

# Structure and Properties of Amylose, Amylopectin, and Intermediate Materials of Oat Starches<sup>1</sup>

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## ABSTRACT

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Starches isolated from three types of oats (*Avena sativa* L.) with a range of lipid contents (6.2, 8.0, and 11.2%) were fractionated into amylose (AM), amylopectin (AP), and intermediate materials (IM) by using 1-butanol precipitation. The structures and physicochemical properties of AM, AP, and IM were characterized by using iodine affinity (IA), blue value, maximum absorbance wavelength, limiting viscosity number, and high-performance size-exclusion chromatography before and after debranching with isoamylase for AM and after debranching for AP and IM. The IA values of AM were 18.4–18.9 g/100 g of starch. The weight-average degree of polymerization (DP<sub>w</sub>) and apparent DP<sub>w</sub> distribution of AM ranged from 939 to 1,208 and from 392 to 2,920 glucose units,

respectively. These values tended to be smaller than those of corn (*Zea mays* L.) and rice (*Oryza sativa* L.) starches reported in literature. The AP had IA values ranging from 0.30 to 0.58 g/100 g of starch. The chain lengths of AP ranged from 204 to 181, 32 to 31, and 20 to 17, by weight of glucose units, for high, intermediate, and low molecular weight fractions, respectively. The chain lengths and chain-length distribution of AP showed differences among oat starch types, with a decreased degree of multiple branching of AP accompanying increased starch-lipid and amylose contents. All structures and properties of IM suggested that the IM contained less highly branched molecules than did AP.

Starch consists of two major chemically distinguishable polysaccharides, amylose (AM) and amylopectin (AP). AM is essentially a linear molecule with a few branches, whereas AP is a highly branched molecule. A third component, intermediate materials (IM), is also found in some starch species (Lansky et al 1949, Banks and Greenwood 1975). Variations in the amounts of AM, AP, and IM, and in their structures and properties, can result in starch granules with very different physicochemical and functional properties, which may affect their utilization in food products or industrial applications (Kobayashi et al 1986, Yuan et al 1993).

The AM content of oat starch ranges from 16 to 33.6%, varying among cultivars and, especially, with the presence of starch-lipid content (MacArthur and D'Appolonia 1979, Paton 1979, Gudmundsson and Eliasson 1989, Sowa and White 1992). Some of the reported variations in percentage of AM could be the result of different analytical methods used. Banks and Greenwood (1967) showed that a pure oat AM, with an iodine affinity (IA) of 19.5 g/100 g of starch, could be produced by repeated crystallization of the first-obtained AM-butanol complex. The structure of oat AP also was studied by Banks and Greenwood (1967), who reported an IA of 0.5 g/100 g of starch and an average chain length that was slightly lower than that of other cereal starches. In addition, MacArthur and D'Appolonia (1979) showed that oat AM tended to have a higher limiting viscosity number [ $\eta$ ] than did wheat (*Triticum aestivum* L.) AM, whereas, oat AP had a slightly lower [ $\eta$ ] than did wheat AP. A third component, IM, also has been identified in oat starch, with IA values being larger than that of AP and molecular weight being less than that of AM (Banks and Greenwood 1967, Paton 1979).

Recently, the structure of AM, AP, and IM has been studied by gel-permeation chromatography (GPC) and high-performance size-exclusion chromatography (HPSEC). The fractions may be debranched with isoamylase before analysis by HPSEC to investigate the chain lengths and chain-length distribution. Starch species that have been studied in this fashion include corn, rice, wheat, tapioca (*Manihot utilissima* L.), sweet potato (*Ipomoea*

*batatas* (L.) Lam.), kuzu (*Pueraria hirsuta* Matsum), lotus (*Nelumbo nucifera* Gaertn.), and potato (*Solanum tuberosum* L.) (Hizukuri and Takagi 1984; Hizukuri 1985, 1986; Takeda et al 1986, 1989). No information about oat starch fractions was found in the literature.

In a previous study, we evaluated the structure and physicochemical properties of three oat starch types isolated from groats containing different lipid contents (L. Z. Wang and P. J. White, unpublished data). From the previous data, the starch-lipid contents of the three types of starches (E77, Dal, and L996) were 1.08, 1.16, and 1.18%, and the amylose contents were 22.1, 25.6, and 26.6%, respectively. The present study was undertaken to characterize the structural and physicochemical properties of AM, AP, and IM fractionated from these same oat-starch types and to study the relationships among these properties and previously determined properties of the starches.

## MATERIALS AND METHODS

### Oat Types

Three types of oat (E77, Dal, and L996) with different oil contents in the whole grains (6.2, 8.0, and 11.2%, respectively) were grown in Ames, IA, in 1991. After harvest, the dehulled oat groats were stored at 4°C and 45% rh until analyzed.

### Starch Isolation

Starches were extracted and purified as described previously (Sowa and White 1992) with modifications by L. Z. Wang and P. J. White (unpublished data). For each starch type, two separate extractions were completed, and the starch was pooled for further preparation.

### Fractionation of Starch

To avoid variations in testing conditions that might cause differences in individual dispersion or fractionation of the starches, the three starch types, individually pooled as just stated, were treated simultaneously. Based on several preliminary fractionation experiments, the complete dispersion of the starch into solution would be likely. The results of IA and blue value (BV) from two preliminary batches of each oat starch type were very similar (data not shown). Additional evidence of the success of starch fractionation was shown by GPC, in which the E77 AM was a pure material uncontaminated by AP or IM (data not shown).

After extraction, the starch was defatted by refluxing in 85% methanol for 24 hr in a Soxhlet extractor and drying overnight at 45°C. Defatted starches were then pregelatinized by: 1) dis-

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persing in 90:10 (v/v) dimethyl sulfoxide (DMSO) and deionized water, 2) stirring in a boiling water bath for 1 hr, and 3) stirring at room temperature for another 24 hr to ensure complete dispersion (Young 1984). Without the pregelatinization to break the strong interchain bonds and remove additional lipids, the subsequent fractionation was unsuccessful.

Each pretreated starch was fractionated into its components (AM, AP, and IM) combining the general procedures of Schoch (1942), Lansky et al (1949), and Takeda et al (1986). Briefly, the pretreated starch (20 g) in DMSO-water solution was precipitated with methyl alcohol; then 1,500 ml of cool water was added; and the mixture was heated with stirring in boiling water until the starch gelatinized. All starch solutions were filtered to remove any insoluble residue. The starch solution was adjusted to pH 5.9–6.3 with a phosphate buffer and autoclaved for 3 hr at 121°C. It was then precipitated with aqueous butanol (15–20% water, v/v). After centrifugation, three layers were evident: an upper supernatant (AP) layer, a bottom precipitate (AM) layer, and a middle layer (IM).

The AM obtained in the first aqueous butanol precipitation was purified further by four recrystallizations from hot aqueous butanol with cooling. Finally, the purified AM fraction was precipitated with methanol, separated by centrifugation, and then redissolved in 90:10 (v/v) DMSO-deionized water. The fraction that did not complex with aqueous butanol, and was in the upper supernatant layer after centrifugation, was considered to be AP and was collected, recrystallized by adding hot aqueous butanol, and then cooled and separated by centrifugation. This purification procedure was repeated four times to successively isolate the AP from contaminating AM and IM. Finally, the purified AP fraction was condensed by vacuum evaporation, precipitated with methanol, separated by centrifugation, and then redissolved in 90:10 (v/v) DMSO-deionized water. The IM fraction was obtained from the middle layer obtained after centrifugation of the complete starch dispersion in an aqueous mixture of 1-butanol as described by Takeda et al (1986). The purification procedure described for AP was used to isolate the IM.

The recovery of each fraction collected from different starch types of the total starch for AM, AP, and IM was about 10, 36, and 4%, respectively. The purity of the fractions was more important than total recovery in our experiments.

#### Physicochemical Properties of AM, AP, and IM

The fractionated AM, AP, and IM in DMSO-water of each oat type were characterized for their physicochemical properties. The IA was determined by standard potentiometric titration (Schoch 1964) at 30°C. The IA was expressed as milligrams of iodine bound to 100 mg of starch. Two separate determinations were done on each sample.

The BV was determined according to Gilbert and Spragg (1964). The same sample was used to measure the wavelength of maximum absorption ( $\lambda_{\max}$ ) from 700 to 500 nm. Three separate determinations were done on each sample.

The  $[\eta]$  (ml/g) was determined with an Ostwald viscometer at 22.5°C, according to the method of Myers and Smith (1964), except that the sample was dissolved in 1N KOH. Three measurements were performed for each sample.

#### High-Performance Size-Exclusion Chromatography

The starch fractions (AM, AP, and IM) were debranched by crystalline *Pseudomonas isoamylase* (Hayashibara Shoji, Inc., Okayama, Japan). The AM and debranched AM, AP, and IM were subfractionated by HPSEC. Briefly, the HPSEC equipment consisted of a Beckman 110 A pump (Beckman Instruments, Inc., Fullerton, CA); 100- $\mu$ l injector loop; Beckman 210 sample injector; two Bio-Sil 125 columns (300  $\times$  7.5 mm); and one guard column (75  $\times$  7.5 mm) (Bio-Rad Laboratories, Richmond, CA); a differential refractometer (Waters, Milford, MA); and a Beckman 10-in. strip-chart recorder. A 100- $\mu$ l sample was injected into the HPSEC system and carried by a flow rate of 0.5 ml/min. The mobile phase (30% DMSO in water) had been filtered through a 0.45- $\mu$ m nylon filter and then degassed with a vacuum

suction device. The columns and the detector were maintained at 30 and 40°C, respectively. The determination of the void volume ( $V_0$ ) was accomplished with oat AP.

For AM, the peak weight-average degree of polymerization ( $DP_w$ ) and apparent  $DP_w$  distribution as defined by  $DP_w$  values at 10% (lowest) and 90% (highest) of the molecular weight range were measured as described by Takeda and Hizukuri (1986). The  $DP_w$  was calculated from the calibration curve generated with pullulan polysaccharide reference standards, and then divided by 162. For AP or IM, the eluted materials were separated into three fractions, with the division being made at minimum points between the fractions according to the detector response of the polysaccharide value and labeled as  $F_1$  (high molecular weight, HMW),  $F_2$  (intermediate molecular weight, IMW), and  $F_3$  (low molecular weight, LMW), in order of elution (Yuan et al 1993). Measurements calculated for AP and IM were: peak  $CL_w$  (weight-average chain length), weight percent (percentages of  $F_1$ ,  $F_2$ , and  $F_3$  within the AP or IM fraction as measured by peak area), moles percent (percentages of  $F_1$ ,  $F_2$ , and  $F_3$  within the AP or IM fraction as calculated by weight percent and  $CL_w$ ), and the ratio of  $F_3$  to  $F_2$  of moles percent (degree of multiple branching of AP or IM).

#### Statistical Analyses

Statistical analyses were performed on all replicated data for each experiment (SAS 1990). Individual analyses on each replicate were used to calculate least significant differences, which were computed at a significance level of  $P < 0.05$ . Correlations were determined, with all replicated data, among structures and physicochemical properties of starch fractions and of starch granules previously characterized (L. Z. Wang and P. J. White, unpublished data).

## RESULTS AND DISCUSSION

#### Structure and Properties of Amylose

Preceding the analyses, the purity of each AM specimen was examined by using HPSEC. No detectable contamination by AP was shown (Fig. 1). Table I summarizes the physicochemical properties of AM isolated from each oat type, including IA, BV,  $\lambda_{\max}$ , and  $[\eta]$ . Their IA ranged from 18.4 to 18.9 g/100 g of starch, with L996 AM being significantly higher than Dal AM. E77 was intermediate in IA. The BV of the AM were significantly different for all oat types, with L996 being the highest (1.437), followed by E77 (1.350), and Dal (1.316). The  $\lambda_{\max}$  value (659 nm) for E77 AM was significantly lower than that of the other two samples (661 and 662 nm, respectively), which were not significantly different from each other. The  $[\eta]$  of AM ranged from 167 to 173 (ml/g) in the order of Dal > E77 > L996 (Table I).

In previous studies, Banks and Greenwood (1967) fractionated oat starch by precipitation and reported an IA of 19.5 g/100 g of starch for oat AM, which was determined by potentiometric titration. When compared with other starch species reported in literature, oat AM in our study had lower IA (18.4–18.9 g/100 g of starch) than did AM from corn (20.1 g/100 g of starch) (Takeda et al 1988) and rice (20.3–21.1 g/100 g of starch) (Takeda and Hizukuri 1986).

Oat AM in our study had lower  $[\eta]$  values than did corn AM (183 ml/g) (Takeda et al 1988) and rice starches (180–208 ml/g) (Takeda and Hizukuri 1986). MacArthur and D'Appolonia (1979), by using an Ubbelohde viscometer, found oat AM to have a  $[\eta]$  value ranging from 246 to 299 ml/g, which was higher than that of wheat AM (233 ml/g). According to Cowie and Greenwood (1957), the relation between degree of polymerization (DP) and  $[\eta]$  is  $DP = 7.4 [\eta]$ . These results suggest that the molecular weight of oat AM is appreciably smaller than those of corn and rice AM.

The differences between IA or  $[\eta]$  values in the current study and those in the literature may be attributed to differences in the starch source and methods used. In general, little information is available about the physicochemical properties of oat starches and its fractions.

Figure 1 shows typical HPSEC profiles of oat AM from each oat type and indicates that the AM was free of AP because there was no carbohydrate in the void volume. The peak  $DP_w$  and apparent  $DP_w$  distribution from the HPSEC analyses are summarized in Table II. The peak  $DP_w$  of oat AM ranged from 939 to 1,208 glucose units, with AM from L996 being significantly lower than that from E77 and Dal. These data are consistent with the  $[\eta]$  analysis in which AM from L996 tended to have a lower  $[\eta]$  than did that of E77 and Dal. This suggests a lower MW for the former starch fraction. The AM of each oat type had a wide distribution of  $DP_w$ , ranging from 392 to 2545 for E77, 568 to 2149 for Dal, and 421 to 2,920 glucose units for L996. Dal AM had the narrowest and L996 had the widest MW distribution among the three samples; however, E77 AM contained compounds of the lowest MW, as also noted in Figure 1. The  $DP_w$  and apparent  $DP_w$  distribution of oat AM is considerably smaller and narrower than those of corn AM (2,270–2,550 and 400–13,400, respectively) (Takeda et al 1988) and rice AM (2,290–2,950 and 210–12,980, respectively) (Takeda and

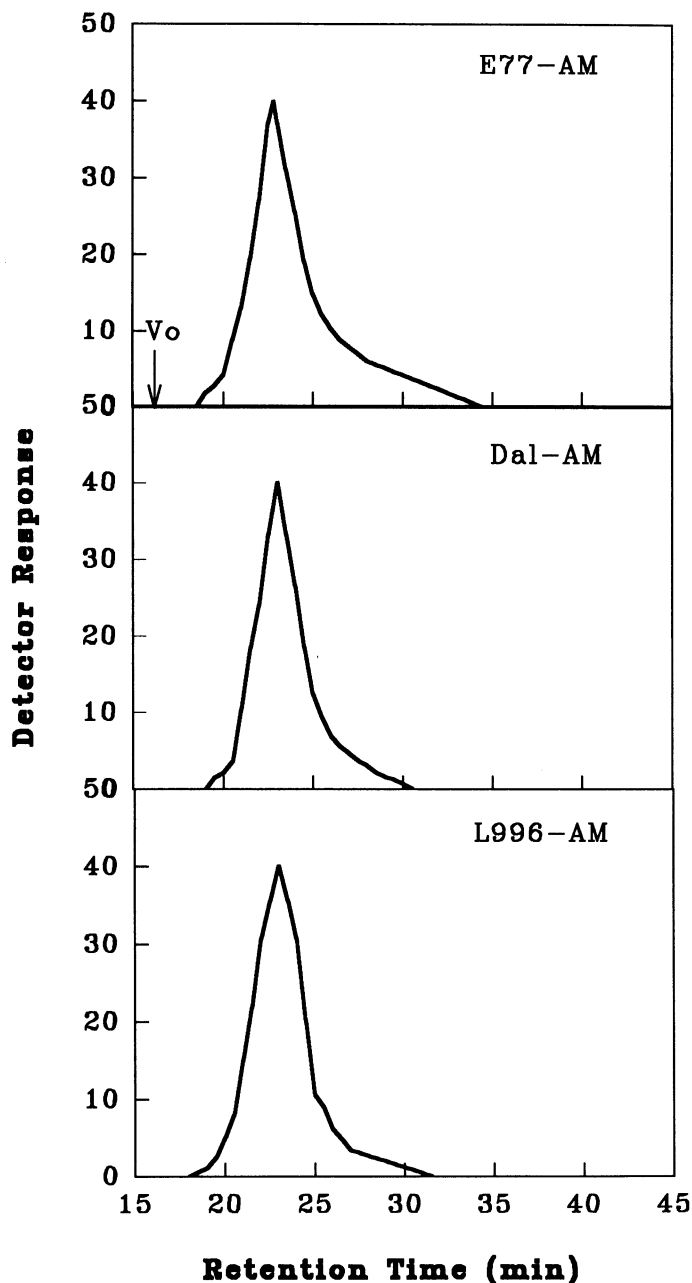


Fig. 1. High-performance size-exclusion chromatography profiles of amylose (AM) from three oat types (E77, Dal, and L996).  $V_0$  = void volume, where amylopectin would elute when present.

Hizukuri 1986). However, it is difficult to compare data from the two studies because differences in fractionation methods may have produced different results.

Amylose is considered to be essentially linear, but it also contains a few branches. This has been confirmed by  $\beta$ -amylase and GPC or HPSEC (Hizukuri et al 1981; Takeda et al 1984, 1988) of starches from many sources. In our study, complete debranching by isoamylase of oat AM was monitored by checking the HPSEC profile of the digest. The branching, achieved in aqueous 30% DMSO on HPSEC, showed a major peak ( $DP_w \sim 700$ ) representing the main amylose chains and a minor peak ( $DP_w \sim 72$ ) representing lower molecular weight materials that were most likely the branches (Fig. 2).

Correlations were determined among features of the structures and physicochemical properties of AM in the current study and starch granules in a previous study (L. Z. Wang and P. J. White, unpublished data). Few significant correlations were found. The  $[\eta]$  of oat AM was negatively correlated with solubility of starch granules at 85°C ( $r = -0.78$ ,  $P < 0.05$ ), indicating that the larger the AM molecular size, the less is the solubility at 85°C. The  $\lambda_{max}$  of oat AM was positively correlated with starch-lipid content ( $r = 0.95$ ,  $P < 0.01$ ) and amylose content ( $r = 0.94$ ,  $P < 0.01$ ).

### Structure and Properties of Amylopectin

The physicochemical properties of AP isolated from the three oat starch types are listed in Table I. All AP samples absorbed little iodine. IA values ranged from 0.30 to 0.58 g/100 g of starch. The BV responses were fairly low. This suggests the presence of short branch chains and freedom from AM. Dal AP had the

TABLE I  
Physicochemical Properties of Oat Components<sup>a</sup>

Sample	Iodine Affinity (g/100 g of starch)	Blue Value	$\lambda_{max}$ (nm)	$[\eta]^b$ (ml/g)
Amylose				
E77	18.7	1.350	659	170
Dal	18.4	1.316	661	173
L996	18.9	1.437	662	167
LSD <sup>c</sup>	0.3	0.015	1	5
Amylopectin				
E77	0.58	0.093	560	140
Dal	0.30	0.087	557	124
L996	0.49	0.080	558	146
LSD	0.06	0.015	1	5
Intermediate Materials				
E77	1.26	0.170	575	145
Dal	0.62	0.100	567	145
L996	0.82	0.106	571	148
LSD	0.04	0.024	1	7

<sup>a</sup>Values are the mean of three determinations, except for IA values, which are the mean of two determinations.

<sup>b</sup>Limiting viscosity number.

<sup>c</sup>Least significant difference at a significance level of  $P < 0.05$ .

TABLE II  
Degree of Polymerization ( $DP_w$ ) and Apparent Distribution of  $DP_w$  for Oat Amylose<sup>a</sup>

Sample	Peak $DP_w^c$	Apparent $DP_w$ Distribution <sup>b</sup>	
		Lowest	Highest (estimated) <sup>d</sup>
E77	1,208	392	2,545
Dal	1,185	568	2,149
L996	939	421	2,920
LSD <sup>c</sup>	106	80	163

<sup>a</sup>Values are the mean of two determinations.

<sup>b</sup> $DP_w$  values at 10% (lowest) and 90% (highest) of the molecular weight range.

<sup>c</sup>Weight-average degree of polymerization, expressed as weight of glucose units.

<sup>d</sup>These values are little larger than the molecular weight of the standards fitting on the straight line portion of the standard curve, so values are estimated.

<sup>e</sup>Least significant difference at a significance level of  $P < 0.05$ .

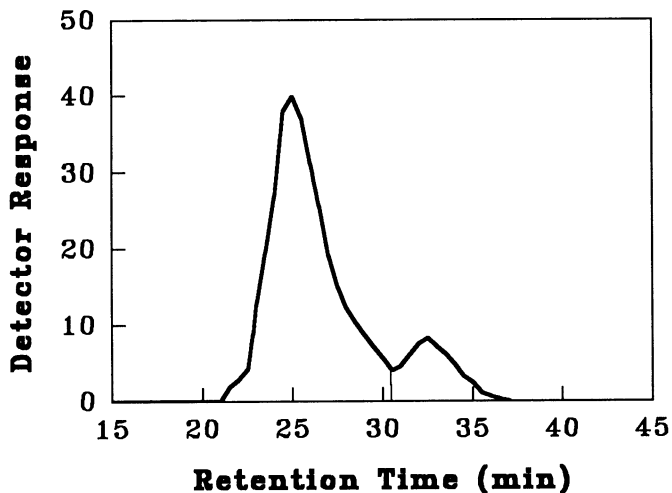


Fig. 2. A typical high-performance size-exclusion chromatography profile of isoamylase-debranched oat amylose.

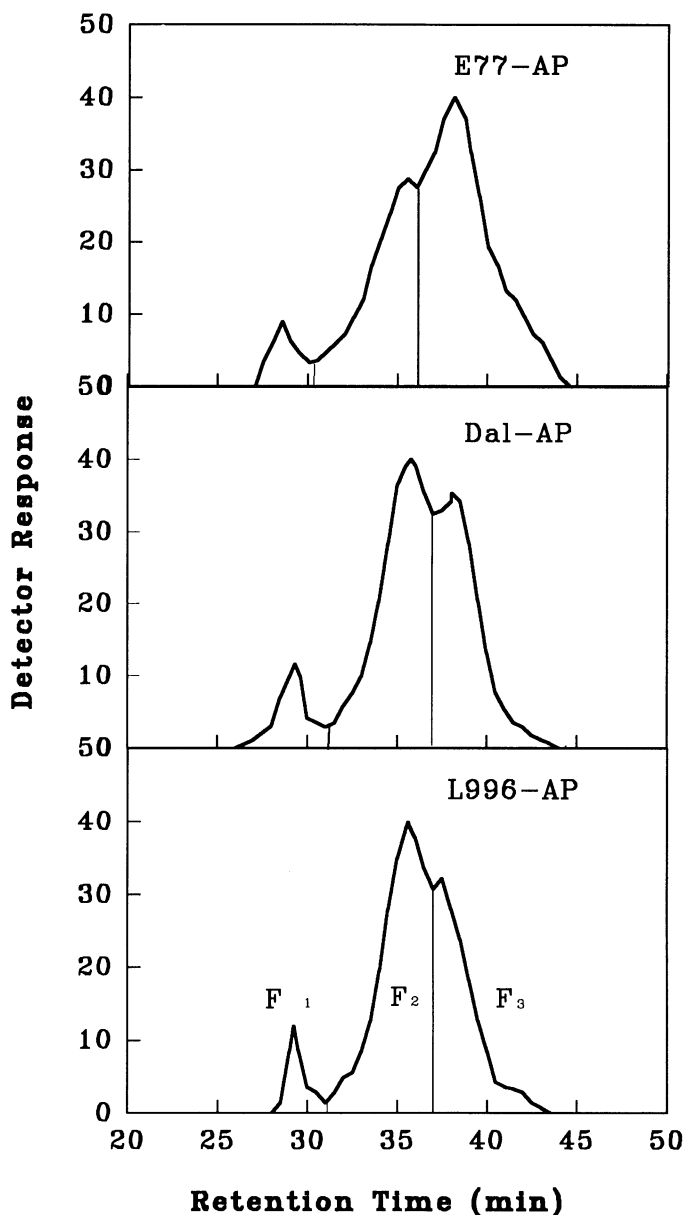


Fig. 3. High-performance size-exclusion chromatography profiles of isoamylase-debranched amylopectin (AP) from three oat types (E77, Dal, and L996). Fractions  $F_1$ ,  $F_2$ ,  $F_3$  contained high molecular weight chains, intermediate molecular weight chains, and low molecular weight chains, respectively.

lowest IA value (0.30 g/100 g), followed by L996 AP (0.49 g/100 g) and E77 AP (0.58 g/100 g). No significant variations ( $P > 0.05$ ) were observed in the BV responses of the AP from the different oat types. The  $\lambda_{\max}$  of E77 AP was significantly higher than those of Dal and L996, which were not significantly different from each other. The  $[\eta]$  of Dal AP was significantly lower than that of E77 AP, which was lower than that of L996 AP. The low  $[\eta]$  of Dal AP suggests a more compact or more spherical molecule with a more compact structure (Takeda et al 1986).

In previous work, Banks and Greenwood (1967) fractionated oat starch by precipitation and reported an IA of 0.5 g/100 g for oat AP, which was similar to that of the present study. MacArthur and D'Appolonia (1979), by using an Ubbelohde viscometer, found a  $[\eta]$  value ranging from 170 to 207 (ml/g) for oat AP, compared with 180 ml/g for wheat AP. These values are higher than  $[\eta]$  values determined in our study, but it is difficult to compare the data because a different method of determination  $[\eta]$  was used for each study. No other data of physicochemical properties on oat AP were found in the literature.

Figure 3 shows typical HPSEC profiles of the debranched AP from oat starches. The shapes of the chromatograms varied among the samples. The profiles were divided into three fractions,  $F_1$  (HMW),  $F_2$  (IMW), and  $F_3$  (LMW). For each HPSEC profile, one minor peak ( $F_1$ ) and two major peaks ( $F_2$  and  $F_3$ ) of carbohydrate components were evident. The  $F_1$ , occasionally observed by other workers, has been considered to be an incompletely debranched material (Lii and Lineback 1977, Atwell et al 1980). Because of the low IA values of AP, it is likely that  $F_1$  contains slightly branched long B chains of AP or IM remaining after fractionation. The peak  $CL_w$ , weight percent, and moles percent of the AP from each oat type, and the ratio of  $F_3$  to  $F_2$  of moles percent, were measured and calculated as shown in Table III.

The  $CL_w$  of  $F_1$  were significantly different among samples, with E77 AP having the highest  $CL_w$  value (204.2) and L996 AP having the lowest  $CL_w$  value (181.7). The  $CL_w$  of  $F_2$  (30.7–31.8) were not significantly different among the samples. The L996 AP had a significantly higher  $CL_w$  for  $F_3$  than did E77 and Dal AP, which were not significantly different from each other. The  $CL_w$  results suggest a structural difference among the AP of the different oat starch types. In comparison with AP of corn starch, oat AP had a lower  $CL_w$  of  $F_2$  (46–47), indicating shorter long-branch chains for oat AP, but oat AP was similar to corn (17–18) in  $CL_w$  of  $F_3$  (Takeda et al 1988).

TABLE III  
Percentage Compositions and Chain Length Distribution of Isoamylase-Debranched Amylopectin (AP) from Oat Starches<sup>a</sup>

Sample	Fraction <sup>b</sup>			$F_3/F_2$
	$F_1$	$F_2$	$F_3$	
Peak $CL_w$ <sup>c</sup>				
E77	204.2	31.8	16.6	
Dal	190.4	31.3	17.1	
L996	181.7	30.7	20.1	
LSD <sup>d</sup>	7.5	3.5	2.9	
Weight % <sup>e</sup>				
E77	7.3	31.8	60.9	
Dal	8.7	46.4	44.9	
L996	5.6	49.2	45.9	
LSD	1.0	7.3	7.0	
Moles % <sup>f</sup>				
E77	0.8	21.2	78.0	3.7
Dal	1.2	39.4	59.4	1.5
L996	0.8	40.9	58.3	1.4
LSD	1.0	8.1	9.9	0.3

<sup>a</sup> Values are the mean of two determinations.

<sup>b</sup> High, intermediate, and low molecular weight fractions.

<sup>c</sup> Weight average chain length.

<sup>d</sup> Least significant difference at a significance level of  $P < 0.05$ .

<sup>e</sup> Percentages of the  $F_1$ ,  $F_2$ , and  $F_3$  within the AP fraction as measured by peak area.

<sup>f</sup> Percentages of the  $F_1$ ,  $F_2$ , and  $F_3$  within the AP fraction as calculated from weight % and  $CL_w$ .

Among the three types of oat AP, the Dal AP had the highest ( $P < 0.05$ ) weight percent of  $F_1$  (8.7%), followed by E77 AP and L996 AP with 7.3 and 5.6% wt%, respectively. The moles percent values of  $F_1$  were similar among samples and were very small because the  $CL_w$  of  $F_1$  was large. Therefore, these values are not discussed. The weight percent and moles percent of  $F_2$  and  $F_3$  of the Dal AP and L996 AP were not significantly different from each other. In contrast, the E77 AP had significantly smaller and larger amounts of  $F_2$  and  $F_3$ , respectively, than did Dal and L996 AP. E77 AP had a higher moles percent ratio ( $F_3/F_2 = 3.7$ ) than did Dal and L996 AP, which were not significantly different from each other. This suggests a more branched structure for the former starch, as described by Biliaderis et al (1981).

Correlations also were analyzed among features of the structures and physicochemical properties of AP determined in this study and of starch granules determined in a previous study (L. Z. Wang and P. J. White, unpublished data). Some correlations

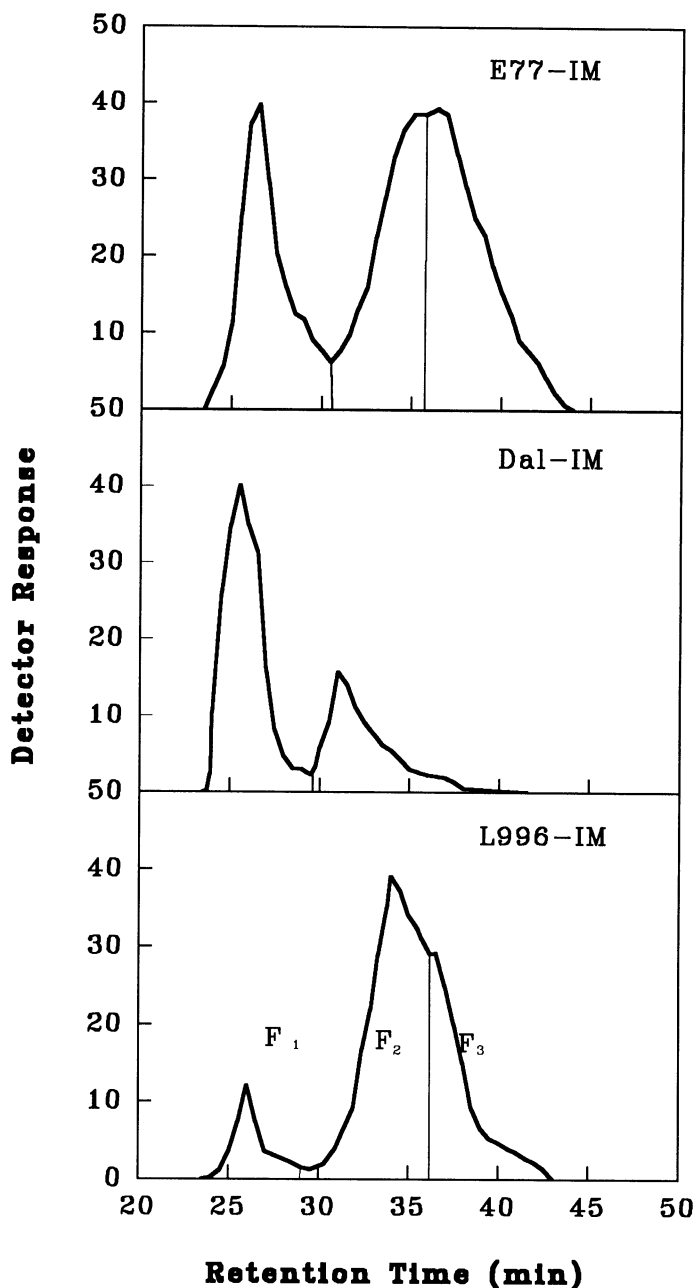


Fig. 4. High-performance size-exclusion chromatography profiles of isoamylase-debranched intermediate materials (IM) from three oat types (E77, Dal, and L996). Fractions  $F_1$ ,  $F_2$ ,  $F_3$  contained high molecular weight chains, intermediate molecular weight chains, and low molecular weight chains, respectively.

were found. The starch-lipid content and amylose content were negatively correlated with weight percent of  $F_3$  ( $r = -0.85$ ,  $P < 0.05$  and  $r = -0.92$ ,  $P < 0.01$ , respectively) and with moles percent ratio ( $r = -0.89$ ,  $P < 0.05$  and  $r = -0.97$ ,  $P < 0.01$ , respectively), indicating that the amount of LMW chains and moles percent ratio of AP decreased with increased starch-lipid and amylose contents.

### IM Structure and Properties

Table I summarizes the properties of the IM fractions of each starch type. All IM fractions gave a deep-blue color with iodine that was visually similar to the AM-iodine color. However, there was less depth to the color, as shown by the lower IA and BV (Table I). For all IM fractions, the IA were about twice those of AP. The BV and  $\lambda_{max}$  for the IM were slightly higher than values for AP (Table I). These results suggested that the IM were close to AP in structure but had longer branch lengths. The  $[\eta]$  of IM were similar among starch types and similar to  $[\eta]$  values for AP, which indicated similar molecular sizes of the two fractions. Takeda et al (1986) studied sweet potato starches and reported that the structure and properties of sweet potato IM were close to those of AP with long-unit chains inasmuch as the BV,  $[\eta]$ , and chain length of IM were very similar to those of AP, and the IA of IM were twice those of the AP.

Typical HPSEC profiles of the debranched IM from the oat starches are shown in Figure 4. The profiles were divided into three fractions ( $F_1$ ,  $F_2$ , and  $F_3$ ) as described for AP. The peak  $CL_w$ , weight percent, and moles percent of the IM from each oat fraction and the ratio of  $F_3$  to  $F_2$  of moles percent are listed in Table IV.

Among the three samples, Dal IM had the longest ( $P < 0.05$ )  $CL_w$  of  $F_1$  and  $F_2$ , followed by L996 IM and E77 IM. The  $F_3$  of E77 IM and L996 IM were not significantly different from each other. It was difficult to determine a separation point between  $F_2$  and  $F_3$  of Dal IM, so all lower molecular weight materials were considered to be in  $F_2$ , and no  $F_3$  was measured. For all IM, the  $CL_w$  of  $F_1$ ,  $F_2$ , and  $F_3$  were longer than the  $CL_w$  of the same fractions for AP, indicating longer branch chains for the IM. These findings also agree with those of previous reports (Banks and Greenwood 1975, Wang et al 1993).

The weight percent and moles percent of Dal IM were greatest for  $F_1$  among starch types, whereas the weight percent and moles percent of L996 IM were least for  $F_1$  and greatest for  $F_2$ . The

TABLE IV  
Percentage Compositions and Chain Length Distribution of Isoamylase-Debranched Intermediate Materials (IM) from Oat Starches<sup>a</sup>

Sample	Fraction <sup>b</sup>			
	$F_1$	$F_2$	$F_3$	$F_3/F_2$
Peak $CL_w$ <sup>c</sup>				
E77	280.0	34.5	22.6	
Dal	341.7	79.9	NA <sup>d</sup>	
L996	310.9	42.2	21.8	
LSD <sup>e</sup>	24.8	5.5	2.3	
Weight % <sup>f</sup>				
E77	24.1	27.1	48.9	
Dal	86.1	14.2	NA	
L996	5.8	59.4	34.9	
LSD	6.2	8.8	3.3	
Mole % <sup>g</sup>				
E77	2.9	26.0	71.2	2.7
Dal	62.3	37.7	NA	NA
L996	0.7	46.7	52.8	1.1
LSD	10.0	6.8	5.1	0.3

<sup>a</sup> Values are the mean of two separate determinations.

<sup>b</sup> High, intermediate, and low molecular weight fractions.

<sup>c</sup> Weight average chain length.

<sup>d</sup> Not available.

<sup>e</sup> Least significant difference at a significance level of  $P < 0.05$ .

<sup>f</sup> Percentages of the  $F_1$ ,  $F_2$ , and  $F_3$  within the IM fraction as measured by peak area.

<sup>g</sup> Percentages of the  $F_1$ ,  $F_2$ , and  $F_3$  within the IM fraction as calculated from weight % and  $CL_w$ .

weight percent and moles percent of  $F_3$  were higher for E77 IM than for L996 IM. The E77 IM and Dal IM had greater weight percent and moles percent of  $F_1$  and lesser weight percent of  $F_2$  and  $F_3$  than did the respective AP fractions. The L996 IM and AP were similar in weight percent and moles percent of  $F_1$ , but  $F_2$  of the IM were higher and  $F_3$  lower in weight percent and moles percent than the same fractions of AP, meaning that the L996 IM contained more IMW chains and fewer LMW chains than did the AP.

The moles percent ratio ( $F_3$  to  $F_2$ ) of E77 IM was higher ( $P < 0.05$ ) than that of L996 IM, and all IM moles percent ratios were lower than their respective ratios for AP. Because  $F_3$  in Dal was negligible, its mole ratio would be very small. These results indicate that the E77 IM fraction was more branched than was L996 IM, and that all oat IM were less branched than were the AP.

Correlations were determined among features of the structures and physicochemical properties of IM reported in this study and of starch granules determined in a previous study (L. Z. Wang and P. J. White, unpublished data). The IA, BV, and  $\lambda_{\max}$  of IM were positively correlated with each other ( $P < 0.01$ ). The  $r$  values were: IA vs. BV,  $r = 0.94$ ; IA vs.  $\lambda_{\max}$ ,  $r = 0.97$ ; BV vs.  $\lambda_{\max}$ ,  $r = 0.88$ . The starch-lipid and amylose content were negatively correlated with weight percent of  $F_3$  of IM ( $r = -0.96$ ,  $P < 0.05$  and  $r = -0.99$ ,  $P < 0.01$ , respectively), indicating that the amount of LMW chains of IM decreased with increased starch-lipid and amylose content. As expected, the moles percent ratio (degree of branching) was negatively correlated with amylose content ( $r = -0.99$ ,  $P < 0.01$ ), meaning that increased amylose content was related to decreased degree of branching of IM.

## CONCLUSIONS

The structures and properties of the AM, AP, and IM fractionated from oat groats with different lipid contents were investigated. The oat AM tended to have a lower IA value, smaller  $DP_w$ , and narrower apparent  $DP_w$  distribution than did corn and rice AM reported in literature. The oat AP had IA values ranging from 0.30 to 0.58 g/100 g of starch and low BV values, suggesting the presence of short branch chains. The HPSEC profiles of debranched oat AP showed distinguishable chain lengths and chain-length distribution patterns among oat starch types. The weight percent of  $F_3$  and moles percent ratio of AP were negatively correlated with starch-lipid and amylose contents, indicating that the amount of LMW chains and moles percent ratio of the oat AP decreased with increased starch-lipid and amylose contents. The oat IM had slightly higher IA, BV,  $\lambda_{\max}$ , and  $CL_w$  of  $F_2$  and  $F_3$  and significantly higher ( $P < 0.05$ )  $CL_w$  of  $F_1$  values than did oat AP, suggesting that the IM were close to AP in structure but with longer branch lengths. A description of the functional properties of the oat starches and the relationships among these functional properties and structures and physicochemical properties of the starch granules and their fractions will be reported later (L. Z. Wang and P. J. White, unpublished data).

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