Characterization of Starch Isolated from Oat Groats with Different Amounts of Lipid[1]

S. M. HARTUNIAN SOWA[2] and P. J. WHITE[2,3]

ABSTRACT

Cereal Chem. 69(5):521-527

Oat starches isolated from three groat types containing different amounts of lipid (6.2, 8.0, and 15.5%) were analyzed for protein, lipid, and phosphorus contents and characterized by iodine affinity, gel permeation chromatography, scanning electron microscopy, and differential scanning calorimetry (DSC). All oat starches contained greater amounts of lipid, phosphorus, and amylose (30–34% by iodine affinity) than did starch from most other native cereal grains reported in the literature. Preliminary results from gel permeation chromatography revealed a large amount of the low-molecular-weight starch fraction, a fact suggesting the existence of intermediate molecular-weight starch. As determined by scanning electron microscopy, the oat starches had small, irregularly shaped granules 3–10 μm in size. As determined by DSC, the starches exhibited gelatinization onset temperatures differing significantly by starch type (ranging from 55.5 to 62.4°C), gelatinization enthalpies ranging from 2.05 to 2.20 cal/g, and a high amylose-lipid peak. After storage for up to 28 days followed by rescanning on the DSC, oat-type starches had retrograded only 32–40%, and the amylose-lipid peak had only partly reformed.

Approximately 78% of the U.S. oat (*Avena sativa* L.) crop is used for livestock feed, and only 22% is used for human consumption (Schrickel 1986). But there is increasing interest in the use of oats and its components for industrial and food purposes. Oat products high in water-soluble fiber offer several dietary benefits. Chen and Anderson (1979), Kirby et al (1981), Klopfenstein and Hoseney (1987), and many others have suggested that the high β-glucan content in oat bran may help reduce serum cholesterol, and Anderson et al (1991) have reported that serum glucose levels and low-density lipoprotein-cholesterol levels are significantly reduced with consumption of oat-hull fiber in men with type II diabetes. Oats may also be used as a source of protein concentrates and isolates (Cluskey et al 1973, Wu et al 1973) and has recently been explored as a possible oil-seed crop (Frey et al 1975, Schipper and Frey, *in press*). Additionally, because starch granules in oats are small (3–10 μm), the starch may be useful for paper sizing, textiles, pharmaceuticals, and other industrial purposes (Caldwell and Pomeranz 1973, Paton 1986, Wilhem et al 1989).

The interest in oats has been accompanied by an increased interest in the study of its starch. Limited research suggests that oat starch does possess some unique chemical, physical, and structural properties. In one study, oat starch had a higher lipid content (1.2%) than did other cereal starches (Clendenning and Wright 1945). Acker and Becker (1971), Youngs (1974), Morrison et al (1984), and Gudmundsson and Eliasson (1989) also reported an unusually high lipid content (1.2–2.3%) for several starches from different oat varieties; a large amount of phosphorus (0.06–0.08%) in the form of lysophospholipids was noted by Paton (1977) and by Morrison et al (1984).

The amylose content of oat starch ranges from a low of 16–18% (Paton 1979) to a high of 26–29% (MacArthur and D'Appolonia 1979, Morrison et al 1984, Doublier et al 1987, Gudmundsson and Eliasson 1989). In addition, Banks and Greenwood (1967) and Paton (1979) reported a relatively large amount of intermediate starch fraction having properties of both amylose and amylopectin; these are termed *anomalous amylose* and *anomalous amylopectin*, respectively.

Some starches from different oat varieties are quite shear-sensitive when held at 95°C, a characteristic typical of waxy maize and potato starches. Oat starch pastes examined with an Ottawa starch viscometer developed unusually high initial setback viscosities within the first 30 sec of cooling and exhibited 75–80% of the final setback viscosity within this short period. These results suggest that the behavior may result from the large amount of intermediate starch fraction found in some oat variety starches (Paton 1979).

Oat starch, in contrast to those of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), contains a relatively large amylose-lipid complex; moreover, as shown by differential scanning calorimetry (DSC), the gelatinization temperature for native oat starch lies between 57 and 62°C (Paton 1987, Gudmundsson and Eliasson 1989). Lineback (1984) reported a slightly lower gelatinization temperature of 53–59°C for oat starch, probably because of structural differences among starches from different oat varieties.

The purpose of the present study was to characterize starches isolated from three different oat cultivars containing a wider range of lipid contents (6.2, 8.0, and 15.5%) than previously reported. This knowledge should lead to improved understanding of the previously reported unique chemical, physical, and structural properties of oat starch.

MATERIALS AND METHODS

Oat Groat Samples and Analyses

Groats (caryopses) of three oat lines were supplied by the Iowa State University Agronomy Department and stored at 4 ± 1°C and 45% rh until analyzed both for lipid content by means of wide-line nuclear magnetic resonance (Conway and Earle 1963) and for protein content by means of near-infrared reflectance (Williams 1975). Additionally, the groats were analyzed for total starch content (Holm et al 1986), and the β-glucan content was determined by the automated flow injection analysis procedure developed by Jørgensen and Aastrup (1988). The weight (in grams) of 100 random whole, unfractured groats was determined in triplicate to compare the groat sizes of the different oat lines.

Starch Extraction

Starch was extracted from the oat groats according to the method of Morrison et al (1984) with certain modifications. The groats were placed in a plastic bag, cracked lightly with a wooden pestle, steeped overnight in 0.02*M* HCl (100 ml) at 4 ± 1°C, and neutralized with 0.2*M* NaOH. The aqueous solution was drained through a sieve, and the solids were recovered from the liquor by centrifuging at 2,000 × *g* for 5 min. The steeped material was rubbed gently with a pestle to expose a large surface area for proteolytic digestion. All solids were collected and suspended in a 0.1*M* Tris-HCl buffer (pH 7.6, 100 ml) containing 0.5% (w/v) $NaHSO_3$ and 0.01% thimerosal and stirred magnetically for 1 hr. Protease (Sigma, type XIV) was added (5 mg/g of original grain), and the suspension was digested overnight at 4 ± 1°C with constant magnetic stirring. On the third day of extraction, the digested material was rubbed gently with a pestle to release the starch, and the starch suspension was passed through a sieve

[1]Journal paper J-14699 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project 2568.

[2]Graduate student and associate professor, respectively, Department of Food Science and Human Nutrition and Center for Crops Utilization Research, Iowa State University, Ames.

[3]Author to whom correspondence should be addressed, 3367 Dairy Industry Building, Iowa State University, Ames, IA 50011.

(75 μm). This process was repeated six to seven times, with distilled water added each time, until no additional starch was extracted. The combined washings were centrifuged for 5 min at 2,000 × *g* to obtain crude starch. The solids obtained were resuspended in distilled water and centrifuged for 20 min at 2,000 × *g*, yielding a dark protein layer that was scraped off the starch surface. To obtain pure starch, that is starch free of protein, the starch was resuspended in distilled water and centrifuged three to four times for 30 min at 18,000 × *g*, steeped for 15 min in 0.02*M* HCl (pH 2.0) to inactivate any residual protease or other enzymes, and washed twice with water. Finally, the starch was collected in a petri dish and air-dried at 4 ± 1°C for a minimum of three days. The isolated starch was stored at 4 ± 1°C and 45% rh until analyzed.

Protein and Moisture Contents of Starch

The percentage of protein remaining in the samples after extraction was determined by means of a Hach Digesdahl Unit, a Flow Through Cell Kit (DR/3A) (Hach Chemical Co., Ames, IA), and the procedure for crude protein analysis as reported by Hach et al (1987).

Moisture was determined by drying the starch samples in aluminum drying pans for 1 hr at 130 ± 1°C (AACC 1983).

Extraction of Starch Lipids

The lipids remaining in the starch after isolation were extracted, and the percentage of lipid was determined according to the method of Morrison and Coventry (1985), with certain modifications: 2 g of oat starch was refluxed under nitrogen in 40.0 ml of 75% *n*-propanol. The sample was refluxed for two 2-hr extractions, and one 1-hr extraction. Additional refluxing for up to 7 hr resulted in no additional lipid removal. After each extraction period, the starch-alcohol solution was cooled in an ice bath for 2 min; the solvent was carefully poured off; fresh solvent was added to the flask; and the starch was liberally washed with 75% *n*-propanol and collected by filtration. Solvent and lipids from the extraction process were collected in a 250-ml round-bottomed flask, and the solvent was removed by rotary evaporation. The lipids were quantitatively transferred to a smaller pre-weighed round-bottomed flask, and the remaining solvent was removed. Finally, the lipids were dried at 100°C for 30 min, desiccated, and weighed. The lipid percentage was determined as the difference in weights of the flask before and after lipid collection. When defatted starch was tested by DSC, no amylose-lipid peak was observed, a fact suggesting complete removal of the starch lipid.

Determination of Starch Fractions

Iodine affinity. Total percentage of amylose in the starch was determined by the standard potentiometric titration of amylose with iodine (Schoch 1964).

Gel permeation chromatography. A Sepharose CL-2B column and the procedure of Jane and Chen (1992) were used to fractionate the oat starch samples by gel permeation chromatography (GPC). Briefly, a completely defatted oat starch sample (1.0 g) dissolved in dimethyl sulfoxide was precipitated from solution with absolute methanol (45 ml). The starch precipitate was collected by centrifugation and dissolved in 40 ml of boiling water. After cooling to room temperature, glucose (8.0 mg) was added to serve as a marker, and water (10.0 ml) was added to make a total of 50.0 ml of solution.

An aliquot of this solution (5.0 ml) was injected onto a 2.6- × 80-cm column (Pharmacia Inc., Piscataway, NJ) packed with Sepharose CL-2B gel. The sample was eluted in an ascending direction, and distilled water containing 0.02% NaCl was used as an eluent, with a flow rate of about 30 ml/hr. Fractions of 4.8 ml per cup were collected and analyzed by using the Autoanalyzer II (Technicon Instruments Corp., Elmsford, NY). Responses of amylose-iodine blue value and total carbohydrate (anthrone-sulfuric acid reaction) were measured at 640 and 630 nm, respectively. The amylose percentage of each starch sample was calculated by totaling the amounts of polysaccharide under the low-molecular-weight peak of the total carbohydrate curve and dividing by the total polysaccharide content in all fractions. One chromatogram for each starch type was evaluated.

Scanning Electron Microscopy

For scanning electron microscopy preparation, a small amount of starch was attached to individual brass discs containing 3M metallic tape (3M Co., St. Paul, MN). The mounted samples were sputter-coated with a platinum-palladium alloy (60:40) for 4 min and then desiccated.

The scanning electron micrographs were taken on a JEOL JSM-35 scanning electron microscope (JEOL, Peabody, MA) at an accelerating voltage of 10–15 kV with Polaroid 665 film. The size of the starch granules was estimated by averaging the diameter of 10–15 granules from each of the replications.

Phosphorus Analysis

Phosphorus contents in the native oat starches and in the isolated starch lipids were determined according to Procedure A in the method described by Morrison (1964).

DSC

Determination of starch thermal properties. The thermal properties of the oat starches were studied by means of a Perkin-Elmer DSC 7 equipped with a Perkin-Elmer 3700 data station (Perkin-Elmer Corp., Norwalk, CT). The instrument was regularly calibrated with indium and zinc standards and operated at a pressure of 14,000 kPa of N_2. The samples were prepared with a water-to-starch ratio of 2:1 (for example, 4.32 mg of starch and 8.6 ml of water by weight), and the samples were hermetically sealed with a volatile sample sealer. Each sample was heated at a rate of 10°C/min from 30 to 120°C, with an aluminum sample pan containing 8.6 ml of distilled water as the reference pan.

Two endothermic peaks were observed for each thermal measurement. The lower-temperature endotherm, M1, corresponded to the melting of starch crystallites, and the higher-temperature endotherm, M2, corresponded to the melting of an amylose-lipid complex. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures and the enthalpy of the transitions (ΔH) were automatically recorded by the data station. The thermal properties statistically analyzed and reported for peaks were T_o, ΔH, range (R), and peak height index (PHI). All endotherms were essentially symmetrical, which allowed the range to be calculated as $2(T_p - T_o)$, as previously described by Krueger et al (1987). Additionally, the PHI for M1 and M2, calculated as $\Delta H/(T_c - T_o)$, was used to define the shape of each peak (Krueger et al 1987). A large PHI indicates a tall, narrow peak; a small PHI indicates a short, wide peak.

Determination of starch retrogradation. Retrogradation of the starches during storage was measured as described by White et al (1989). After the initial DSC run described previously for day 0, sample pans were stored at 4°C for either 7, 14, 21, or 28 days. Each storage time required an initial day 0 scan; thus, each day 0 measurement was coupled with a retrogradation measurement. No significant differences existed among storage days for starch from a single oat type; thus, values for all storage days were averaged.

Stored samples were equilibrated at room temperature for approximately 1 hr before rescanning by DSC. The reappearance of M1 was recorded as the retrogradation peak, and the values for the M2 were noted and calculated. The DSC parameters were reported as previously mentioned. Also, for M1, the ΔH of retrogradation divided by the ΔH of gelatinization was reported as the percentage of retrogradation. The results are the average of one to three DSC scans for two replications of each starch type.

Moisture determination of DSC samples. To calculate the ΔH values accurately on a starch dry basis, each pan was carefully punctured, dried at 105°C for 16 hr, and reweighed to determine moisture contents of the rescanned starch samples (Eliasson et al 1988).

Statistical Analyses

The analysis of variance "Proc" procedure of the Statistical Analysis System computer program (SAS Institute 1990) was used to perform statistical analyses on all data. Least significant differences were noted. For all analyses, one replication represents the averaged data from an individual starch isolation. Unless otherwise stated, results are the average of three replications.

RESULTS AND DISCUSSION

Oat Groat Analyses

The composition of the three oat lines is shown in Table I. Both E77 and DAL oats are commercial varieties commonly grown in the United States, and the MO42-17 oat is a high-lipid experimental line. As the percentage of lipids in the groats increased, the starch percentage decreased and the β-glucan percentage increased. All β-glucan amounts were within the normal expected range; it was noted in our laboratory, however, that oats containing high lipid contents also contain high β-glucan contents (Lim et al 1992). Moreover, all of the groats used in this study contained relatively high but similar protein contents. Generally, groat protein contents average 15–20% (Peterson and Brinegar 1986). The MO42-17 high-lipid groats were much smaller and weighed less (1.28 g/100 groats) than did the E77 and DAL groats (2.43 g/100 groats). Branson and Frey (1989) reported that seed weight (weight of 100 random oat seeds) was negatively correlated with groat oil content. Finally, the color of the groat flour increased in darkness as the lipid content of the starches increased, perhaps because of greater amounts of pigment in the oat oil or because of the increased β-glucan contents in these groats (β-glucans are dark gray).

Starch Extraction

In the present study, the amount of starch isolated from the groats ranged from 60 to 80% based on total starch content. As expected, higher percentages of starch were recovered from groats with higher total starch contents. Moreover, the starch percentage recovered showed no relationship to the protein percentage in the oat groats.

Protein Content

The protein contents of the isolated starches ranged from 0.85 to 0.95%. Such a small amount of protein is probably the result of the residual protein inside the starch granule (Morrison et al 1984). Previously, White and Abbas (1989) reported that protein percentages of up to 5% remaining in the isolated starches had no significant influence on the thermal properties of starches. Thus, these starches were sufficiently pure for further analyses.

Starch Lipids

Compared with other cereal grains, oat starch is the starch containing the greatest amount of lipids (Acker and Becker 1971, Morrison et al 1984, Gudmundsson and Eliasson 1989), and oats contain the greatest percentage of total oil (Youngs 1986). In the current study, all oat starches contained a greater amount of starch lipid (2.1–2.5%, Table II) than did other cereal starches, which typically are between 0.5 and 1.2% lipid (Morrison 1978).

TABLE I
Pertinent Composition of Groats from Three Oat Lines with Different Lipid Contents[a]

Oat Line	Lipid (%, db)	Protein (%, db)	Starch (%, db)	β-Glucan (%, db)	Groat Weight (g/100 groats)	Groat Color[b]
E77	6.2	18.3	57.8 a	4.1	2.43 a	Cream
DAL	8.0	21.4	50.8 b	4.6	2.43 a	Light brown
MO42-17	15.5	21.2	39.3 c	5.7	1.28 b	Medium brown

[a]Different letters in columns indicate significance at a probability level of $P < 0.05$. Values are the mean of three replicates, except for protein values, which are the mean of two replicates.
[b]Determined by the subjective appearance of the groat flour.

Additionally, the groat type with the least overall lipid content, E77 (6.2%, Table I), had significantly less starch lipid than did other groat types (Table II). The line MO42-17 had nearly twice as much total oil (15.5%, Table I) as did the DAL oat variety (8.0%, Table I); however, the oat starch isolated from the MO42-17 groats contained the same amount of lipid as did the DAL starch. Gudmundsson and Eliasson (1989), who isolated starch lipids with 75% *n*-propanol for 5 hr, reported that the oat starch isolated from the oat varieties containing the greatest oil content (7.5% oat oil) contained the greatest starch lipid content (2.3% starch lipid). But no relationship was found between starch lipid and oat lipid contents for the other oat starches they examined.

Extraction with propanol-water may have overestimated the lipid percentages, as this extraction procedure removes material soluble in alcohol-water in addition to the lipids. Further extraction of the propanol-water residues with chloroform-methanol as described by Takahashi and Seib (1988) would exclude this portion. Similar values were obtained in our laboratory by acid hydrolysis of the E77 and DAL starches followed by an ether extraction, yielding lipid values of 1.08 and 1.16%, respectively. Thus, the lipid values of oat starch reported in this study and by Gudmundsson and Eliasson (1989) may be slightly higher than the actual amount.

Determination of Starch Fractions

Iodine affinity. Significant differences existed among the three oat starch types in terms of iodine affinity (Table II). The percentage of amylose shown in the same table was determined by dividing the iodine affinity by a factor of 19 for pure amylose. As the lipid content in groats increased (Table I), the amylose

TABLE II
Percent Lipid, Iodine Affinity, and Percent Amylose of Starch from Three Oat Lines[a]

Oat Line	Starch Lipid (%, db)	Iodine Affinity (db)	Amylose[b] (%, db)	Starch Granule Size and Range[c] (μm)
E77	2.09 b	5.76 c	30.3 c	6.3 (3.0–10.4)
DAL	2.43 a	6.16 b	32.4 b	5.3 (3.0–7.5)
MO42-17	2.45 a	6.38 a	33.6 a	3.8 (3.2–5.5)

[a]Different letters in columns indicate significance at a probability level of $P < 0.05$. Values are the mean of three replicates.
[b]Calculated from the iodine affinity results, by using an amylose iodine affinity value of 19.
[c]Measured from scanning electron micrographs.

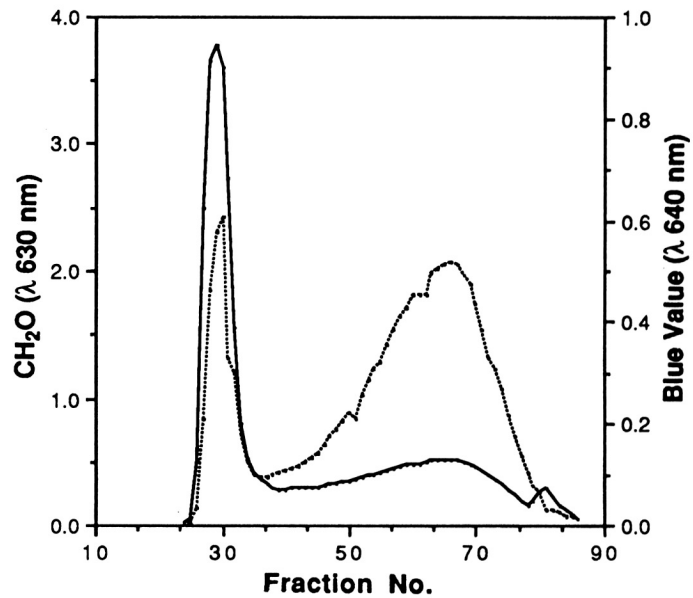

Fig. 1. Fractionation of E77 oat starch by gel permeation chromatography. Dashed line = amylose-iodine blue value, solid line = total carbohydrate value.

content in the oat starches also increased. Moreover, the isolated oat starch containing the least starch lipid (E77) contained the smallest percentage of amylose. Morrison et al (1984) and Gudmundsson and Eliasson (1989) also found a similar relationship between the amylose and lipid contents in different oat variety starches. Morrison (1988) has suggested that lipids may play a role in the regulation of starch biosynthesis, and this may contribute to the positive relationship between the amylose and lipid contents in cereal starches.

GPC. GPC was used to fractionate starch molecules by molecu-

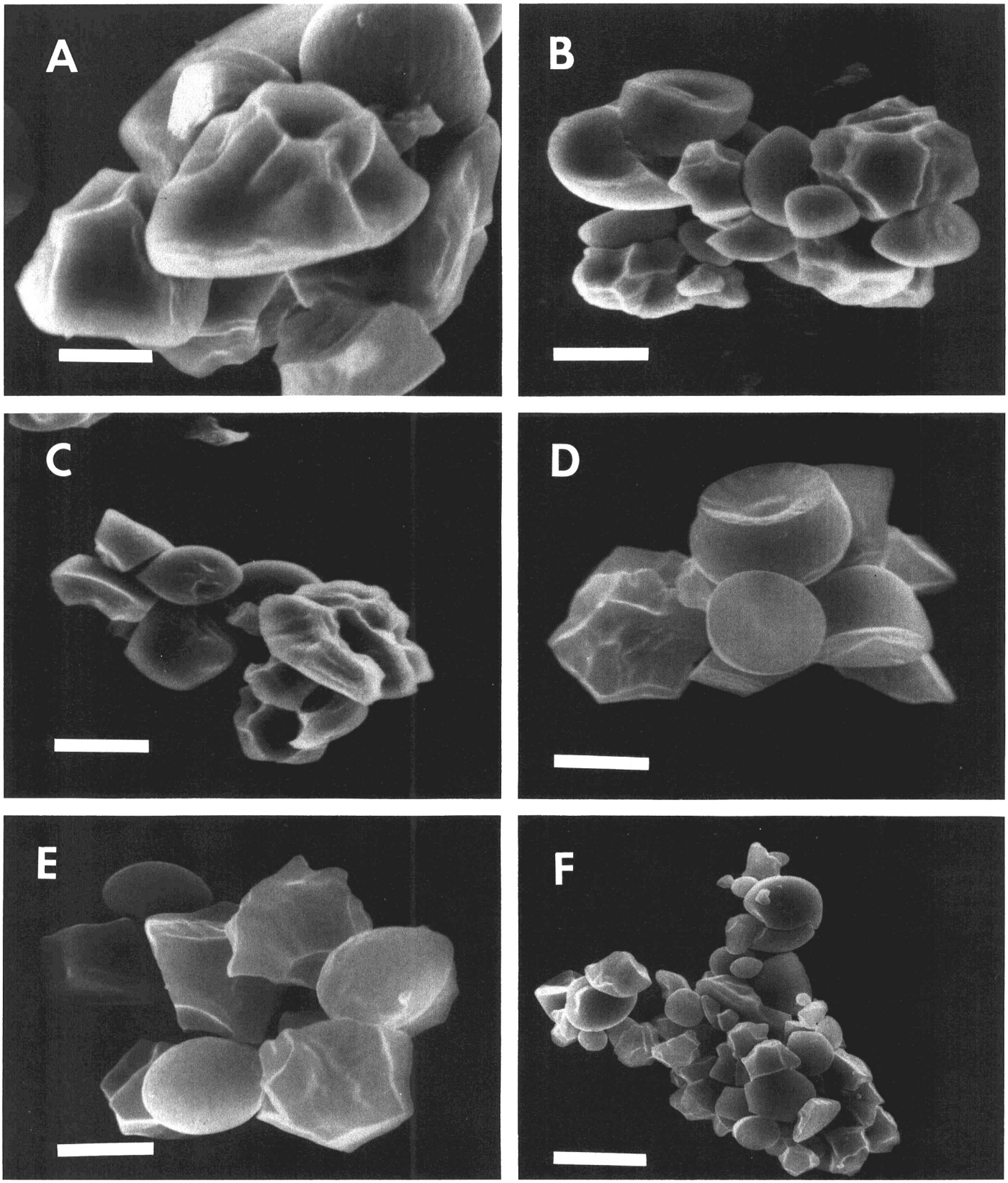

Fig. 2. Scanning electron micrographs illustrating the irregularly shaped oat starch granules. **A,** E77 (bar = 2.8 μm), 6,000×; **B,** E77 (bar = 4.4 μm), 4,000×; **C,** DAL (bar = 4.6 μm), 3,600×; **D,** DAL (bar = 3.0 μm), 6,000×; **E,** MO42-17 (bar = 2.3 μm), 6,600×; **F,** MO42-17 (bar = 8.5 μm), 2,200×.

lar weight, as previously reported by Matheson (1971), Yamada and Taki (1976), and Jane and Chen (1992). Because of the limited availability of equipment, it was possible to do only one replication for starch from each oat type; thus these results are only preliminary. A GPC chromatogram of E77 starch is shown in Figure 1. Two peaks were observed from the GPC chromatograms for each oat starch type. A high-molecular-weight peak was observed between fraction numbers 25 and 39 and a low-molecular-weight peak between fraction numbers 40 and 80. The amylose percentages estimated from the low-molecular-weight starch fraction were 41.2, 45.8, and 53.5% for E77, DAL, and MO42-17, respectively. Amylose content increased with groat oil content, a relationship also noted by iodine affinity tests; much greater amylose percentages were estimated by GPC than by iodine affinity, however. This great discrepancy suggests that the low-molecular-weight peak fraction for all oat starches may contain not only amylose but also an intermediate-molecular-weight starch fraction unable to bind iodine in the amylose-iodine potentiometric titrations, which GPC separated in the low-molecular-weight peak fraction. Paton (1979) used GPC to show that the oat variety starch Hinoat contained a large "anomalous" intermediate starch fraction (26%) separated as a clearly defined intermediate peak located between the high-molecular-weight fraction and the low-molecular-weight fraction. In the present study, a clearly defined intermediate-molecular-weight peak was not observed by GPC. These results are only preliminary, and further investigation of oat starch is currently in progress to improve understanding of the molecular components.

Scanning Electron Microscopy

In one of the earliest studies of oat starch, its histological properties were examined by means of light microscopy (Reichert 1913). Since this study, other researchers have shown that oats contain compound starch granules that develop in clusters about 60 μm in diameter (Reichert 1913, Winton and Winton 1932, Matz 1969). Figure 2 displays scanning electron micrographs of the three oat starches. Characteristically, these starches were irregular in shape and had several pointed oval-shaped granules (Reichert 1913, Matz 1969). That many of the granules were round on one side and polygonal on the opposite side indicates growth of the granules in clusters, where they acquired the shape of the neighboring starch granules.

Oat starch granules averaged 3–10 μm in size (Table II), which agrees with previously reported data (Reichert 1913, Winton and Winton 1932, Matz 1969, Lineback 1984, Paton 1986). As the groat oil content increased from 6.2 to 15.5% (Table I) and the amylose content increased from 30.3 to 33.6% (Table II), the average size of the oat starch granules decreased from 6.3 to 3.8 μm (Table II and Fig. 2).

Phosphorus Content in Oat Starches

The DAL oat starch contained a significantly greater ($P < 0.05$) percentage of starch phosphorus (0.19%) than did the starch of either E77 or MO42-17 (both contained 0.15%, Table III). Preliminary investigations indicated that lipid-free starch samples contained negligible starch phosphorus (results not shown), a fact indicating that most starch phosphorus was probably found in the lipid. The phosphorus content in the bound starch lipids after removal from the starch was only slightly less than that in the native starches, based on the total weight of starch lipid (Table III). These results confirm that nearly all the phosphorus was bound to the starch lipid. Similar results were reported for the phosphorus found in wheat starch (Morrison and Gadan 1987). Paton (1977) reported a phosphorus content in several Canadian and U.S. oat starches of only 0.063–0.081%, but the fact that these starches also contained less lipid (<0.3%) may have accounted for the smaller phosphorus contents.

TABLE III
Phosphorus (P) Content of Starches and Isolated Starch and Lipids from Three Oat Lines[a]

Oat Line	Starch P (%)[b]	P in Starch Based on Total Native Starch Lipid (μg/0.05 mg of lipid)[c]	P in Lipid Isolated from Starch (μg/0.05 mg of lipid)[d]
E77	0.15 b	3.59	3.12 b
DAL	0.19 a	3.91	3.51 a
MO42017	0.15 b	3.06	2.26 c

[a]Different letters in columns indicate significance at a probability level of $P < 0.05$. Values are the means of three replicates.
[b]Determined from 6.0-mg starch samples.
[c]Amount of phosphorus in starch calculated from total starch-lipid weight reported in Table II.
[d]Determined from 0.05-mg samples of isolated starch-lipid.

Thermal Properties of Oat Starches

Day zero. Table IV shows the DSC properties of the M1 and M2 transitions for all oat starches evaluated on day 0 and after storage. On day 0, there were significant differences in the T_o and R values of the M1 peaks among the oat starches. The E77 starch, having the smallest groat oil (Table I), amylose, and starch lipid contents (Table II), also had the lowest T_o and tended to have the smallest R values on day 0. In contrast, the MO42-17 starch, with the greatest groat oil (Table I) and amylose (Table II) contents, and containing a starch lipid percentage equal to that of DAL oat starch, exhibited the highest T_o values. In a similar study among starches from four oat lines, starch isolated from the oat with the greatest lipid content (7.5%) and the greatest starch lipid content (2.3%) also exhibited the highest T_o values (61.2–62.0°C) (Gudmundsson and Eliasson 1989). Even though the lipid content of groats in that study was similar to that of the DAL oats, the T_o of the DAL starch (59°C) was somewhat lower than the T_o of the starch in that study. This discrepancy might reflect differences in variety or in growing conditions.

As indicated by PHI values, the shapes of the M1 endotherms were similar for all starches. The ΔH values for the M1 endotherm

TABLE IV
Thermal Properties[a] of Oat Starches Evaluated on Day 0 and After Storage[b,c]

Time	Groat Line	M1 Transition[d] T_o (°C)	ΔH (cal/g)	R (°C)	PHI	Percent Retro	M2 Transition[d] T_o (°C)	ΔH (cal/g)	R (°C)	PHI
Zero days of storage	E77	55.5 c	2.05 a	8.8 b	0.49 a	...	90.3 a	0.84 a	18.3 a	0.10 a
	DAL	59.0 b	2.19 a	10.8 a	0.41 a	...	90.6 a	0.72 a	19.7 a	0.08 a
	MO42-17	62.4 a	2.20 a	9.8 ab	0.45 a	...	91.1 a	0.61 a	17.7 a	0.07 a
After storage[b]	E77	45.1 a	0.76 a	15.5 a	0.10 a	37 a	97.4 a	0.45 a	15.4 a	0.06 b
	DAL	46.9 a	0.70 a	14.1 a	0.10 a	32 a	98.4 a	0.54 a	13.9 a	0.08 a
	MO42-17	44.0 a	0.89 a	15.7 a	0.11 a	40 a	95.7 a	0.24 b	12.0 a	0.04 c

[a]T_o = peak onset temperature, ΔH = enthalpy of the phase transition, R = range of the phase transition, PHI = peak height index = $\Delta H/(R/2)$, percent retro = retrogradation percentage = ΔH (retrogradation)/ΔH (gelatinization) × 100.
[b]Means of all storage days (days 7, 14, 21, and 28).
[c]Different letters in columns (for 0 days of storage or for after storage) indicate significance at a probability level of $P < 0.05$. Values are the means of two replications of one to three differential scanning calorimetry scans.
[d]M1 = low-temperature endotherm, M2 = high-temperature endotherm.

on day 0 were similar for all the oat starches examined and were slightly lower than what has been reported for maize, wheat, waxy maize, or rice (*Oryza sativa* L.) (Paton 1987, Gudmundsson and Eliasson 1989). The low ΔH value for oat starch may be due to the high lipid content in oat starch (Biliaderis et al 1986, Paton 1987). In their study of native and waxy rice starches, Biliaderis et al (1986) noted that the waxy starches (containing essentially no lipid) exhibited a higher ΔH value than did the nonwaxy starch samples (containing a high percentage of lipid). Also, Kugimiya et al (1980) showed that lysolecithin-saturated potato starch exhibited a lower ΔH value than did native potato starch.

There are actually two processes occurring during the gelatinization phase transition: first, the melting of the starch crystallites, which is an endothermic process, and second, the formation of the amylose-lipid complex, which is an exothermic process (Biliaderis et al 1986). A net endothermic process is detected by DSC, and the net energy required to form this peak is less when lipids are present in the starch-water system than when they are not. On the other hand, the low ΔH value noted for oat starch may simply be a reflection of the high amylose content. Both Stevens and Elton (1971) and Inouchi et al (1984) reported higher ΔH values for waxy starch than for normal and high-amylose starches and concluded that in gelatinization amylopectin makes a more important contribution than does amylose.

On day 0 the T_o, ΔH, R, and PHI values of the M2 transition were not significantly different among all oat starch types, likely a reflection of the small differences found in their starch-lipid percentages (Table II). The oat starches contained larger amylose-lipid complexes (ΔH = 0.84, 0.72, and 0.61 cal/g for E77, DAL, and MO42-17 starches, respectively) than did wheat and maize starches (ΔH = 0.3 and 0.4 cal/g, respectively), as reported by Acker and Becker (1971). Paton (1987) and Gudmundsson and Eliasson (1989) also reported a large amylose-lipid complex ranging between 0.57 and 1.43 cal/g for starches from various oat varieties. The high ΔH values for the M2 endotherm observed in oat starch likely reflected the high lipid content found in native oat starch.

After storage. A DSC rescan of the stored starches (4°C for up to 28 days) resulted in the reappearance of both M1 and M2 endotherms. For all starches, the M1 peak had lower T_o, lower ΔH, higher R (broader transition), and higher PHI values after storage than on day 0 (Table IV). White et al (1989) also reported similar trends in the T_o, ΔH, and R values for stored potato, maize, wheat, and waxy maize starches. Donovan and Mapes (1980) suggested that after starches have been fully gelatinized, the disordering of the crystalline structure causes a lower regelatinization temperature, and the heterogeneity of the crystalline structure in gelatinized starch creates broader transitions. This trend was also seen in the smaller PHI values after storage.

After storage, there were no significant differences among the oat starch types in the DSC parameters of the M1 peak. In addition, no direct relation was seen between the percentage of retrogradation of the starches and the lipid content of the oat starches. Oat starches retrograded less than did maize, rice, and potato starches, as reported by White et al (1989). Paton (1987) observed that oat starches retrograded more slowly than did other cereal starches and that, after 10 days of storage, oat starch had retrograded only 50% compared with wheat and waxy maize starches, which had retrograded 70–75%. The generally high lipid content in oat starches may play a role in decreasing the amount and extent of retrogradation, as noted by Doublier et al (1987).

The T_o values for the M2 transition were slightly higher for all starch varieties after storage (96–98°C) than on day 0 (90–91°C). The ΔH, R, and PHI values generally were lower after storage, and MO42-17 oat starch, with the highest amylose percentage and starch lipid content (Table II), exhibited significantly lower ΔH and PHI values than did either E77 or DAL oat starches.

To investigate further the changes in the DSC values of M2 after storage, each starch type was run in duplicate on day 0, cooled, and immediately rescanned. Data are not shown. The reheated samples showed no M1 endotherm, whereas the M2 transition had a similar or slightly higher T_o value and a similar ΔH value compared with values measured on day 0, thus affirming the reversibility of the formation and remelting of the amylose-lipid complex in oat starch, which was previously reported for other cereal starches (Eliasson 1980, Kugimiya et al 1980). But the PHI values were slightly lower after the rescan, a fact suggesting that the amylose-lipid complex reformed in a somewhat different crystalline or molecular form upon cooling (Kugimiya et al 1980). In our study, the ΔH values for the remelting of the amylose-lipid complex greatly decreased after storage, as noted in Table IV, indicating that the amylose-lipid complex was altered during storage. Perhaps fine structural differences in the amylose fractions of oat starch or the presence of an anomalous intermediate fraction influenced these changes through their retrogradation.

CONCLUSIONS

Oat starches isolated from groats containing 6.2–15.5% lipid showed unique chemical, physical, and structural properties, perhaps related to the high lipid content in the groats and isolated starches. All oat starches contained greater lipid amounts (2.09–2.45%), as determined by the propanol-water extractions, than reported for other cereal starches (0.5–1.2%). The starch isolated from the groats with the lowest lipid content (E77) had significantly less lipid and a smaller percentage of amylose than did the starches isolated from groats containing higher lipid contents (DAL and MO42-17). The MO42-17 starch, isolated from groats containing the greatest amount of groat oil, contained the highest amylose percentage. Scanning electron micrographs showed irregularly shaped starch granules and a tendency for starch granule size to decrease with increased amylose content. GPC revealed possible molecular differences among the three oat starch types and the existence of a possible intermediate starch fraction. All oat starches had high phosphorus contents (0.15–0.19%), likely in the form of lysophospholipids. By DSC, gelatinization T_o differed significantly among starch types, ranging from 55.5 to 62.4°C, and increased with increased amylose content. Starches exhibited a large amylose-lipid complex similar to that observed in other oat variety starches (Paton 1987, Gudmundsson and Eliasson 1989). The high lipid content found in the oat starches may play a role in decreasing the rate and the amount of starch retrogradation inasmuch as the oat starches retrograded only 32–40% over a 28-day storage period.

ACKNOWLEDGMENTS

We thank K. J. Frey (Agronomy Department, Iowa State University) for supplying the oats, K. Guilchrist (Oat Quality Laboratory, University of Wisconsin) for protein determinations of the groats, D. E. Alexander (Agronomy Department, University of Illinois) for lipid determinations of the groats, H. Lim (Food Science and Human Nutrition Department, Iowa State University) for β-glucan analyses of the ground groats, and J.-L. Jane (Food Science and Human Nutrition Department, Iowa State University) for advice on gel permeation chromatography and the use of equipment.

LITERATURE CITED

ACKER, V. L., and BECKER, G. 1971. Recent studies on lipids of starches. Part II. Lipids of various types of starch and their bond to amylose. Starch/Staerke 23:419.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 44-15A, approved October 1975, revised October 1981. The Association: St. Paul, MN.

ANDERSON, J. W., HAMILTON, C. C., HORN, J. L., SPENCER, D. B., DILLON, D. W., and ZEIGLER, J. A. 1991. Metabolic effects of insoluble oat fiber on lean men with type II diabetes. Cereal Chem. 68:291.

BANKS, W., and GREENWOOD, C. T. 1967. The fractionation of laboratory isolated cereal starches using dimethyl sulfoxide. Starch/Staerke 19:394.

BILIADERIS, C. G., PAGE, C. M., MAURICE, T. J., and JULIANO, B. O. 1986. Thermal characterization of rice starches: A polymeric approach to phase transitions of granular starch. J. Agric. Food Chem. 34:6.
BRANSON, C. V., and FREY, K. J. 1989. Recurrent setection for groat oil content in oat. Crop Sci. 29:1382.
CALDWELL, E. F., and POMERANZ, Y. 1973. Industrial uses of cereals: Oats. Pages 393-411 in: Industrial Uses of Cereals. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
CHEN, W. L., and ANDERSON, J. W. 1979. Effects of plant fiber in decreasing plasma total cholesterol and increasing high-density lipoprotein cholesterol. Proc. Soc. Exp. Biol. Med. 162:310.
CLENDENNING, K. A., and WRIGHT, D. E. 1945. Polarimetric determination of starch in cereal products. III. Composition and specific rotatory power of starches in relation to source and type. Can. J. Res. 23B:131.
CLUSKEY, J. E., WU, Y. V., WALL, J. S., and INGLETT, G. E. 1973. Oat protein concentrates from a wet-milling process: Preparation. Cereal Chem. 50:475.
CONWAY, T. F., and EARLE, F. R. 1963. Nuclear magnetic resonance for determining oil content of seeds. J. Am. Oil Chem. Soc. 40:265.
DONOVAN, J. W., and MAPES, C. J. 1980. Multiple phase transitions of starches and nageli amylodextrins. Starch/Staerke 32:190.
DOUBLIER, J.-L., PATON D., and LLAMAS, G. 1987. A rheological investigation of oat starch pastes. Cereal Chem. 64:21.
ELIASSON, A.-C. 1980. Effect of water content on the gelatinization of wheat starch. Starch/Staerke 32:270.
ELIASSON, A.-C., FINSTAD, H., and LJUNGER, G. 1988. A study of starch-lipid interactions for some native and modified starches. Starch/Staerke 40:95.
FREY, K. J., and HAMMOND, E. G., and LAWRENCE, P. K. 1975. Inheritance of oil percentage in interspecific crosses of hexaploid oats. Crop Sci. 15:94.
GUDMUNDSSON, M., and ELIASSON, A.-C. 1989. Some physico-chemical properties of oat starches extracted from varieties with different oil content. Acta Agric. Scand. 39:101.
HACH, C. C., DOWDEN, B. K., KOPELOVE, A. B., and BRAYTON, S. V. 1987. More powerful peroxide Kjeldahl digestion method. J. Assoc. Off. Anal. Chem. 70:783.
HOLM, J., BJORCK, I., DREWS, A., and ASP, H.-G. 1986. A rapid method for the analysis of starch. Starch/Staerke 38:224.
INOUCHI, N., GLOVER, D. V., SUGIMOTO, Y., and FUWA, H. 1984. Developmental changes in starch properties of several endosperm mutants of maize. Starch/Staerke 36:8.
JANE, J., and CHEN, J. 1992. Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. Cereal Chem. 69:60.
JØRGENSEN, K. G., and AASTRUP, S. 1988. Quantification of high molecular weight (1-3)(1-4)-β-D-glucan using calcofluor complex formation and flow injection analysis. II. Determination of total β-glucan content of barley and malt. Carlsberg Res. Commun. 53:287.
KIRBY, R. W., ANDERSON, J. W., SIELING, B., REES, E. D., CHEN, W. J., MILLER, R. E., and KAY, R. M. 1981. Oat-grain intake selectively lowers serum low-density lipoprotein cholesterol concentrations in hypercholesterolemic men. Am. J. Clin. Nutr. 34:824.
KLOPFENSTEIN, C. F., and HOSENEY, R. C. 1987. Cholesterol-lowering effect of β-glucan-enriched bread. Nutr. Rep. Int. 36:1091.
KRUEGER, B. R., KNUTSON, C. A., INGLETT, G. E., and WALKER, C. E. 1987. A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. J. Food Sci. 52:715.
KUGIMIYA, M., DONOVAN, J. W., and WONG, R. Y. 1980. Phase transitions of amylose-lipid complexes in starches: A colorimetric study. Starch/Staerke 32:265.
LIM, H. S., WHITE, P. J., and FREY, K. J. 1992. Genotypic effects on β-glucan content of oat lines grown in two consecutive years. Cereal Chem. 69:262.
LINEBACK, D. R. 1984. The starch granule organization and properties. Baker's Dig. 13:16.
MacARTHUR, L. A., and D'APPOLONIA, B. L. 1979. Comparison of oat and wheat carbohydrates. II. Starch. Cereal Chem. 56:458.
MATHESON, N. K. 1971. Amylose changes in the starch of developing wheat grains. Phytochemistry 10:3213.
MATZ, S. A. 1969. Oats. Pages 78-96 in: Cereal Science. AVI Publ. Co.: Westport, CT.
MORRISON, W. R. 1964. A fast, simple and reliable method for the microdetermination of phosphorus in biological materials. Anal. Biochem. 7:218.
MORRISON, W. R. 1978. Cereal lipids. Page 221-348 in: Advances in Cereal Science and Technology. Vol. 2. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
MORRISON, W. R. 1988. Lipids in cereal starches: A review. J. Cereal Sci. 8:1. MORRISON, W. R., and COVENTRY, A. M. 1985. Extraction of lipids from cereal starches with hot aqueous alcohols. Starch/Staerke 37:83.
MORRISON, W. R., and GADAN, H. 1987. The amylose and lipid contents of starch granules in developing wheat endosperm. J. Cereal Sci. 5:263.
MORRISON, W. R., MILLIGAN, T. P., and AZUDIN, M. N. 1984. A relationship between the amylose and lipid contents of starches from diploid cereals. J. Cereal Sci. 2:257.
PATON, D. 1977. Oat starch. Part I. Extraction, purification, and pasting properties. Starch/Staerke 29:149.
PATON, D. 1979. Oat starch: Some recent developments. Starch/Staerke 31:184.
PATON, D. 1986. Oat starch: Physical, chemical, and structural properties. Pages 93-120 in: Oats: Chemistry and Technology. F. H. Webster, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
PATON, D. 1987. Differential scanning calorimetry of oat starch pastes. Cereal Chem. 64:394.
PETERSON, D. M., and BRINEGAR, A. C. 1986. Oat storage proteins. Page 153-203 in: Oats: Chemistry and Technology. F. H. Webster, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
REICHERT, E. T. 1913. Starches of Graminaceae, genus Avena. Pages 374-375 in: The Differentiation and Specificity of Starches in Relation to Genera, Species, etc. Carnegie Institute Washington Publications: Washington, DC.
SCHIPPER, H., and FREY, K. J. In press. Three recurrent selection regimes to increase groat-oil content of oat. Crop Sci.
SCHOCH, T. J. 1964. Iodometric determination of amylose. Potentiometric titration: Standard method. Pages 157-160 in: Methods in Carbohydrate Chemistry. Vol. 4. R. L. Whistler, ed. Academic Press: New York.
SHRICKEL, D. J. 1986. Oats production, value, and use. Page 1-11 in: Oats: Chemistry and Technology. F. H. Webster, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
SAS INSTITUTE. 1990. Pages 891-996 in: SAS User's Guide: Statistics. The Institute: Cary, NC.
STEVENS, D. J., and ELTON, G. A. H. 1971. Thermal properties of the starch/water system. Starch/Staerke 23:8.
TAKAHASHI, S., and SEIB, P. A. 1988. Paste and gel properties of prime corn and wheat starches with and without native lipids. Cereal Chem. 65:474-483.
WHITE, P. J., and ABBAS, I. R. 1989. Effect of protein on gelatinization of cornstarch as measured by differential scanning calorimetry. Cereal Foods World 34:778.
WHITE, P. J., ABBAS, I. R., and JOHNSON, L. A. 1989. Freeze-thaw stability and refrigerated-storage retrogradation of starches. Starch/Staerke 41:176.
WILHELM, E., DETMOLD, K., RAJAMÄKI, A. L., and CARANSA, A. 1989. Process technology for the production of starch, protein and fiber from oats. Starch/Staerke 41:372.
WILLIAMS, P. C. 1975. Application of near infrared reflectance spectroscopy to analysis of cereal grains and oilseeds. Cereal Chem. 52:561.
WINTON, A. L., and WINTON, K. B. 1932. Oats. Pages 158-176 in: The Structure and Composition of Foods. John Wiley & Sons: New York.
WU, Y. V., CLUSKEY, J. E., WALL, J. S., and INGLETT, G. E. 1973. Oat protein concentrates from a wet-milling process: Composition and properties. Cereal Chem. 50:481.
YAMADA, T., and TAKI, M. 1976. Fractionation of maize starch by gel-chromatography. Starch/Staerke 11:374.
YOUNGS, V. L. 1974. Extraction of a high-protein layer from oat groat bran and flour. J. Food Sci. 39:1045.
YOUNGS, V. L. 1986. Oat lipids and lipid-related enzymes. Pages 205-226 in: Oats: Chemistry and Technology. F. H. Webster, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

[Received October 28, 1991. Revision received April 13, 1992. Accepted April 18, 1992.]