Isolation of Oat Starch from Oat Flour[1]

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ABSTRACT

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Oat starch was isolated from oat flour by 1) high shear in water after a presoak, 2) low shear after digestion with protease, and 3) low shear at alkaline pH. Oat flour was soaked in water (25% solids) at 20°C for 6 hr and subjected to high shear using a tissue homogenizer. After 1 min of shearing followed by centrifugation, the yield of starch (1.3% protein) was 70%, based on oat flour. Two bases were used to isolate oat starch at different concentrations and stirring times. Stirring for 60 min at pH 10.5 with 0.01*M* sodium hydroxide (pH 10.5) or 0.03*M* calcium hydroxide (pH 11.0) led to 72-75% isolated starch with 0.3% protein. Digestion of oat flour (25% solids) with *Aspergillus oryzae* protease at pH 7.5 and 37°C for 6 hr gave 78% starch with 1.1% protein. Oat starch isolated with sodium hydroxide showed a delayed pasting temperature and increased paste consistency in the amylograph compared to starch isolated by high shear or calcium hydroxide. The same phenomena was observed in wheat starch isolated with 0.01*M* sodium hydroxide but not in corn or rice starch.

Dry milling of oat groats yields approximately 35% oat bran and 65% oat flour. When the demand for oat bran is high, a surplus of oat flour results.

Separating oat flour into starch and protein is complicated by strong binding between the two components and by the presence of β-glucan. Oat starch has been isolated with sodium carbonate (Paton 1977) or sodium hydroxide (Wu et al 1973) at low shear and with water at high shear (Meuser et al 1985). No reports have appeared on the use of protease or cellulase to release oat starch, nor has a comparison been made of the use of sodium and calcium hydroxides.

The objectives of this investigation were to compare 1) the yield and purity of oat starch isolated from oat flour using low shear with alkali, protease, and/or cellulase treatment and high shear with water and 2) the pasting properties of oat, wheat, corn, and rice starches isolated with sodium and calcium hydroxides.

MATERIALS AND METHODS

General

Oat flour was obtained from the ConAgra Flour Milling Co., Omaha, NE. The flour contained 10.3% moisture, 6.8% protein, 80.0% starch, 3.1% lipid, 0.6% ash, and 0.5% β-glucan. Hard red winter wheat, dent corn, and long-grain rice were commercial samples. Moisture, protein, ash, lipid, and starch were determined by AACC methods (1983) 44-18, 46-13, 08-01, 30-26, and 76-11, respectively. The β-glucan content was determined using a β-glucan test kit (Biocon, Inc., Rockville, MD).

Starch lipids were determined gravimetrically after extraction with propanol/water (3:1, v/v) at 100°C (Morrison and Coventry 1985). All chemicals were reagent grade.

Protease (from *Aspergillus oryzae, Bacillus subtilis, Streptomyces griseus,* and *Rhizopus* sp.) and cellulase (from *Penicillium funiculosum*) were purchased from Sigma Chemical Co. (St. Louis, MO). Cellulase from *Trichoderma reesei* (Celluclast) was from Novo (Danbury, CT). One unit of protease activity was defined as the amount of enzyme that liberated 1.0 μmol of tyrosine per minute from casein at pH 7.5 and 37°C. One unit of cellulase activity was defined as the amount of enzyme that released 1.0 μmol of glucose from cellulose in 1 hr at pH 5.0 and 40°C.

Isolation of Starch Using Low Shear at Alkaline pH

Oat flour (100 g) was mixed with 500 ml of 0.005-0.025*M* sodium hydroxide or 0.003-0.068*M* calcium hydroxide, stirred for 30 min at 25°C, and then centrifuged (1,400 × *g*). The supernatant was discarded, the sediment was slurried with water (500 ml), and the mixture was filtered through a nylon bolting cloth (50 μm). The filtrate was neutralized with 1*M* hydrochloric acid, and the mixture was centrifuged. The supernatant was discarded, along with the tailings layered on top of the starch, which were carefully removed by scraping. The starch was washed with water (3 × 200 ml), collected by centrifugation, and dried overnight in a forced-convection oven at 40°C.

Corn, wheat, and rice starches were isolated from whole-grain flours using 0.01*M* NaOH or 0.03*M* $Ca(OH)_2$. Before rice starch isolation, the rice flour was presoaked in 0.64*M* NaOH for 24 hr or in 0.03*M* $Ca(OH)_2$ for 1 hr.

Isolation of Starch Using High Shear in Water

Oat flour (10 g) was soaked in 30 ml of water in a large test tube for 6 hr at 20°C. The tube was placed in an ice bath, and the slurry was subjected to high shear for different times using a tissue homogenizer (model SDT99078, 170W, Tekmar Co., Cincinnati, OH). The sheared slurry, with a final temperature of

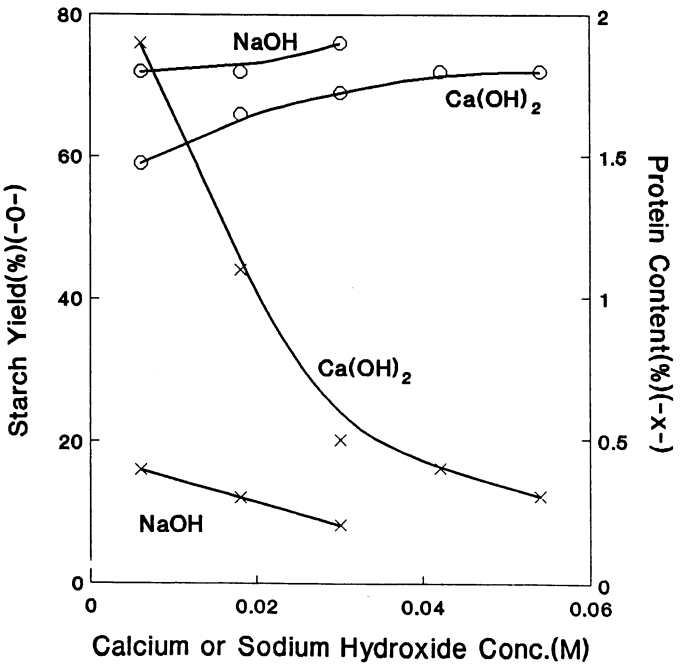

Fig. 1. Yield and protein content of oat starch isolated from oat flour using calcium or sodium hydroxide. Oat flour (17% solids) was stirred in an alkaline medium for 30 min at 25°C. Starch yield is calculated based on oat flour (dry basis).

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4–10°C, then was centrifuged, and the supernatant and tailings were discarded. The starch sediment was resuspended in water, the mixture was filtered through a nylon bolting cloth (50 μm), and the filtrate was centrifuged. The supernatant and tailings were discarded, and the starch was dried at 40°C overnight.

Isolation of Starch Using Low Shear in Water

Corn and wheat starches were isolated with low shear in water by the methods of Watson (1964) and Wolf (1964), respectively.

Isolation of Starch Using Protease and Cellulase at Low Shear

A mixture of oat flour (10 g), protease (50 units) or cellulase (500 units), and water (30 ml) was adjusted to pH 7.5 and 37°C for protease or to pH 5.0 and 40°C for cellulase. The pH was adjusted with 0.1*M* NaOH or HCl, respectively. In another test, successive digestion was done with cellulase (500 units) at pH 5 and 40°C for 3 hr followed by protease at pH 7.5 and 37°C for 3 hr. Each mixture was centrifuged, and the supernatant and tailings fractions were discarded. The starch fraction was resuspended and washed with water (100 ml) and then isolated and dried in the usual manner.

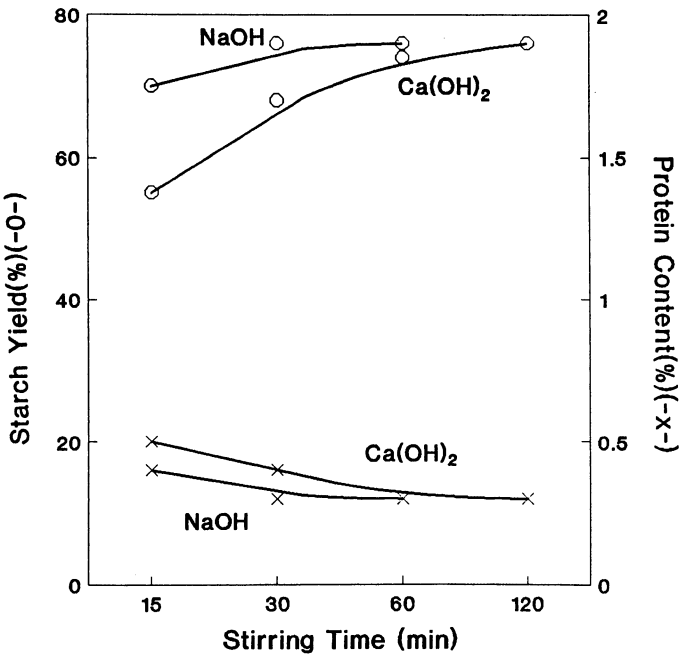

Fig. 2. Effect of stirring time on the yield and protein content of oat starch. Oat flour (17% solids) in 0.03*M* $Ca(OH)_2$ or 0.01*M* NaOH was stirred at 25°C.

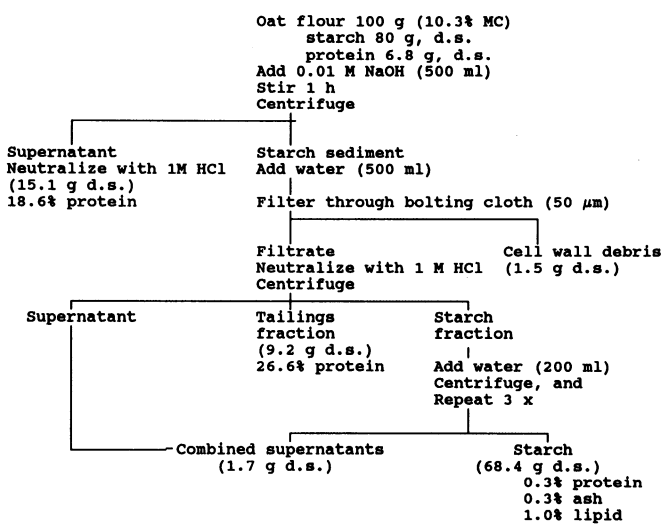

Fig. 3. Isolation of oat starch using 0.01*M* sodium hydroxide at low shear; mass balance. MC = moisture content; d.s. = dry solids.

RESULTS AND DISCUSSION

Isolation of Oat Starch Using Low Shear at Alkaline pH

Oat flour at a solids level of 17% was mixed for 30 min at 25°C in an alkaline medium, and the starch then was isolated. Figure 1 shows that sodium hydroxide was more effective than calcium hydroxide in releasing low-protein oat starch, especially at concentrations below 0.03*M*. At a concentration above 0.01*M* NaOH (pH 10.5) or 0.03*M* $Ca(OH)_2$ (pH 11.0), the yield of starch was 72–76% based on the dry weight of oat flour, which equals a recovery of 90–95% of the starch. The starch contained 0.3–0.4% protein on a dry-solids basis (Fig. 1).

In 0.01*M* NaOH or 0.03*M* $Ca(OH)_2$, stirring beyond 60 min did not increase the yield or remove additional protein from the starch (Fig. 2). Figure 3 gives a mass balance of the process to isolate oat starch using low shear in 0.01*M* NaOH. The process gave 76% starch (86% of theory) based on oat flour, and the starch had a protein content of 0.3%. The protein recovered in the combined alkaline extracts and the starch tailings amounted to 5.3 g (78% of theory) on a dry-solids basis. Total solids recovered were 107%. When 0.03*M* $Ca(OH)_2$ was used in place of NaOH, the total recovery of solids was 105%, whereas the starch and protein yields and their purities were almost the same as with 0.01*M* NaOH.

Isolation of Oat Starch Using High Shear in Water

Presoaking of oat flour in water was an essential step for the successful isolation of starch using high shear in water. During

Pasting of Starches

Starch pasting properties were measured (Deffenbaugh and Walker 1989) with the Rapid Visco-Analyser (Foss Food Technology Co., Eden Prairie, MN). The total weight of water and starch was constant at 28 g, whereas the dry solids concentration was generally 10 g/100 ml. After the starch and water was introduced into the sample holder, the paddle was inserted, and the mixture was stirred by hand to eliminate lumps. Viscograms were recorded at 0–100% relative consistency at full scale using a chart speed of 1 cm/min. In the 18-min test, the sample was held at 50°C for 2 min, heated to 95°C in 3 min, held at 95°C for 5 min, cooled to 50°C in 4 min, and held at 50°C for 4 min.

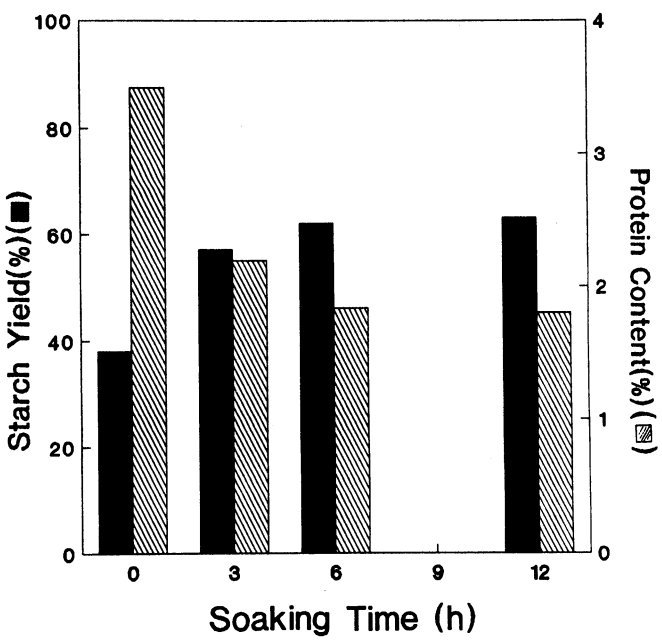

Fig. 4. Effect of soaking time on the yield and protein content of oat starch isolated using high shear. Oat flour (25% solids) in water was soaked at 20°C and sheared for 30 sec.

soaking, the binding between oat starch and protein was weakened, either by wetting or by enzyme-catalyzed reactions. Increasing the soaking time of oat flour (25% solids) from 3 to 6 hr at 20°C, followed by shearing for 30 sec, improved starch yield and purity (Fig. 4), but there was no further improvement after soaking for an additional 6 hr. The yield of starch increased with increasing soaking temperature from 5 to 20°C during a 6-hr soak, but it leveled off at 20 to 35°C (data not given). However, during all high-shear treatments, the starch slurry was cooled to maintain temperature below 10°C.

Figure 5 shows the effect of shearing time on the yield and protein content of oat starch after soaking in water for 6 hr at 20°C. An increase in the shearing time from 15 to 60 sec was accompanied by a marked increase in starch yield and in purity, whereas after a 90-sec shearing, the starch yield and purity improved only marginally. No separation of starch was observed in water without high shear.

Figure 6 shows the mass-balance for the process in which oat flour was soaked at 25% solids in water at 20°C for 6 hr, and the mixture then was subjected to high shear for 60 sec. The yield of starch was 70%, based on oat flour with a protein level

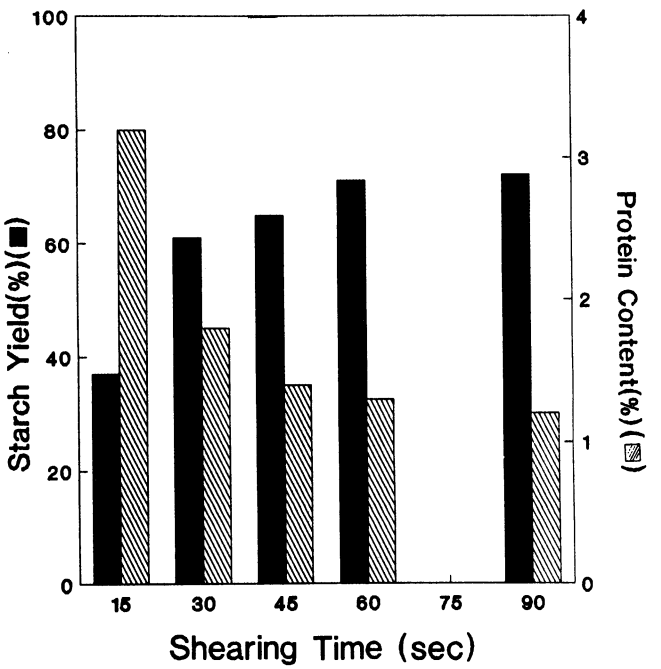

Fig. 5. Effect of shearing time on the yield and protein content of oat starch isolated using high shear. Oat flour (25% solids) in water was soaked at 20°C for 6 hr and sheared to isolate oat starch.

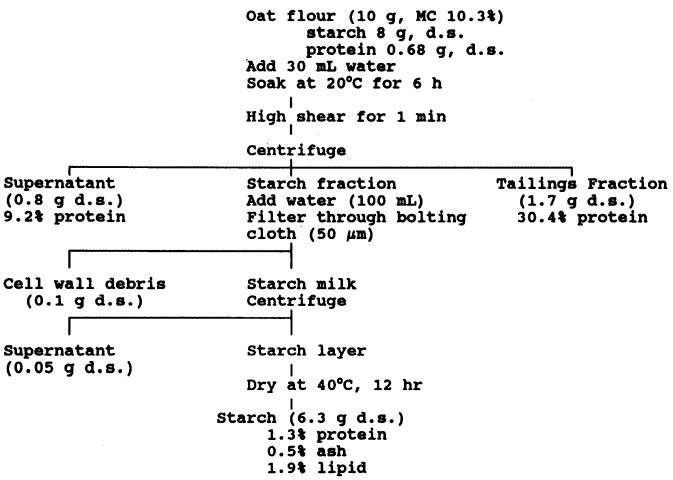

Fig. 6. Isolation of oat starch using high shear in water; mass balance.

of 1.3%. The oat starch also contained almost twice the lipid and ash levels of starch isolated at alkaline pH (compare Figs. 6 and 3). The protein recovery in the combined supernatant and tailings fractions totaled 0.6 g (88% of theory) on a dry-solids basis. Total solids recovery was 100%.

Isolation of Oat Starch Using Protease or Cellulase at Low Shear

Proteolytic enzymes might be expected to weaken the intimate association of protein and starch in oat flour and facilitate the isolation of starch. In addition, the fibrous cell walls in oat endosperm contain mixed β-glucan, which may inhibit the effective separation of oat starch. Cellulase might digest and weaken any intact cells.

Table I shows the effect of microbial proteases or cellulases on the isolation of oat starch after a 3-hr digestion at optimum pH and temperature. At the low shear used in these experiments, no separation of oat starch was observed without enzymes (blank). The protease from *A. oryzae* gave a 72% yield of starch containing 1% protein. All of the proteolytic enzymes were more effective than the two cellulases.

As shown in Table II, incubation of a 25% slurry of oat flour with 50 units of protease (*A. oryzae*) in 30 ml of buffer for 6 hr at 37°C gave 78% starch (based on oat flour) with a protein content of 1.1%. Increasing the level of protease from 50 to 100 units per 30 ml of buffer gave a slight improvement in yield but no reduction in protein content. Successive digestion with 50 units of the *A. oryzae* protease, followed by 500 units of cellulase, gave no benefit.

A method to isolate approximately 86% of the starch from oat starch using *A. oryzae* protease is shown in Figure 7. The isolated starch contained 1.1% protein, 0.5% ash, and 1.7% lipid.

TABLE I
Yield and Protein Content of Oat Starch Isolated from Protease or Cellulase Digests of Oat Flour in Water[a]

		Incubation		Starch	
Enzyme	Source	pH	Temperature (°C)	Yield (%)	Protein (%)
None		7.5	37	NS[b]	NS
		5.0	40	NS	NS
Protease	*Aspergillus oryzae*	7.5	37	72	1.0
	Bacillus subtilis	7.5	37	60	1.9
	Streptomyces griseus	7.5	37	58	2.5
	Rhizopus sp.	7.5	37	55	2.0
Cellulase	*Penicillium funiculosum*	5.0	40	40	2.5
	Trichoderma reesei	5.0	40	34	2.5

[a] All reactions were done on a slurry of oat flour (25% solids). The slurry (25% solids) was adjusted to pH 7.5 using 0.1*M* NaOH and to pH 5.0 using 0.1*M* HCl. Cellulase (500 units) or protease (50 units) was added, and the digest was gently stirred for 3 hr at constant temperature.
[b] No separation.

TABLE II
Yield and Protein Content of Oat Starch after Digestion with Cellulase and/or Protease[a]

		Incubation		
Protease (units)	Cellulase (units)	Time (hr)	Yield (%)	Protein (%)
10	0	6	70	1.4
50	0	3	72	1.0
50	0	6	78	1.1
100	0	3	80	1.1
100	0	6	81	1.0
0	500	3	38	2.5
0	500	6	40	2.2
0	1,000	3	45	2.2
50	500	9[b]	78	1.1

[a] All reactions were done in 30 ml of a 25% slurry of oat flour at 40°C and pH 5.0 (cellulase from *Pennicillium funiculosum*) or 37°C and pH 7.5 (protease from *Aspergillus oryzae*).
[b] Protease at pH 7.5 for 3 hr, followed by cellulase at pH 5.0 for 6 hr.

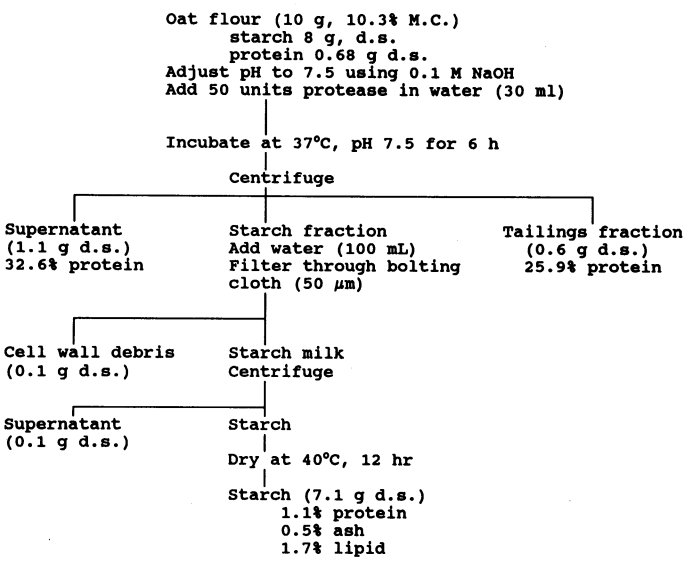

Fig. 7. Isolation of oat starch using protease from *A. oryzae*; mass balance.

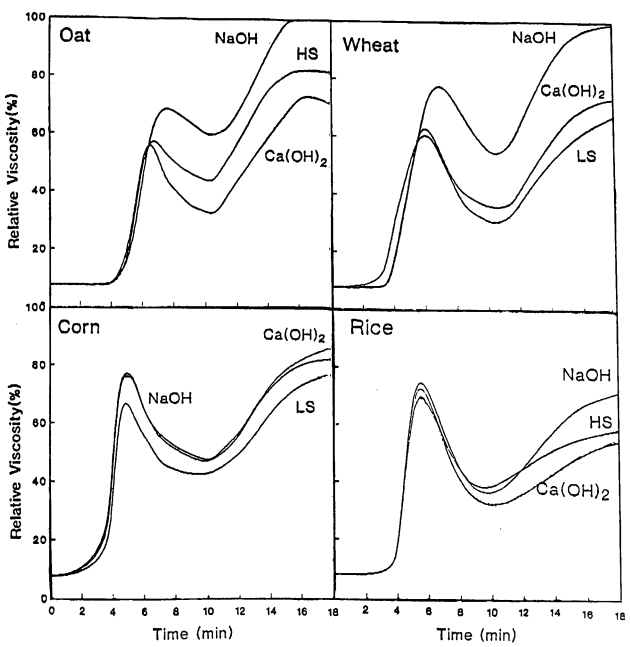

Fig. 8. Pasting curves of oat, wheat, corn, and rice starches isolated using sodium hydroxide, calcium hydroxide, or high shear (HS) or low shear (LS) in water. All starches contained approximately 1% lipid and 0.3% protein, except the oat starch isolated using high shear, which contained 1.9% lipid and 1.3% protein. Starch slurries were held at 50°C for 2 min, heated to 95°C in 3 min, held at 95°C for 5 min, cooled to 50°C in 4 min, and held at 50°C for 4 min.

The sum of the protein recovered in the supernatant and tailings fractions was 75%, and the total recovery of dry solids was 100%.

Pasting Properties of Starches Isolated Under Different Conditions

The pasting curves of oat starches isolated under three different conditions were measured using the Rapid Visco-Analyser. Oat starch isolated in aqueous sodium hydroxide gave a different pasting curve than starch isolated in calcium hydroxide or by high shear in water (Fig. 8). Isolation in sodium hydroxide caused a delayed pasting peak and an increased paste consistency. The same effects were observed when wheat starch was isolated in sodium hydroxide but not when corn starch was. Rice starch isolated in sodium hydroxide showed some increase in paste consistency but no delay of its pasting peak.

The reason for the pasting curve difference is unknown. We speculate that the calcium salts of the lysophospholipids in wheat and oat starches, and to some extent in rice starch, are less soluble than their sodium salts. The soluble sodium salts of the lysophospholipids form a complex with amylose during the early stages of starch pasting. The amylose-lipid complex delays the swelling of the starch granule, because the complex does not begin to dissociate until the temperature of the paste exceeds 85°C (Ghiasi et al 1982, Biliaderis et al 1985). That delay in swelling delays the occurrence of the pasting peak in the curve. The delay in swelling also gives more shear stability to the granules during stirring at 95°C, which yields a higher paste consistency.

Of incidental interest is the fact that the isolation of rice starch using 0.03*M* $Ca(OH)_2$ was difficult. Brief stirring of rice flour in the base gave low yields because of limited diffusion of the base into the endosperm, whereas prolonged stirring gave a mixture that resisted separation by centrifugation.

CONCLUSIONS

The isolation of low-protein oat starch from oat flour is achieved rapidly with sodium or calcium hydroxide or slowly with protease digestion or water soaking followed by high shear. Oat starch and wheat starch isolated with sodium hydroxide had delayed pasting peaks and increased paste consistencies compared with starch isolated with calcium hydroxide.

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