intermediate, and short branch chains, respectively. [j] See Table 1 for full description. [k] Least significant difference ($P < 0.05$). [l] Percent crystallinity was determined by X-ray analysis.

Table 3. Correlation Coefficients between Molecular Weight (M_w) and Molecular Structure Variables of Amylose and Amylopectin Fractions Fractionated from Corn Starch

	amylose		amylopectin	
correlation parameters	M_w[a]	crystallinity (%)	M_w[a]	crystallinity (%)
branch ratio	−0.5865*	−0.6824**	0.1331	0.3797
LCBF[b]	−0.1514	0.4972		
DP[c]	0.8699**	0.4594	0.7149**	−0.8380*
crystallinity (%)	0.6660*		0.5201	

[a] Weight-average molecular weight. *Represents significance at $P < 0.05$. **Represents significance at $P < 0.01$. [b] Long-chain branch frequency per 100 glucose units [c] Number-average and weight-average degree of polymerization for amylose and debranched amylopectin, respectively; reported value was calculated using data for short branched chains.

(Mua and Jackson, 1995), while HPSEC/DRI gives a wider polydispersity than HPSEC/MALLS (Takeda et al., 1988, 1992). Literature values were obtained using amylose and amylopectin components obtained via aqueous dispersion and in some cases characterized by HPSEC/DRI.

In this study, amylose analyzed via HPSEC/MALLS had lower M_w (1.03−4.89E+05 vs 1.40−5.68E+05) and M_w/M_n (1.4−1.9 vs 2.1−4.7) values when compared to those calculated using HPSEC/DRI (Table 2 vs Table 4). Nevertheless, M_w data from both methods were correlated; M_w/M_n was also correlated for both techniques (Table 5). Although branching ratio (HPSEC/MALLS, Table 2) and relative branching (BPNI obtained via "wet chemistry," Table 4) values are not interchangeable, both parameters were correlated (Table 5).

Amylopectin. Results on amylopectin M_w and branching attributes are shown in Table 2. There were significant differences ($P < 0.05$) among some amylopectin fractions in M_w and DP_w of branched chains (F-1, F-2, F-3). Polydispersity and branching ratios were not statistically different (Table 2).

The branched chains decreased in DP_w from F-1, the long branched chain, through F-3, the short branched chain (Table 2 and Figure 1). The long branch chain (F-1) of the low M_w amylopectin fraction (AP5) was longer ($P < 0.05$) than that of an intermediate M_w fraction (AP2) but did not differ from those of its other

Table 4. HPSEC Molecular Weight Distribution, Polydispersity, Purity, and Branch Point Number Index (BPNI) Values[a] of Amylose (AM) and Amylopectin (AP) Fractionated by Aqueous Leaching from Four Different Corn Starches

sample[b]	M_w[c]	M_n[d]	M_w/M_n	purity (%)	BPNI
AP1	5.43E+07	4.18E+07	1.3	100	1.32E−03
AP2	3.92E+07	3.56E+07	1.1	100	7.30E−04
AP3	3.75E+07	2.68E+07	1.4	96	1.54E−03
AP4	3.71E+07	2.65E+07	1.4	92	8.70E−04
AP5	2.88E+07	1.92E+07	1.5	93	1.00E−03
AM1	3.78E+05	1.80E+05	2.1	99	3.79E−05
AM2	5.68E+05	2.10E+05	2.7	99	4.14E−05
AM3	3.30E+05	1.14E+05	2.9	92	2.91E−04
AM4	4.44E+05	1.11E+05	4.0	91	2.10E−04
AM5	1.40E+05	5.38E+04	2.6	94	3.60E−04
AM6	2.90E+05	6.17E+04	4.7	93	1.93E−04
LSD[e]	2.31E+07	2.19E+06	1.05	1.2	4.00E−04

[a] Values are means of three analyses. [b] See Table 1 for sample identification. [c] Weight -average molecular weight. [d] Number-average molecular weight. [e] Means in the same column that are greater than the LSD value are significantly different ($P < 0.05$).

Table 5. Correlation Coefficients between HPSEC/MALLS[a] and HPSEC/Pullulan Standard[b] Molecular Structure Attributes

	HPSEC/pullulan standards					
	amylose			amylopectin		
HPSEC/MALLS	M_w[c]	M_w/M_n[d]	BPNI[e]	M_w	M_w/M_n	BPNI
M_w	0.8266*			0.8948*		
M_w/M_n		0.7662*			0.7989*	
branch ratio			0.7856*			0.6748*

[a] High performance size-exclusion chromatography and multiple-angle laser light scattering. [b] High performance size-exclusion chromatography using pullulan standards of known molecular weights. [c] Weight-average molecular weight. *Represents significance at $P < 0.05$. [d] Polydispersity. *Represents significance at $P < 0.05$. [e] Branch point number index. *Represents significance at $P < 0.05$.

counterparts (AP1, AP3, AP4). Except for the high M_w fraction (AP1), the DP_w for the intermediate and short branch chains (F-2) and F-3, respectively), were not different ($P > 0.05$).

Table 6 shows the molar percentages of the various amylopectin fraction branched chains. The quantity of the short branched chain (F-3) was significantly higher ($P < 0.05$) than those of the long (F-1) and intermediate (F-2) branched chains, for all amylopectin fractions

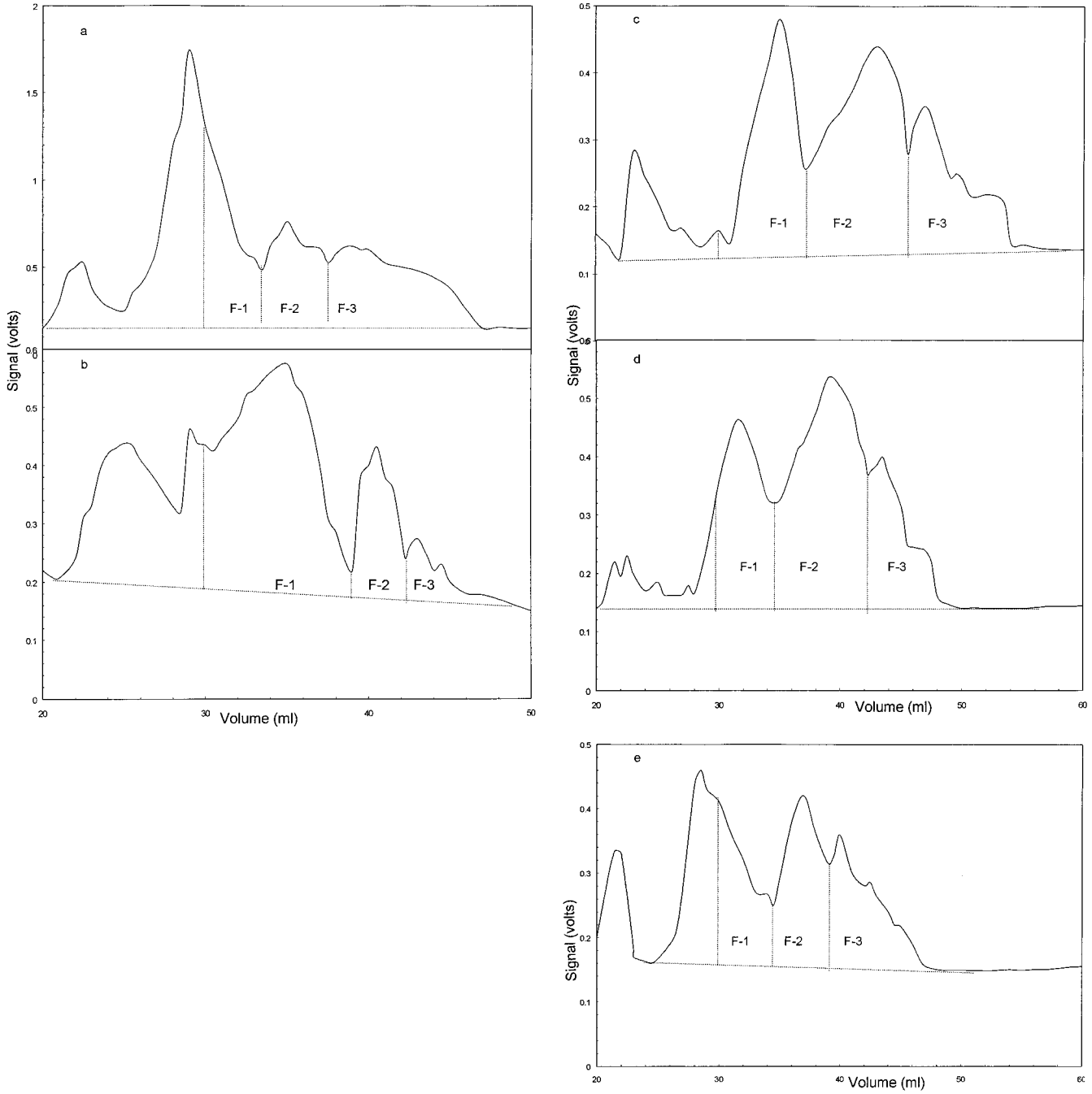

Figure 1. MALLS chromatograms of amylopectin fractions (AP) debranched using pullulanase enzyme and showing different chain length divisions for (a) AP1, (b) AP2, (c) AP3, (d) AP4, and (e) AP5 fractions. Chain lengths decrease in the order F-1 > F-2 > F-3. See Table 1 for detailed description of fractions.

(AP1-AP5). Amylopectin fraction M_w was proportional to DP_w for the short branched chains (Table 3). Except for amylopectin fraction AP1 and AP4 that were higher and lower in F-1 and F-2 branched chain concentrations, respectively, the remaining fractions were not significantly ($P < 0.05$) different (Table 6).

Generally, our amylopectin findings agree with those of other researchers (Hizukuri, 1985; Takeda et al., 1988; Bradbury et al., 1993; Wang et al., 1993; Yuan et al., 1993). Amylopectin DP_w values for this study's intermediate (59−86 for waxy or 37−56 for regular corn starch) and short (12−36 for waxy or 15−18 for regular corn starch) branched chains are close to the intermediate (39−60) and short (15−21) branched chain values reported in the literature. The dominance in quantity of short branched chains is also evident in literature values. Amylopectin from A-type starches (regular and waxy cereals) has been reported to have shorter average chain lengths than B-type (high amylose corn, pea, potato, and canna) starches (Hizukuri, 1985; Shi and Seib, 1992). Studies also show that regular corn amylopectin contains longer branch chains than waxy starch (Takeda et al., 1988; Wang et al., 1993). Some regular corn amylopectins had longer chains than waxy amylopectins in our study. The use of aqueous leaching, which discretely releases polymers of a narrow MW range, may be the reason why we obtained long and short chains for each starch type.

Amylopectin M_w values determined by HPSEC/MALLS were higher (7.08−9.88E+07 vs 2.88−5.43E+07) and M_w/M_n was not different (1.1−1.2 vs 1.1−1.5) when values were compared to those determined by HPSEC/DRI (Table 4). There was a correlation in M_w and M_w/M_n between the two methods (Table 5). Also, branching

Table 6. Percentage Distribution of Amylopectin Fraction Branched Chains Calculated from Molar Concentrations[a]

	molar percentage of branch chains			
sample	F-1 (long)[b]	F-2 (intermediate)[b]	F-3 (short)[b]	LSD
AP1[c]	8	26	72	12.00
AP2	4	20	74	9.29
AP3	2	21	76	7.13
AP4	3	10	78	15.26
AP5	1	16	80	2.54
LSD[d]	3.3	13.8	1.2	

[a] Values are means of two analyses; each value was calculated by dividing the molar concentration of material eluted under each chain's chromatogram by that for the entire chromatogram. [b] See Figure 1 for detailed description. [c] See Table 1 for full description. [d] Least significant difference ($P < 0.05$).

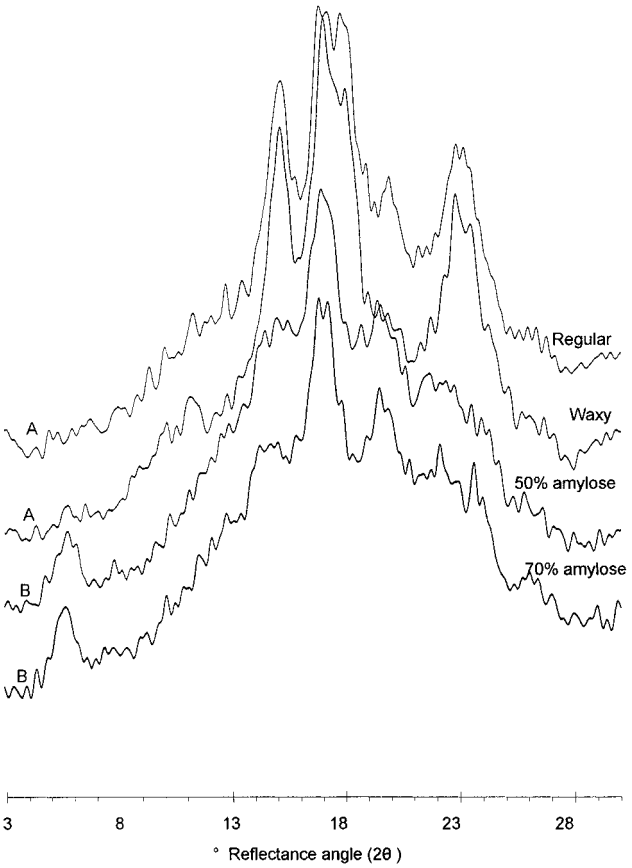

Figure 2. X-ray diffraction patterns of regular, waxy, and 50 and 70% high-amylose (hAM) corn starches. Pattern types are shown on the left-hand side.

ratio (HPSEC/MALLS) and relative branching (determined by wet chemistry) were correlated.

X-ray Analysis. Figure 2 shows the X-ray patterns of native corn (<1, 30, 50, and 70% amylose) starches having 34, 29, 21, and 19% crystallinity, respectively. As expected, the regular and waxy corn starches show an A-type diffraction, while the two high-amylose starches show a B-type pattern. The diffraction patterns of amylose and amylopectin fractions are also shown (Figures 3 and 4).

The fractions gave X-ray patterns not reflective of their parent starch. The high M_w amylose (AM1) fraction and one intermediate one (AM2) gave V-type patterns typical of helical amylose complexes as reported by Zobel (1988). The low M_w fraction (AM3) had a diffraction with both B- and V-type characteristics; the intermediate fraction close to it in M_w (AM6) had a

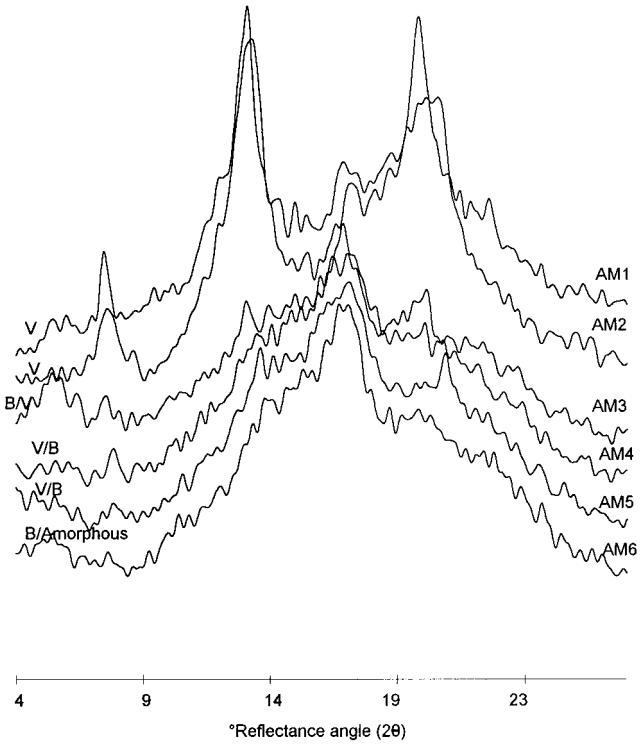

Figure 3. X-ray diffraction patterns of amylose fractions. Pattern types and sample identifications are shown on the left- and right-hand sides, respectively.

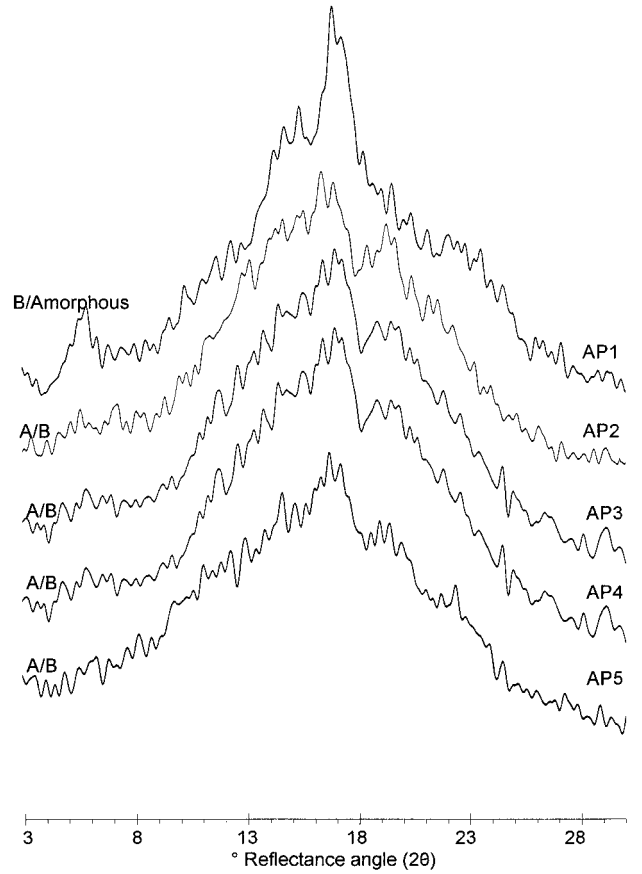

Figure 4. X-ray diffraction patterns of amylopectin fractions. Pattern types and sample identifications are shown on the left- and right-hand sides, respectively.

B/amorphous appearance. The other intermediate fractions (AM4, AM5) were crosses between B- and V-type patterns. All of the amylopectin fraction images were

A/B combinations, except for the high M_w fraction with a B/amorphous complex.

Significant differences in percent crystallinity were observed among amylopectin fractions, whereas no differences were found among amyloses (Table 2). The low M_w fraction (AP5) had a higher crystalline ratio value than one intermediate fraction (AP2), but AP5 did not differ from the other samples. Correlation coefficients between amylose molecular weight and molecular structure parameters (Table 3) revealed a positive relationship between crystallinity and M_w. Meanwhile, there was an inverse relationship between crystallinity and branch ratio.

For amylopectin, crystallinity was inversely related to DP_w (Table 3). Crystallinity increased with decreasing DP_w for amylopectin short branched chains (F-3). Hizukuri (1985) and Zobel (1988) reported that the production of crystalline varieties depended on amylopectin chain length. In his research, Hizukuri (1985) determined the short branched chain of native corn starch to be between 17 and 18, while Zobel (1988) reported a length of 26. These values agree with the values found in this study.

Conclusion. Amylose M_w as determined by HPSEC/MALLS (this study) was 10^5; that of amylopectin was 10^7. The same magnitudes were obtained when samples were analyzed via HPSEC/DRI, in a previous study, using pullulan standards of known molecular weights, but values in this study are slightly lower and higher than those for amylose and amylopectin, respectively. This study also showed lower amylose polydispersities (M_w/M_n) than those we found in the previous study. However, the absolute M_w, (M_w/M_n), and branching ratios for HPSEC/MALLS were correlated to the apparent values for HPSEC/DRI (Table 5).

Polydispersity (1.4−1.9) and branch points per chain of 1000 glucose units (0.6−3.2) were each not different among the amylose fractions, while branching ratios differed (0.3−1.0) and seemed to increase with a decrease in M_w (4.89−1.03E−05). Amylose DP_n increased proportionally to M_w (1.03−2.47E+05). Neither amylopectin polydispersity (1.1−1.2) nor branching ratios (1.5−4.3) were different among the fractions. The high M_w amylopectin fraction (AP1) had longer long (F-1), intermediate (F-2), and short (F-3) branched chains than the other fractions (AP2−AP5). The short branch chains (F-3) were more abundant for each amylopectin fraction, and the chains decreased proportionally for DP_w and M_w. High to low M_w amylopectin fractions with branching values of 1.5 and above, that had short branched chains with DP_w between 15 and 18, showed high crystalline ratios (>28%).

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