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Studies on isolation and characterization of starch from oat (*Avena nuda*) grains

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Starch from AC Hill oat grains (*Avena nuda*) was isolated and some of the characteristics determined. The yield of starch was 23·4% on a whole grain basis. The shape of the granule was polyhedral to irregular, with granules 6–10 μm in diameter. Lipids were extracted by acid hydrolysis and by selective solvent extraction with chloroform–methanol 2:1 v/v (CM) at ambient temperature, followed by *n*-propanol–water 3:1 v/v (PW) at 90–100°C. The acid hydrolyzed extracts which represented the total starch lipids (TSL) was 1·13%. The free lipids in the CM extract (1% TSL) was 6·2%, whereas the free and bound lipids in the PW extracts was 93.0%. Neutral lipids formed the major lipid class in the CM and PW extracts. The monoacyl lipid content in both CM and PW extracts was 61·0%. The total amylose content was 19·4%, of which 13·9% was complexed by native lipids. X-ray diffraction was of the 'A' type. Oat starch differed from wheat starch in showing a higher swelling factor, decreased amylose leaching, co-leaching of a branched starch component and amylose during the pasting process, higher peak viscosity and set-back, low gel rigidity, greater susceptibility towards acid hydrolysis, greater resistance to α-amylase action and a higher freeze–thaw stability. Furthermore, in comparison to wheat starch, the amylose chains of oat starch appear to be more loosely arranged in the amorphous regions, whereas in crystalline regions, oat starch chains are more compactly packed. Lipid removal from oat and wheat starches decreased their swelling factor, peak viscosity, set-back, gelatinization temperatures, freeze–thaw stability and paste clarity (at pH > 4·0), and increased their thermal stability, amylose leaching, enthalpy of gelatinization, susceptibility towards α-amylase and paste clarity (at pH < 4·0). The results also showed that the properties of AC Hill oat starch is not representative of oat starch in general.

INTRODUCTION

Research on the structure and physicochemical characteristics of the main cereal starches of commerce such as rice, wheat and corn has resulted in their extensive utilization in the food industry. However, oat starch has not been subjected to detailed study. Although there have been some reports on the rheological, gelatinization, textural and swelling characteristics of oat starch pastes and gels (Paton, 1977, 1979, 1981; MacArthur & D'Appolonia, 1979; Doublier *et al.*, 1987; Gudmundsson & Eliasson, 1989), relatively little information is available on granule crystallinity, starch-lipid composition, granular susceptibility to hydrolysis (acid and α-amylase), effect of pH on paste clarity and rheological properties, freeze–thaw stability, and the effect of lipid removal on physicochemical properties.

In the relatively few studies conducted to date, considerable difference has been observed between the physicochemical properties of oat starch and other cereal starches. Doublier *et al.* (1987) reported that the swelling power and amylose leaching during pasting, was higher in oat starch than in corn and wheat starches. However, Gudmundsson & Eliasson (1989) reported that although oat starches exhibited higher gel volumes, the extent of amylose leaching was much lower than in other cereal starches.

Doublier *et al.* (1987) observed that in oat starch, both amylose (alone or in complexed form) and amylopectin are co-leached from native granules throughout the pasting process, while in other cereal

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starches, amylose is preferentially leached. However, these authors did not provide an explanation for this difference. Pasting curves monitored on a Brabender Viscoamylogram (MacArthur & D'Appolonia, 1979), and on an Ottawa starch viscometer (Paton, 1977), showed that oat starch exhibits higher hot paste consistencies and dramatic consistency increases on cooling, compared to similar paste concentration of other cereal starches. Paton (1977) observed that, although oat starch exhibits high set-back torque values, the cooled gel is clearer, less firm, more elastic, more adhesive and less susceptible to retrogradation than those of other cereal starches. These results are similar to those reported by Doublier *et al.* (1987), using the rheometric concentric cylindrical viscometer, where oat starch pastes displayed a marked thixotropy in their shear stress/shear rate flow curves in contrast to wheat and corn starches pasted at the same concentration. On the basis of this experiment, the above authors concluded that the structural network within hot oat starch paste is stronger than is found for wheat and corn starches. The retrogradation of oat starch gels has also been studied (Paton, 1987; Gudmundsson & Eliasson, 1989) by differential scanning calorimetry (DSC). These authors have shown that native oat starch retrogrades to a lesser extent than wheat or corn starches.

Removal of bound lipids was found to increase the retrogradation of oat starch (Paton, 1987; Gudmundsson & Eliasson, 1989). The former author reported that the rate of retrogradation of defatted oat starch was similar to those of normal wheat and waxy corn starches. However, the latter authors showed that defatted oat starches retrograded to a lesser extent than normal wheat or maize starches.

DSC has also been used to study the thermal transitions of native and defatted oat starches. Paton (1987) showed that oat starch (*variety sentinel*) exhibited two endotherms, one around 66°C (melting of starch crystallites) and a second at 102–104°C (melting of amylose–lipid complexes). The transition enthalpies associated with these two endotherms were respectively 9·1 J/g and 3·6 J/g. The former value was slightly lower than those reported for rice and wheat starches (Paton, 1987), but the latter value was about twice as high than those of wheat and rice starches (Paton, 1987). Similar findings were reported by Gudmundsson & Eliasson, (1989) on other varieties of oat starch.

The present study was undertaken to obtain more information on the physicochemical properties of native and defatted oat starches that might assist in explaining some of the properties of oat starch which are not similar to those of other cereal starches. Wheat starch was used for comparison purposes, since its lipid composition is fairly similar to that of oat starch. It is hoped that this investigation would increase opportunities for utilizing this starch as an ingredient in food preparations.

EXPERIMENTAL

Cereal grain

AC Hill oat grains (*Avena nuda, var chinensis, Fish. ex link*) which is a spring type, day length sensitive, naked seeded cultivar that has been known to perform well in Eastern Canada (Burrows, 1986) were obtained from the central experimental farm at Ottawa.

Isolation and purification of the starch

Oat grains (500 g) were steeped in water (at 50°C) for 3 h. A ratio of 1 part soaked grains to 3 parts distilled water was mixed for 3 min in a Waring blender at low speed. The resultant slurry was then passed through a cheese cloth and then centrifuged at 5000*g* for 15 min. The supernatant was discarded, and the sediment suspended in excess 0·02% NaOH. After standing for 1 h, the supernatant was removed. This procedure was repeated six times. The final sediment was suspended in distilled water, and then subjected to sequential filtration through 70 and 20 μ polypropylene screens, neutralized to pH 7·0 with HCl, filtered on a Buchner funnel and thoroughly washed on the filter with distilled water. The filter cake was dried overnight at 30°C.

Chemical composition of starch

Quantitative estimations of moisture, ash, nitrogen and starch damage were performed by the standard AACC (1984) procedures. Starch lipids were analyzed as follows: at ambient temperatures (25–27°C), lipids were extracted from oat starch (5 g dry basis) with 100 ml of $CHCl_3$-CH_3OH (CM) (2:1 v/v) under vigorous agitation in a wrist action shaker for 1 h. At elevated temperatures (90–100°C) lipids were obtained by soxhlet extraction (7 h) with 100 ml of *n*-propanol–water (PW) (3:1 v/v). Lipids were also extracted, after acid hydrolysis of oat starch with 24% HCl at 70–80°C for 30 min and the hydrolyzate then extracted three times with *n*-hexane (Goshima *et al.*, 1985). The purification and fractionation of extracted lipids and quantification of lipid classes were carried out by procedures that have been described elsewhere (Vasanthan & Hoover, 1992).

Apparent and total amylose content were determined by the blue value method of Gilbert & Spragg (1964). Calculation for amylose content was by the method of Kawamura (1969).

Different scanning calorimetry

Gelatinization temperatures were measured and recorded on a Perkin-Elmer DSC-2 differential scanning calorimeter, with a heating rate of 10°C/min, and a chart speed of 20 mm/min. Water (8·0 μl) was added

with a microsyringe to starch (2·5 mg) in the DSC pans, which were then sealed, reweighed and allowed to stand overnight at room temperature. The scanning temperature range was 20–120°C. The thermogram was recorded with water as reference. The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c) of the gelatinization endotherm. Indium was used for calibration.

Relative crystallinity by X-ray diffraction

X-ray diffractograms were obtained with a Rigaku RU 200R X-ray diffractometer with a chart speed of 20 mm/min. The starch powder was scanned through the 2θ range of 3–35°C. Traces were obtained using Cu-Kα radiation detector with a nickel filter and a scintillation counter operating under the following conditions: 40 kV, 50 mA, 1°/1° divergence slit/scattering slit, 0·30 mm receiving slit, 1 s time constant, and scanning rate of 3°/min. Relative crystallinity was measured by the method of Hermans & Weidinger (1948). Quartz was used as the 100% reference crystal.

Swelling factor

The swelling factor of the starches when heated to 50–95°C in excess water was measured according to the method of Tester and Morrison (1990). This method measures only intragranular water and hence the true swelling factor at a given temperature. The swelling factor is reported as a ratio of the volume of swollen starch granules to the volume of the dry starch.

Extent of amylose leaching

Various concentrations of native and defatted starch (15–20 mg) in water were heated in volume calibrated sealed tubes (50–95°C) for 30 min. The tubes were then cooled to ambient temperature and centrifuged at 3500 rev/min for 10 min. The supernatant liquid (1 ml) was withdrawn and its amylose content was determined by the method of Chrastil (1987).

Iodine-absorption spectra

Slurries (0·2% (w/v)) of starch (native and defatted), potato amylose and potato amylopectin in distilled water were heated in sealed tubes at 70°C for 30 min. The tubes were cooled to ambient temperature and centrifuged at 2500 rev/min for 10 min. The iodine–polysaccharide complexes were prepared by mixing 0·5 ml of the supernatant with 0·5 ml of iodide solution containing 0·0002 mg of iodine and 0·002 mg of potassium iodide per ml. The absorption was measured as a function of wavelength using a Hewlett Packard spectrophotometer with water as a blank.

Pasting behavior

A Brabender viscoamylograph, Model VA-V equipped with a 700 cmg cartridge was used to study pasting properties at a concentration of 6% (w/v). The slurry pH was adjusted where necessary by addition of 0·1 N HCl or NaOH as required.

Light transmittance of starch pastes

The following procedure adapted from Craig *et al.* (1989) was used to prepare 1% starch pastes. Starch (50 mg db) was suspended in water (5 ml) in screwcap tubes and the pH adjusted as described above. The tubes were then heated in a boiling water bath (with occasional shaking) for 30 min. After cooling to ambient temperature, the percentage transmittance (%T) at 650 nm was determined against a water blank in a Hewlett Packard spectrophotometer.

Acid hydrolysis

The starches were hydrolyzed with 2·2 N HCl at 35°C (1·0 g starch/40 ml acid) for 25 days. The starch slurries were shaken by hand daily to resuspend the deposited granules. At 24 h intervals, aliquots of the reaction mixtures were neutralized and centrifuged (3500 rev/min) and the supernatant liquid was assayed for total carbohydrate (Bruner, 1964). The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial starch.

Enzymatic digestibility

Enzymatic digestibility studies on native and defatted starches were done using a crystalline suspension of porcine pancreatic α-amylase in 0·5 M saturated sodium chloride containing 3 mM calcium chloride (Sigma Chemical Co., USA), in which the concentration of α-amylase was 23·9 mg/ml and the specific activity was 1240 units per milligram of protein. One unit was defined as the α-amylase activity which liberated 1 mg maltose in 3 min at 20°C at pH 6·9. The extent of hydrolysis was determined following previously described methods (Hoover *et al.*, 1991).

Microscopy

Granule morphology of native starch and the mode of action of α-amylase on native and defatted starches were studied by scanning electron microscopy (SEM). Starch samples were mounted on circular aluminum stubs with double sticky tape and then coated with 20 nm of gold and examined and photographed in a Hitachi (S570) scanning electron microscope at an accelerating potential of 20 kV. Enzyme digested granules were prepared for SEM by rapidly freezing in

liquid nitrogen and freeze drying at −80°C. The dried samples were prepared for viewing as described above.

Degree of syneresis

Aqueous suspensions of starches (6% w/v) were rapidly heated to 95°C under constant agitation to prevent sedimentation. These suspensions were then kept at 95°C for 30 min before being cooled to 25°C. The gels so obtained were subjected to cold storage at 4°C for 16 h (to increase nucleation) and then frozen at −16°C. To measure freeze-thaw stability, the gels frozen at −16°C for 24 h were thawed at 25°C for 6 h and then refrozen at −16°C. Five cycles of freeze-thaw were performed. The exuded water was determined by centrifuging the tubes at 1000 g for 20 min after thawing.

RESULTS AND DISCUSSION

Morphological granular characteristics of the starch

Starch granules tend to exist in clusters of individual granules (Fig. 1(a)). The granules ranged from polygonal to irregular in shape with an average granule diameter of 6–10 μm (Fig. 1(b)). The surfaces appeared to be smooth with no evidence of fissures (Fig. 1(a)).

Chemical composition of the starch

The data on composition and yield are presented in Table 1. The nitrogen content of isolated starches represents the contributions from endosperm storage proteins, lysophospholipids (LPL) and proteins located inside starch granules (Morrison, 1981). The nitrogen content of the purified oat starch was 0·02% (dry basis) (Table 1), indicating the absence of endosperm proteins and by implication, most of the non-starch lipids (Morrison, 1981).

The total starch lipids (TSL) obtained by acid hydrolysis was 1·13% (Table 1). This value was higher than those reported (Vasanthan & Hoover, 1992) for wheat (0·7%), rice (0·76%) and corn (0·79%) starches. The solvent extracted starch lipids (SEL), which refers to those lipids obtained by the combined action of CM and PW was 1·12% (Table 1). This level was lower than the range (1·3–2·3%) reported by Morrison *et al.* (1984), Doublier *et al.* (1987) and Gudmundsson and Eliasson (1989) for starches isolated from other varieties of oat grains.

Lipid composition

CM extracted lipids

The CM extracted lipids probably represent to a large extent the free surface lipids. However, since the granules were slightly damaged, the probability of

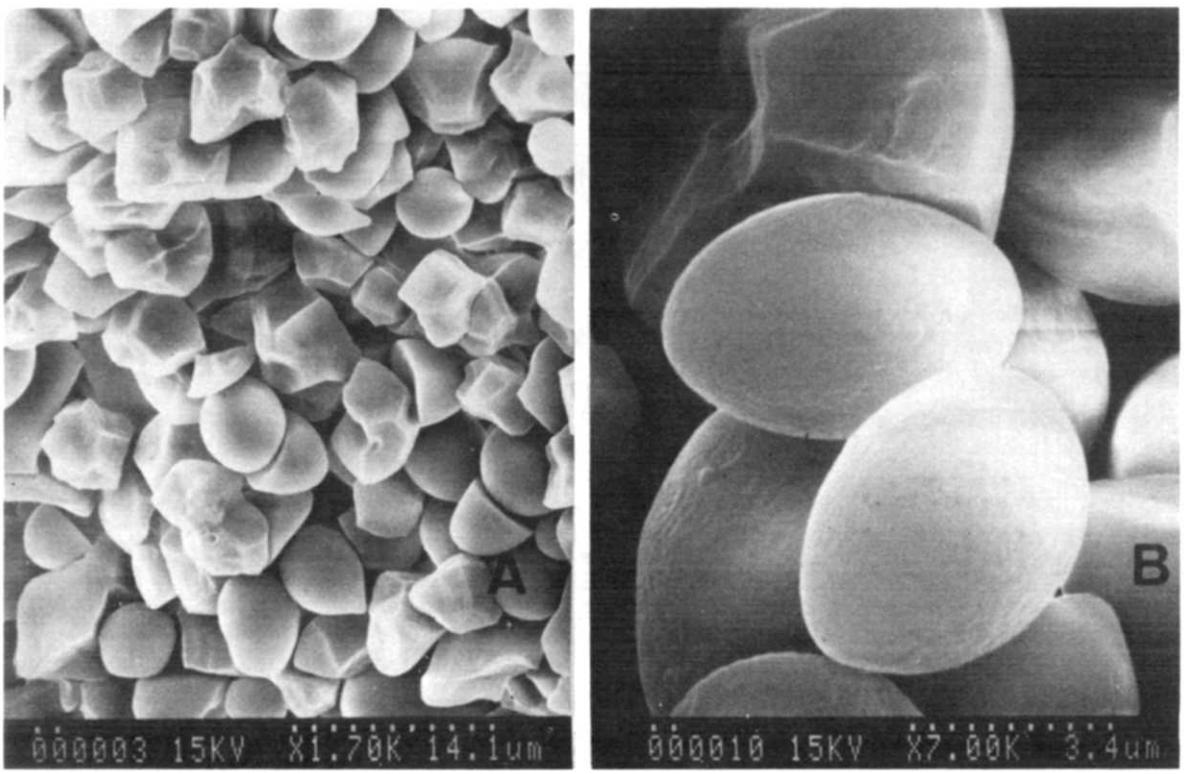


Fig. 1. Scanning electron photomicrographs at different magnifications of native oat starch granules: (A) 1700×; (B) 7000×, (original magnifications).

Table 1. Chemical composition (%) and some of the properties of oat starch[a]

Characteristics	Composition (%)
Yield (% initial material)	23·4
Moisture	10·2
Ash	0·03
Nitrogen	0·05
Starch damage	13·0
Lipid	
Acid hydrolysed[b]	1·13
Solvent extracted	
CM[c]	0·07
PW[d]	1·05
Amylose content (% of total starch)	
Apparent[e]	16·7
Total[e]	19·4
Amylose complexed with native lipid[f]	13·9
Starch granule characteristics	
Granule shape	Polyhedral to irregular
Granule size	6–10 μm

[a]All data reported on dry basis and represent the mean of three determinations.
[b]Lipids obtained by acid hydrolysis (24% HCl) of the native starch (total lipids).
[c]Lipids extracted from native starch by CM 2:1 (v/v) at 25°C (mainly unbound lipids).
[d]Lipids extracted by hot PW 3:1 (v/v) from the residue left after CM extraction (mainly bound lipids).
[e]Apparent and total amylose determined by I_2-binding before and after removal of bound lipids by hot PW extraction.
[f]$\frac{\text{Total amylose} - \text{apparent amylose}}{\text{Total amylose}} \times 100$.

Table 2. Lipid classes in chloroform–methanol and *n*-propanol–water extracts

Extraction method	Lipid class[a] (mg/100 g dry starch)		
	Neutral	Phospholipid	Glycolipid
CM	56	17	tr
PW	434	413	200

[a]Values are average of three determinations.

contamination with free internal lipids cannot be ruled out. The CM extracted lipids amounted to 6·2% of TSL (Table 2). This value was higher than the range (1·8–5·4%) generally reported for cereal starches (Youngs *et al.*, 1977; Morrison, 1978; Vasanthan & Hoover, 1992). The lipid classes followed the order: NL > PL > GL. The concentration (% SEL) of NL and PL were respectively 5 and 1·5%. GL occurred only in trace quantities (Table 2). The major NL (% total NL) and PL (% total PL) (Table 3) were respectively, free fatty acids (FFA) (39·6%) and lysophosphatidylcholine (LPC) (93%). C18:2 and C16:0 acids were respectively the predominant fatty acids in the NL and PL fractions (Table 4).

Table 3. Lipid class component composition of oat starch

	Lipid class[a] (mg/100 g dry starch)	
	CM extract	PW extract
Neutral lipids[b]		
FFA	22·2	273·4
MG	8·7	tr
DG	5·6	25·6
TG	8·9	134·5
FS	7·8	tr[d]
SE	2·7	tr
Glycolipids		
MGMG	tr	5·6
MGDG	tr	10·6
DGDG	tr	166·6
Cer I	tr	4·2
Cer II	tr	12·8
Unknown	tr	tr
Phospholipids		
LPC	15·8	291·2
LPE	tr	54·9
PC	1·2	21·5
PE	tr	36·7
PS	tr	4·1
PG	tr	4·5
PA	tr	tr

[a]Based on densitometric absorbance.
[b]FFA, free fatty acid; MG, monoacylglycerol; DG, diacylglycerol; TG, triacylglycerol; FS, free sterol; SE, sterol ester; MGMG, monogalactosylmonoglyceride; MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; Cer I, cerebroside I; Cer II, cerebroside II; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; PA, phosphatidic acid.
[c]Values are average of three determinations.
[d]Trace = less than 0·5% of total lipid class.

Table 4. Fatty acid distribution of the major lipid classes in CM and PW extracts of oat starch

Lipid class and extraction medium	Fatty acid composition (area %)						
	16:0	18:0	18:1	18:2	18:3	20:0	Other[a]
Neutral lipids							
CM	35·3	3·6	11·7	40·9	8·2	tr[b]	0·3
PW	38·7	4·3	8·9	41·5	4·8	1·6	0·2
Glycolipids							
CM	tr	tr	tr	tr	tr	tr	tr
PW	46·6	0·5	10·1	40·2	tr	21·1	0·5
Phospholipids							
CM	72·4	tr	2·9	13·8	10·2	tr	0·7
PW	74·2	0·5	5·4	17·1	1·1	0·8	0·9

[a]Includes 14:0 and 22:0.
[b]Trace = less than 0·1%.

PW extracted lipids

Lipids obtained by extraction of the CM residue with hot PW are presented in Table 2. They probably represent most of the free and bound lipids in the granule interior and some bound lipids that may have been present on the granule surface. They amounted to 97·0% of TSL. This meant that 0·8% of TSL were not extractable by solvents and were released only on hydrolysis by acid (Table 1). The lipid classes followed the order NL > PL > GL. The NL content (% SEL) was 38·7% (Table 4). The major NL (% total NL) was FFA (63%). Unlike in other cereal starches, where the TG content of the PW extract is only about 3·3–5·2% of the total NL (Vasanthan & Hoover, 1992), the TG in this sample of oat starch was 33·1% of the total NL. The GL content (% SEL) was 17·8% (Table 2). This value was higher than the range (2·8–10·4%) generally reported for cereal starches (Vasanthan & Hoover, 1992). The major GL (Table 3) fraction (% total GL) was digalactosyldiglyceride (DGDG) (83·3%). The PL level (% SEL) was 36·8% (Table 2). The major PL fraction (% total PL) was LPC (70·5%). The major fatty acids in the NL fraction was C18:2, whereas C16:0 was the major fatty acid in PL and GL fractions (Table 4).

The monoacyl lipid content (% TSL) in both CM and PW extracts was 61% (Table 3). This value was lower than those reported for wheat (85·2%), rice (83·2%) and corn (78·2%) starches (Vasanthan & Hoover, 1992).

The results obtained above provide the first comprehensive survey of the lipids in oat starch, classified according to their degree of extractability with cold and hot solvent systems.

Amylose content

The total amylose contents of oat starches have generally been reported to be in the range 25·2–29·4% (Morrison *et al.*, 1984; Gudmundsson & Eliasson, 1989), of which 28·3–32·0% is complexed by native lipids (Morrison *et al.*, 1984). However, the corresponding values for AC Hill oat starch were respectively 19·4 and 13·9% (Table 1). These major discrepancies in data are probably due to varietal differences.

X-ray diffraction

The X-ray spectra of native oat starch was of the A type (Fig. 2) representative of cereal starches, with spacing at 3·8 Å, 4·8 Å, 5·2 Å and 5·8 Å. At approximately the same moisture content, the relative crystallinity of oat starch (0·90) was higher than those of wheat starch (0·67). This seems to suggest a closer packing of double helices (formed from adjacent branches of amylopectin) in oat starch granules. However, Paton (1987) reported that native oat and wheat starches have similar X-ray diffraction patterns. It is difficult to explain this discrepancy, since the above author did not indicate whether comparisons of crystallinities were made by visual assessment of the X-ray traces or by calculation.

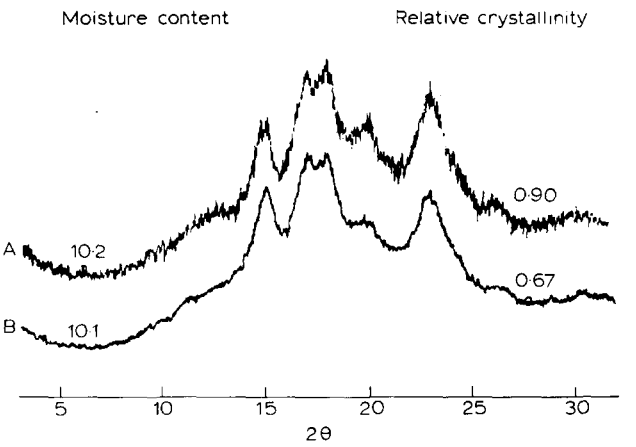


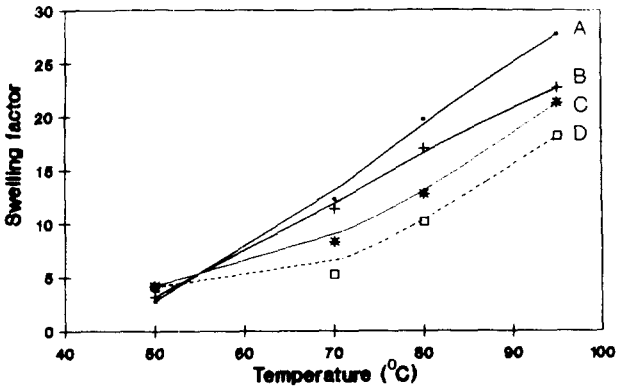
Fig. 2. X-ray diffraction patterns of native starches: (A) oat; (B) wheat.

Swelling factor and amylose leaching

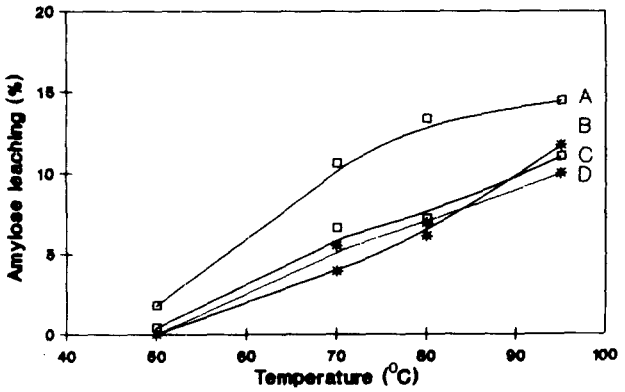
The swelling factor and amylose leaching of oat and wheat starches in the temperature range 50–95°C, and the spectral behavior of their solubles at 70°C are presented in Figs 3(a) and 3(b).

The swelling factor of native and defatted starches increased with rise in temperature (Fig 3(a)). These values were higher in oat starch. The swelling factor of native starches were generally higher than those of their defatted counterparts (Fig. 3(a)). The above differences were fairly negligible at temperatures below 55°C in wheat starch and 70°C in oat starch. The difference in swelling factor between native and defatted starches remained fairly constant at temperatures above 70°C in wheat starch, but continued to increase in oat starch. At 95°C, the swelling factor of native and defatted starches were respectively 27·4 and 22·4 in oat, and 20·1 and 17·1 in wheat. The swelling factor at 95°C for native oat starch was close to those reported by Gudmundsson and Eliasson (1989) for oat varieties *Svea* (28·0) and *Chicauhau* (27·5), but lower than that of *sentinel* (30·8) (Doublier *et al.*, 1987).

Leach *et al.* (1959) postulated that the bonding forces within the starch granule would influence the manner of swelling. Thus a highly associated starch with an extensive and strongly bonded micellar network would be resistant to swelling. Furthermore, Tester and Morrison (1990) have shown, by studies on waxy and normal starches, that swelling is a property of amylopectin, and that in normal cereal starches amylose and lipids actively inhibit swelling under conditions where amylose–lipid complexes are likely to form. From the above considerations, the differences in the extent of swelling shown by native oat and wheat starches can be attributed to differences in relative crystallinity (Fig. 2)



(a)



(b)

Fig. 3. (a) Swelling factor of native and defatted starches: (A) native oat; (B) defatted oat; (C) native wheat; (D) defatted wheat; (b) Amylose leaching in native and defatted starches; (A) defatted oat; (B) native oat; (C) defatted wheat; (D) native wheat.

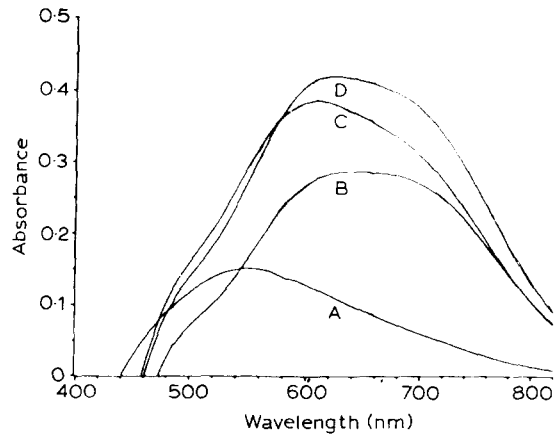
and amylose complexed lipids (Table 1; Morrison & Laignelett, 1983).

Amylose leaching in native oat starch (Fig. 3(b)) was generally about 52% less than that of other oat starch varieties (Paton, 1979; Gudmundsson & Eliasson, 1989), and was also generally less than that of wheat starch (Fig. 3(b)). These differences probably reflect the lower amylose content of AC Hill oat starch.

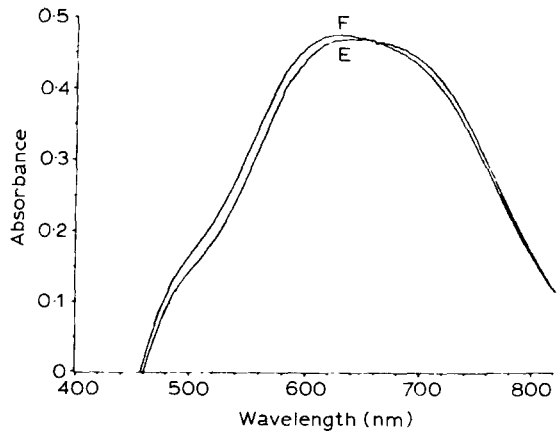
Amylose leaching was enhanced on defatting (Fig. 3(b)). This was expected, since removal of bound lipids is known to increase amylose solubilization (Hoover & Hadziyev, 1981; Eliasson, 1985). However, the difference in amylose leaching between native and defatted starches was much higher in oat than in wheat (Fig. 4(b)). This was rather surprising, since a higher percentage (33·1%) of amylose molecules are complexed by native lipids in wheat starch (Morrison & Laignelett, 1983). We postulate that, during lipid removal, an increase in amylose chain mobility (due to moisture and thermal energy) occurs in the amorphous regions of the starch granule, enabling them to interact with each other via hydrogen bonding. The results suggest that this interaction decreases amylose leaching, and is much stronger in wheat starch. Presumably, the amylose chains of native oat starch occur in a highly disorganized manner in the amorphous regions, and are thus not properly aligned for maximum interaction.

The wavelength of maximum absorption (λ_{max}) of the solubles leached at 70°C from native oat and wheat starches occurred respectively, at 600 (Fig. 4(a)) and 630 nm (Fig. 4(b)). The λ_{max} of pure amylose and pure amylopectin were respectively, 640 and 550 nm (Fig. 4(a)). These observations indicate that amylose, and probably a molecule less branched than amylopectin, are co-leached from granules of oat, but preferential leaching of amylose occurs in wheat starch. Doublier *et al.* (1987) have stated that amylopectin co-leaches with amylose in oat starch. However, it is difficult to envisage the leaching of a highly branched molecule such as amylopectin from intact starch granules at 70°C.

Banks and Greenwood (1967) demonstrated the existence of fractions intermediate in behavior to



(a)



(b)

Fig. 4. (a) Spectral behavior of: (A) potato amylopectin; (B) potato amylose; (C) native oat starch; (D) defatted oat starch. (b) Spectral behavior of: (E) native wheat starch; (F) defatted wheat starch.

amylose and amylopectin. An amylopectin sub-fraction termed anomalous amylopectin was identified for all cereal starches. Paton (1979) showed by means of gel chromatography that oat starch (Hinoat cultivar) contains 56% amylopectin, 26% intermediate material and 18% amylose. The corresponding values for wheat were 56, 16 and 28%. It is likely that anomalous amylopectin (in the intermediate material) may be less branched in oat than in wheat, and is, therefore, able to co-leach with oat amylose during the pasting process.

Pasting properties

The pasting properties of native and defatted oat and wheat-starches at pH values of 5·5 and 3·0 are presented in Fig. 5.

pH 5·5

Native oat and wheat starches showed wide differences in peak viscosity and set-back. These values were higher in oat. However, both starches showed identical pasting temperatures (Figs 5(a, b)). Similar observations have been made by MacArthur and D'Appolonia (1979) on three varieties (*Dal, Froker, Cayuse*) of oat starch. The difference in peak viscosity can be attributed to higher quantities of amylose-bound lipids in wheat starch. Lipid removal decreased pasting temperature, peak viscosity and set-back, and increased the thermal stability for both oat and wheat starches (Figs 5(a, b)). However, these changes were more marked in the former. These results suggest that defatting increases granular strength, being of a higher order of magnitude in oat starch. The rigidity of native and defatted starch gels (obtained on cooling the hot starch paste to 30°C) was greater in wheat than in oat. This was rather surprising, since high set-back values are generally associated with greater starch paste retrogradation. It has been proposed by Doublier *et al.* (1987) that starch pastes can be described as suspensions of swollen particles dispersed in a macromolecular medium. Swollen particles are composed of remaining swollen granules and the continuous phase is a solution of macromolecules of the soluble fraction as estimated in swelling-solubility experiments. Recently, Hansen *et al.* (1991) showed by means of oscillatory rheometry measurements, that corn starch gelation is hindered in the presence of amylopectin. Furthermore, Miles *et al.* (1985) postulated that amylose gelation requires network formation, and network formation requires polymer chain entanglement. Therefore, the low rigidity of oat starch gels could be attributed to interaction between the exudates (branched component and amylose chains) in the continuous phase. Most likely, this interaction restricts the ability of amylose chains to form a strong network by lateral association of double helical junction zones.

We postulate that set-back values reflect the extent

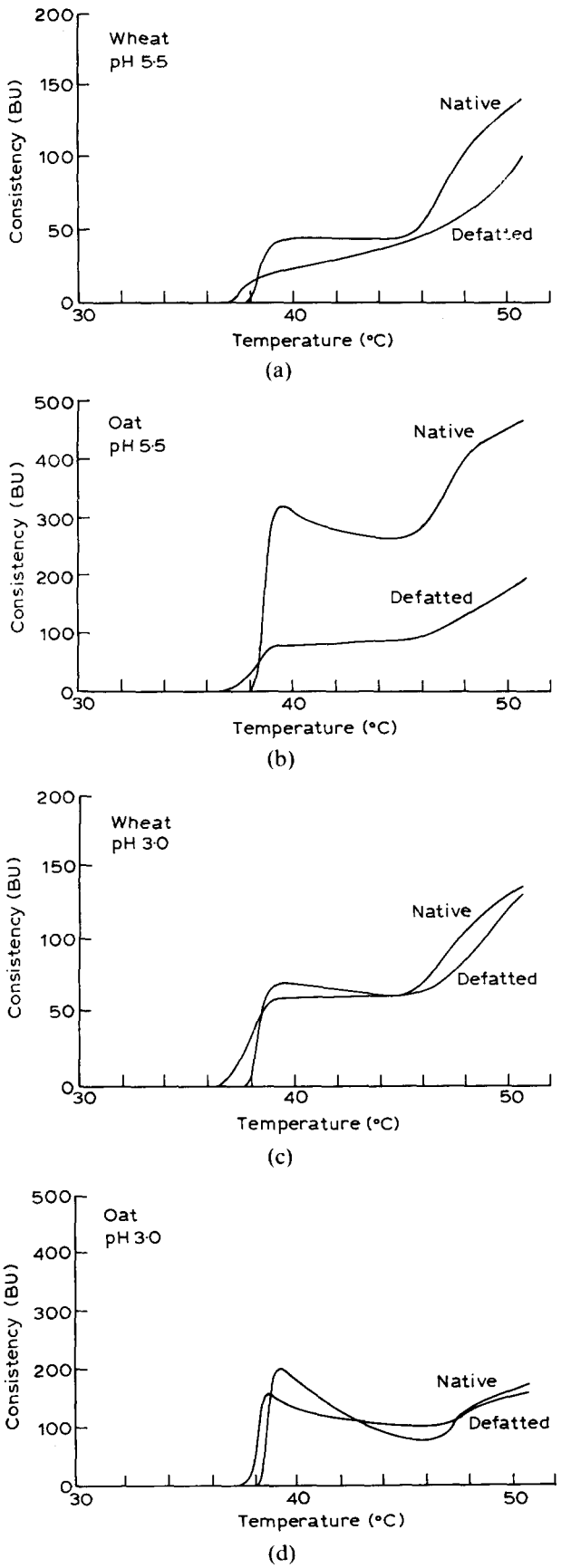
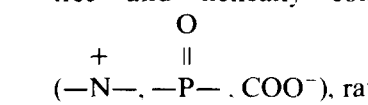


Fig. 5. Pasting characteristics of native and defatted starches: (a) wheat starch, pH 5·5; (b) oat starch, pH 5·5; (c) wheat starch, pH 3·0; (d) oat starch, pH 3·0.

of water immobilization around the charged centers of starch components ($O^{\delta-}$—$H^{\delta+}$) and those of free and helically complexed lipid molecules



(—N—, —P— , COO^-), rather than starch paste retrogradation. The association of water molecules with these charged centers would decrease the effective water concentration in the continuous phase, resulting in a rise in viscosity during the cooling cycle. The decreased set-back on lipid removal thus stands explained. Among native starches the extent of water immobilization (set-back) is higher in oat starch (Figs 5(a, b)), due to the presence of more charged centers (provided by the leached branched component) in the continuous phase.

pH 3·0

The thermal stability and set-back of wheat and oat starches at pH 3·0 were lower than at pH 5·5 (Figs 5(c, d). However, the peak viscosity of native oat starch was lower than at pH 5·5, while that of native wheat starch was higher. These results indicate that amylose chains in the amorphous regions of oat and wheat starch granules are more extensively hydrolyzed by H_3O^+ at pH 3·0. The magnitude of this hydrolysis is greater in oat starch. This seems to suggest that the amylose chains of oat starch are less compactly packed than those of wheat starch, and are therefore more accessible to hydrolysis by H_3O^+. The difference in set-back values at pH 3·0 and 5·5 can be attributed to greater neutralization of the negatively charged centers of lysophospholipid molecules, and to decreased ionization of the hydroxyl groups of starch components at pH 3·0.

The difference in paste consistencies between defatted starches at pH 3·0 (Figs 5(c, d)) were also less marked than at pH 5·5 (Figs 5(a, b)). The peak viscosities of both defatted oat and wheat starches were higher, and the thermal stabilities and set-backs were lower than at pH 5·5 (Figs 5(c, d)). The magnitude of these differences were slightly higher in defatted oat starch. The effect of pH on paste consistencies were less marked with defatted starches due to the absence of helically complexed charged lipids.

Acid hydrolysis

The solubilization pattern of native oat starch is presented in Fig. 6. The extent of hydrolysis during the first 12 days (corresponding to hydrolysis of the amorphous regions of the granule), was generally about 8% higher than that of wheat starch (Fig. 6). These observations suggest that amorphous regions of oat starch granules are more accessible to penetration

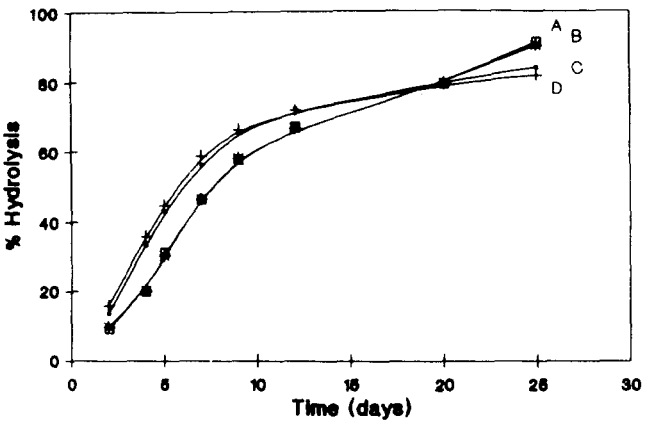


Fig. 6. Acid hydrolysis of native and defatted starches: (A) native wheat; (B) defatted wheat; (C) native oat; (D) defatted oat.

by hydrated protons (H_3O^+), presumably due to a lower state of aggregation of amylose chains in these regions.

The second stage, which involves hydrolysis of the crystalline regions, was slower in oat than in wheat starch (Fig. 6). For instance, between the 12th and 20th day, hydrolysis increased only by 7·5% in oat starch, whereas the corresponding value for wheat starch was 18·0%. On the 25th day, oat starch was 83·1% hydrolyzed and wheat starch 91%. This seems to suggest that packing of starch chains in the crystalline regions may have been more compact in oat starch than in wheat starch granules. Hydrolysis decreased only slightly when lipids were removed from oat starch (Fig. 6). However, native and defatted wheat starch granules were hydrolyzed to the same extent.

Enzymatic digestibility

The extent of hydrolysis (24 h) of native and defatted oat and wheat starches by porcine pancreatic α-amylase are presented in Table 5. Native oat starch was hydrolyzed (31·6%) to a lesser extent than native wheat starch (42·0%). This could be attributed to differences in amylose content (Table 1; Morrison & Laignelett, 1983). Lipid removal increased the digestibility of both oat and wheat starches by 25·6 and 30·4% respectively. This indicates that a change in amylose conformation (V helix → random coil) occurs on lipid removal, which increases the surface area available for enzyme action. Thus, the extent of increase in digestibility on lipid removal probably reflects the amount of amylose chains complexed by native lipids in wheat (22·7%) and oat (13·9%) starch granules.

Microscopy

The mode of attack by α-amylase on native and defatted oat starch granules was investigated using

Table 5. *In-vitro* hydrolysis of native and defatted oat and wheat starches by porcine pancreatic alpha amylase[a]

Source	% hydrolysis[b]	
	Native	Defatted
Oat	31·6 ± 1·2	57·2 ± 1·5
Wheat	42·0 ± 1·0	72·4 ± 1·4

[a]24 h hydrolysis.
[b]Values are mean of three replicates ± SD.

SEM (Fig. 7). After 24 h of hydrolysis, defatted granules (Fig. 7(b)) were more eroded than those of native granules (Fig. 7(a)). Most of the defatted granules had roughened surfaces, suggesting that these granules were hydrolyzed by surface erosion. The roughened surfaces were quite different from the still smooth surfaces of many of the native granules. The mode of attack of α-amylase on native oat starches was quite different from that seen with other cereal starches (MacGregor & Ballance, 1980; Knutson *et al.*, 1982), where pitting and pinhole effects are generally observed after degradation with α-amylase.

Gelatinization temperatures

The DSC thermograms of native and defatted starches are presented in Table 6. The transition temperatures (61–73°C) and the gelatinization enthalpy (10·4 J/g) of native oat starch was within the range reported for other varieties (Paton, 1987; Gudmundsson & Eliasson, 1989). The corresponding values for native wheat starch were 57–67°C and 11·5 J/g. The higher gelatinization temperature of oat starch indicates a greater degree of order in the crystalline structure. This is supported by

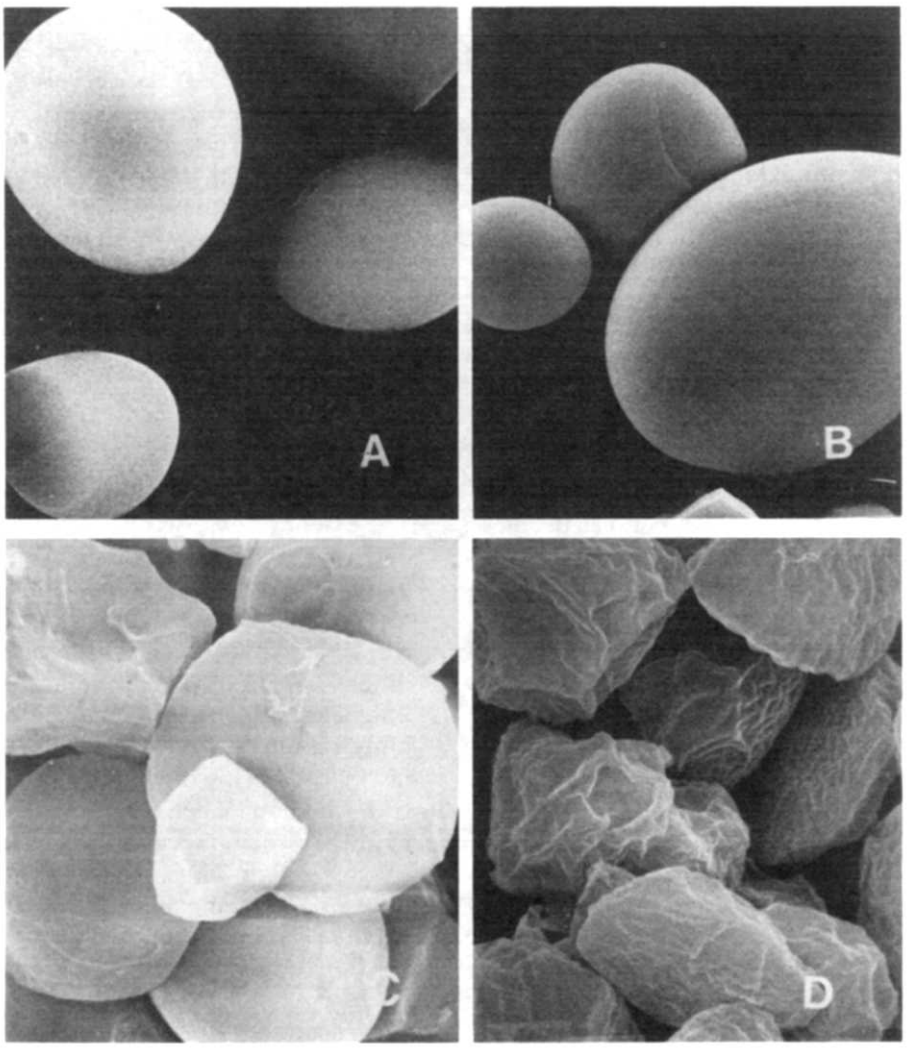


Fig. 7. Scanning electron micrographs of native and defatted oat starches before and after attack by porcine pancreatic α-amylase: (A) native starch; (B) defatted starch; (C) native starch attacked by α-amylase; (D) defatted starch attacked by α-amylase.

Table 6. DSC characteristics of native and defatted oat and wheat starches[a]

Starch	Water (%)	T_o[b]	T_p (°C)	T_c	ΔH_g[c] (J/g)	T_{cx}[d] (°C)	ΔH_{cx}[e] (J/g)
Oat							
Native	75	61	66	73	10·4	106	0·84
Defatted	75	58	64	71	11·1	—	—
Wheat							
Native	75	57	62	67	11·5	105	1·30
Defatted	75	56	61	65	12·2	—	—

[a]Average standard deviation = 0·1 (n = 3).
[b]T_o T_p and T_c indicate the temperature of the onset, midpoint and end of gelatinization respectively.
[c]Enthalpy of gelatinization.
[d]Midpoint of amylose-lipid complex melting temperature.
[e]Enthalpy of amylose-lipid complex melting.

X-ray diffraction data (Fig. 2). The transition enthalpy (ΔH_{cx}) of the amylose-lipid complex in oat starch (0·84 J/g) was slightly lower than that of wheat starch (1·30 J/g) (Table 6), but much lower than the range reported (2·4–3·7 J/g) for other oat starch varieties (Paton, 1987; Gudmundsson & Eliasson 1989). This is indicative of decreased amylose binding by native lipids in AC Hill oat starch (Table 1). Defatting decreased transition temperatures and increased gelatinization enthalpies. These changes were of the same order of magnitude in both oat and wheat starches.

Syneresis

The freeze–thaw stability of the starches are presented in Fig. 8. The stability of a starch gel is evaluated by the amount (%) of water released when starch chains reassociate (via hydrogen bonds) during the freeze–thaw cycles. Among native starches, the degree of syneresis was higher in wheat (Fig. 8). Syneresis decreased on lipid removal. The extent of this decrease was similar in both starches. As discussed earlier, at ambient temperature water molecules form an orderly immobilized layer around the hydroxyl groups ($O^{\delta-}—H^{\delta+}$) of starch chains and the positive (nitrogen) and negative (phosphate) charges on the helically complexed lysophospholipid molecules. In this form the water is unavailable as a solvent and does not freeze. During the sol to gel transformation, a progressive increase in hydrogen bonding occurs between adjacent starch chains, resulting in exclusion of the immobilized water molecules. The quantity of immobilized water released is thus a measure of the extent of retrogradation. Among native starches, the degree of syneresis was lower in oat, due to retardation of amylose aggregation by the branched component. The decrease in syneresis on defatting probably reflects a decrease in water immobilization, due to removal of helically complexed lysophospholipid molecules.

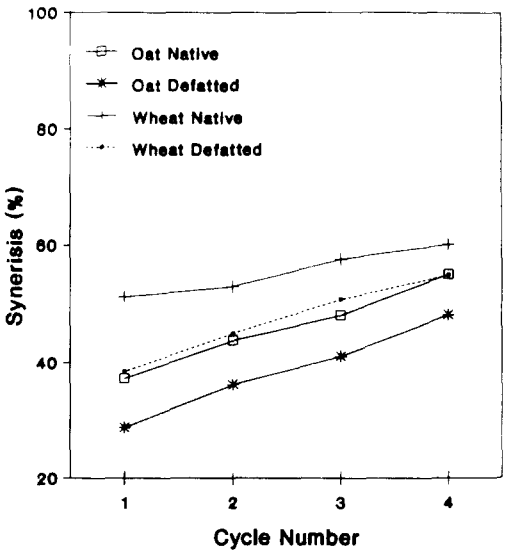
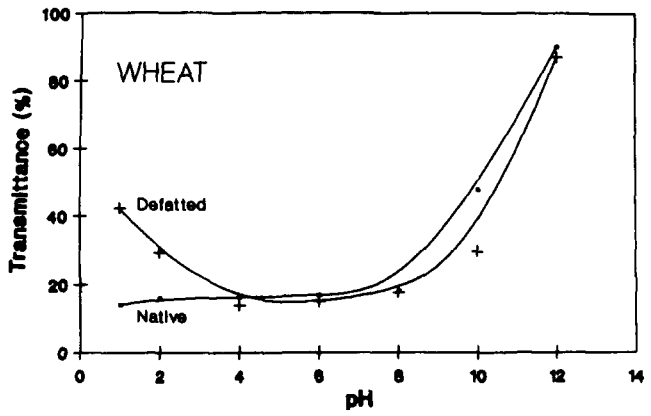


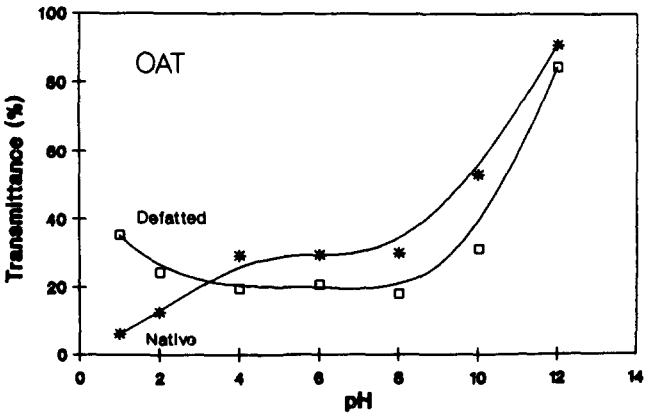
Fig. 8. Freeze–thaw stability of native starches.

Paste clarity

The light transmittance of oat and wheat starch pastes at different pH values are presented in Fig. 9. At pH values less than 4·0, the clarity of defatted starches were higher than those of their native counterparts. However, this trend was reversed beyond pH 4·0. Furthermore, the differences in clarity between native and defatted starches were higher in oat. In native starches, at pH values below 4·0, the negatively charged phosphate groups on helically complexed lysophospholipid molecules would be neutralized, and the ionization of hydroxyl groups suppressed. Therefore, lysophospholipid complexed amylose chains would contain only electropositive nitrogens. Coulombic repulsion between these positive nitrogens on adjacent amylose chains would decrease the compactness of the amorphous region, thus enabling H_3O^+ to penetrate and rapidly degrade the amylose chains into smaller chain length units. These small molecular weight amylose chains



(a)



(b)

Fig. 9. Paste clarity of native and defatted starches at different pH values. (a) wheat; (b) oats.

could then aggregate rapidly forming junction zones (Gidley *et al.*, 1986). These junction zones, being larger compared to the wavelength of illumination, would scatter the incident light, and thus reduce transmittance (Craig *et al.*, 1989). Since the above effects would be less pronounced in defatted starches (due to absence of charged centers), the percentage transmission would consequently be higher. This seems plausible, since starches devoid of helically complexed lysophospholipid molecules such as those from lentil (Vasanthan & Hoover, 1992) show identical transmittance values for both native and defatted starches (unpublished results). At pH values greater than 4·0, light transmittance continued to increase, due to an increase in granular swelling, resulting from repulsion between adjacent negative charges centered on the hydroxyl groups of amylose chains and phosphate groups of helically complexed lysophospholipid molecules.

CONCLUSION

This study has shown that the properties of AC Hill oat starch are not typical of oat starch in general. This could be attributed to low amylose content and decreased amylose binding by native lipids. Further work is now in progress on the fine structure to obtain a more precise insight into the granular structure.

ACKNOWLEDGEMENTS

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