Gelatinization of Wheat Starch. I. Excess-Water Systems[1]

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ABSTRACT

The surfactants sodium stearoyl lactylate (SSL) and monoglycerides inhibited swelling and solubility of wheat starch at temperatures below 85°C. At higher temperatures, SSL did not affect solubility, but monoglycerides effectively reduced solubility up to at least 120°C. Scanning electron microscopic pictures of starch heated in an amylograph also showed that SSL and monoglycerides kept starch granules intact at temperatures up to 85°C, whereas untreated starch was grossly deformed at that temperature. At 95°C the difference between the SSL-treated and the untreated starch had disappeared. Thus, SSL apparently is effective in retaining granule shape and starch solubility only at temperatures of 85°C or lower. Iodine affinity values, β-amylolysis limits, and gel filtration on Sepharose 2B-CL columns all showed that amylose was leached from untreated starch at temperatures below 95°C. The surfactants effectively stopped the leaching of amylose.

Starch heated in water changes little until an energy level high enough to dissociate the relatively weak bonding is reached. Then the granule swells tangentially, and fully hydrated starch molecules separate from the intricate micellar network and diffuse into the surrounding medium (Leach et al 1959).

According to Savage et al (1958), an increase in amylose content tends to raise the gelatinization temperature. Such a relationship implies that linear starch molecules are more firmly bonded than branched ones; however, the linear molecules are solubilized first and the branched molecules remain undissolved (Montgomery and Senti 1958, Reeve 1954).

Banks and Greenwood (1967) reported that low molecular weight linear amylose is leached from starch granules at low temperatures; as the extraction temperature is increased, higher molecular weight and branched amylose are obtained. Our objective was to characterize the structure of material leached from wheat starch at various temperatures and to study effects of certain surfactants on swelling and solubilization of wheat starch.

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MATERIALS AND METHODS

Prime starch was isolated from hard wheat flour (cultivar Cloud), milled on a Buhler experimental mill by the dough kneading procedure (Wolf 1964). Sodium stearoyl lactylate (SSL) was obtained from C. J. Patterson Company, Kansas City, MO. Distilled monoglycerides (MG) (Dimodan PV) were obtained from Grinsted Products, Overland Park, KS. The fatty acid compositions of SSL and MG were determined by gas chromatography. Fatty acids were esterified according to the AOAC method (1970), and the esters were identified on a Hewlett Packard 5750 gas chromatograph with a flame ionization detector. The column was 6 ft × 1/8 in. and was packed with 10% SP2330 on 100/120 chromosorb WAW obtained from Supelco, Inc. The column temperature was 195°C. Both surfactants had similar fatty acid profiles, containing mainly C_{18} (80%) and C_{16} (16%) with small amounts of C_{14}, $C_{18:1}$, and C_{20}.

Starch Swelling and Solubility

Starch swelling was determined by the method of Leach et al (1959). The level of leached solubles was determined by suspending

5 g of starch in 180 ml of water, placing the sample in a water bath adjusted to the desired temperature, and mechanically stirring. For samples above 100° C, the heating was under pressure. If the starch was to be chemically characterized, the sample was purged with nitrogen and held at the desired temperature for 30 min. After centrifugation at 1,000 × *g*, the carbohydrate in the supernatant was determined by the phenol-sulfuric acid method (Dubois et al 1956). The level of SSL and MG used (2% of starch) was determined by the maximum effect obtained in an amylograph. Higher levels showed no further change in viscosity.

Characterization of Carbohydrates Leached from Starch

Iodine affinity of defatted leached carbohydrate was determined by Schoch's method (1964). β-Amylolysis (crystalline, sweet potato, Sigma) and debranching with pullulanase (*Enterobacter aerogenes,* Sigma) were performed on nondefatted samples as described by Ghiasi et al (1979). Reducing sugars were measured by the colorimetric copper procedure (Nelson 1944) and total carbohydrate, by phenol-sulphuric acid (Dubois et al 1956). Gel filtration of the samples was performed on columns (2.6 × 70 cm) packed with Sepharose 2B-CL or Biogel P-10. The columns were eluted in an ascending direction with 0.01*N* NaOH for the Sepharose and phosphate buffer (pH 7) for the Biogel.

TABLE I
Effect of Temperature and Added Surfactant—Sodium Stearoyl Lactylate (SSL) or Monoglycerides (MG)—on Starch Solubility and Iodine Affinities of Leached Solubles

Pasting Temperature (° C)	Solubility (%)			Iodine Affinity of Leached Solubles (%)		
	Control	MG	SSL	Control	MG	SSL
75	5.9	1.4	1.1	18.5	4.4	3.9
85	10.1	1.7	1.7	17.8	7.3	9.2
95	34.5	4.8	34.3	15.5	11.7	15.7
120	80.0	20.1	79.0	7.4	6.3	7.0

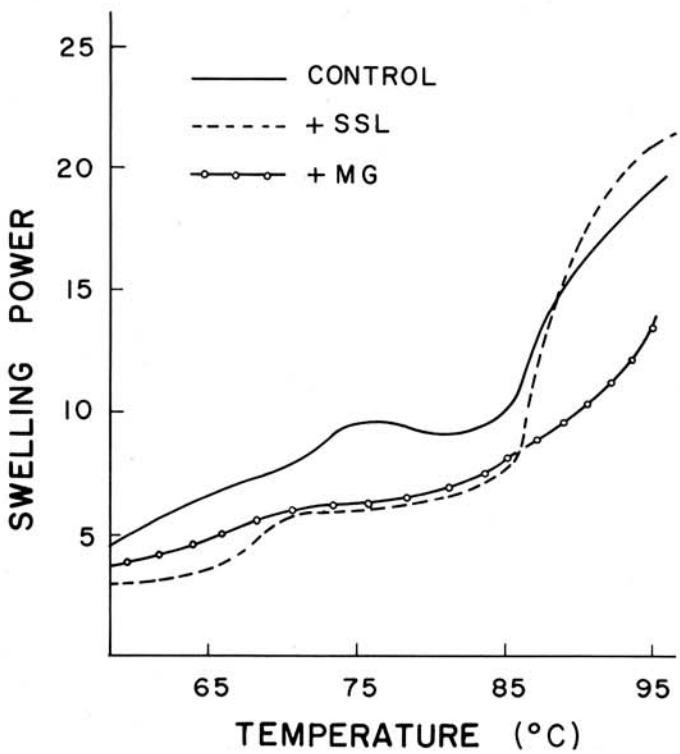

Fig. 1. Effect of monoglycerides (MG) or sodium stearoyl lactylate (SSL) on swelling patterns of wheat starch.

Scanning Electron Microscopy

Aliquots of dilute starch suspension (0.25%) heated in an amylograph were removed at certain temperatures and centrifuged to remove solubles. The sediment was rapidly frozen in liquid N_2-cooled isopentane and dried in an Edwards freeze drier at −60° C for 48 hr. Dried samples were sprinkled onto double-sided adhesive tape, which was attached to specimen stubs. Samples were coated with gold-palladium, and micrographs were taken on an ETEC U-1 scanning electron microscope at an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

Effect of SSL and Distilled Monoglyceride on Swelling and Solubility

Swelling and solubility patterns of starches have been used to study the associative bonding in granules and the influence of such factors as surfactants on the properties of starch (Collison 1968). The influence of MG and SSL on the swelling power of wheat starch is shown in Fig. 1. Wheat starch's swelling power was slightly repressed by those additives at temperatures below 85° C. But at temperatures above 85° C, SSL had no effect on solubility, whereas MG was effective at temperatures up to at least 120° C (Table I). Gray and Schoch (1962) observed that sodium lauryl sulphate repressed the sediment weight and the solubility of corn and potato starch below 85° C, with the effect reversed at higher temperatures.

The surfactants affected the solubility of wheat starch (Table I) more than its swelling power (Fig. 1). Comparison of the solubilization and swelling of samples showed that solubility paralleled swelling for the SSL and control samples. But swelling increased much more dramatically than did solubility for the MG samples, particularly at temperatures between 85 and 95° C.

Scanning Electron Microscopy

To visualize swelling of starch granules, aliquots of a amylograph-heated starch suspension were removed at certain temperatures and the granules recovered by centrifugation. The sediment was instantly frozen in isopentene cooled by liquid N_2 and dried in a freeze drier. The scanning pictures of heated starch, starch plus MG, and starch plus SSL are presented in Figs. 2, 3, and 4, respectively. Untreated starch granules are swollen and slightly folded at 65° C (Fig. 2a). Folding becomes more pronounced at higher temperatures (Fig. 2b). Folding of starch granules treated

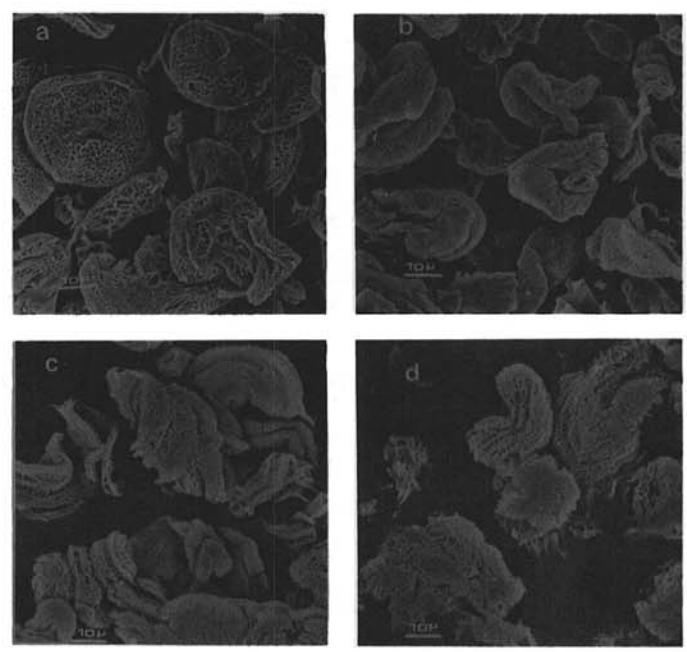

Fig. 2. Scanning electron micrographs of wheat starch heated to: **a**, 65° C; **b**, 75° C; **c**, 85° C; **d**, 95° C.

with SSL and MG at the same temperature was not significant (Figs. 3b and 4b). Starch granules heated with MG and SSL were relatively intact even when heated to 85°C (Figs. 3c and 4c), whereas starch granules of the control sample heated to the same temperature were grossly deformed.

At 95°C, the difference between the SSL-treated sample and the control sample had disappeared; the granules were fully deformed and had lost their integrity (Figs. 2d and 4d). The MG-treated samples heated to 95°C still retained their shape (Fig. 3d). Those data indicate that SSL is effective in retaining granule integrity only up to 85°C, whereas MG is effective even at 95°C. Both results support our earlier data showing those surfactants' effects on swelling and solubility patterns (Fig. 1 and Table I).

Iodine Affinity of Carbohydrate Leached from Starch

Iodine affinity values for carbohydrates leached from untreated starch at various temperatures are high, indicating that those samples are primarily amylose (Table I). As the leaching temperature was increased, the iodine affinity decreased. Therefore, at lower pasting temperatures, amylose is preferentially leached from the granules, whereas, at higher temperatures, amylopectin is gradually solubilized. Samples treated with SSL and MG and leached at low temperatures had low iodine affinities, which increased as the leaching temperature increased to 95°C. However, in the presence of surfactants, the level of extractable solubles at temperatures below 95°C was small. So one probably should not conclude that at low temperatures amylopectin is preferentially leached from the granules in a surfactant-treated sample.

β-Amylolysis of Carbohydrate Leached from Starch

β-Amylolysis limits of starch (solubles and insolubles) heated to different temperatures, with and without added MG, are given in Table II. As expected, β-amylolysis values of starch increased as pasting temperature increased. Prime starch heated to 45°C (essentially a nonheated control) gave low β-amylolysis values. As starch was heated to higher temperatures, it became more digestible by β-amylase, probably because at higher temperatures, starch becomes more soluble and the granules more porous and thus more susceptible to β-amylase attack. With a starch slurry heated to 85°C, cooled, and incubated with β-amylase (Fig. 5), the hydrolysis rate was rapid at short incubation times, showing that both soluble starch and remnants of starch granules are available to the enzyme. The slow rate of hydrolysis at longer incubation times indicates that β-amylase cannot attack the more ordered areas in the starch remnants.

The β-amylolysis limit was lower for the MG-treated sample. MG keeps the granules intact at a much higher temperature (Fig. 3) and delays solubilization of amylose (Table I).

Molecular Size of Carbohydrates Leached from Soluble Starch

Molecular size of carbohydrates leached from starch was investigated by gel filtration of the leached starch and its pullulanase-debranched products. The elution profile of the leached starch isolated at 75°C and of the pullulanase-debranched sample on a Sepharose 2B-CL column is presented in Fig. 6. The lack of material eluting at the void volume of this column indicates that no amylopectin is present, which agrees with the high iodine affinity values for that sample (Table I).

Molecular size of leached starches isolated at 85 and 95°C and of their debranched products on Sepharose 2B-CL are given in Figs. 7 and 8, respectively. As the pasting temperature increased, the amount of higher molecular weight components eluted at the void volume of the column also increased. Elution profiles of the debranched sample and the nondebranched sample leached at a high temperature (Fig. 8) differ widely from those leached at low temperature (Fig. 6). The elution patterns of the sample leached at 95°C (Fig. 8) show the presence of a higher molecular weight fraction eluted at the void volume and a fraction that is separated on the 2B column. After treatment with pullulanase, both fractions eluted much later on the column.

TABLE II
Effects of Pasting Temperatures and with Monoglyceride (MG) on β-Amylolysis Limits of Starch (%)

Pasting Temperature (°C)	β-Amylolysis (%)	
	Control	MG
65	24.4	...
75	29.8	15.3
85	35.7	25.5
95	40.0	35.7
120	44.5	40.4

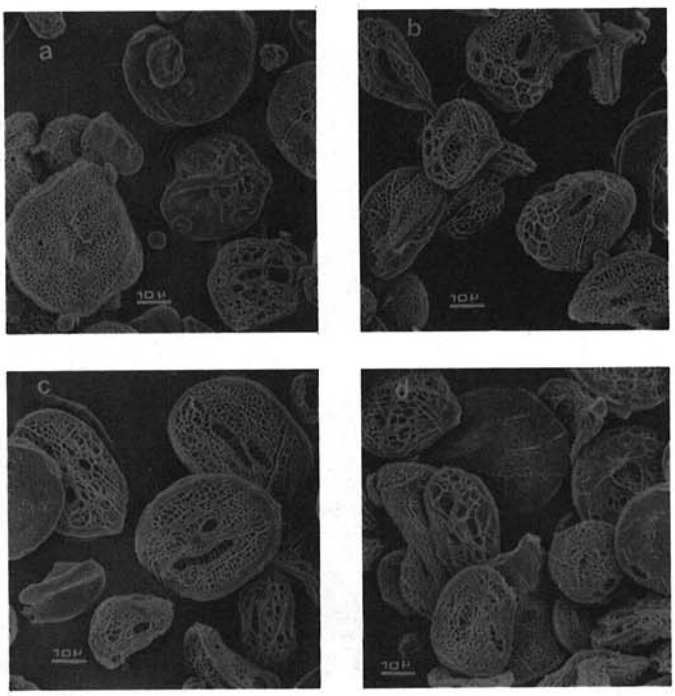

Fig. 3. Scanning electron micrographs of wheat starch heated with monoglycerides to: **a**, 65°C; **b**, 75°C; **c**, 85°C; **d**, 95°C.

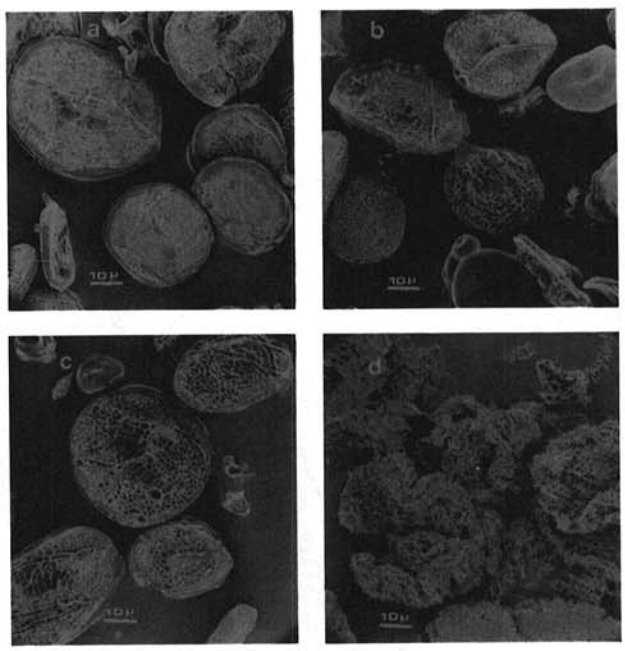

Fig. 4. Scanning electron micrographs of wheat starch heated with sodium stearoyl lactylate to: **a**, 65°C; **b**, 75°C; **c**, 85°C; **d**, 95°C.

From the relatively high iodine affinity of the sample heated to 95°C (Table I), one would expect less branched material than is indicated by Fig. 8. Others (Banks and Greenwood 1967, Greenwood 1960) reported limited branching in amylose. So one explanation for the high degree of branching and the relatively high iodine affinity is a branched amylose fraction. The branched amylose, composed of long chains (Banks and Greenwood 1967) acts as straight chain amylose for binding iodine.

The debranched leached starches were fractionated on a Biogel P-10 column. The samples were eluted mainly at the void volume of the column. If the material debranched by pullulanase were amylopectin, one would expect much more material eluted after the void volume. That data from Biogel P-10 supports our hypothesis that the higher molecular weight fractions in Fig. 8 are branched amylose rather than amylopectin. The small amount of amylopectin also present is enough to lower the iodine affinity of the sample to 15.5 (Table I).

Leached starch produced by heating starch to 95°C in the presence of MG and SSL was also chromatographed. Despite a large difference in solubility between the control and MG-treated

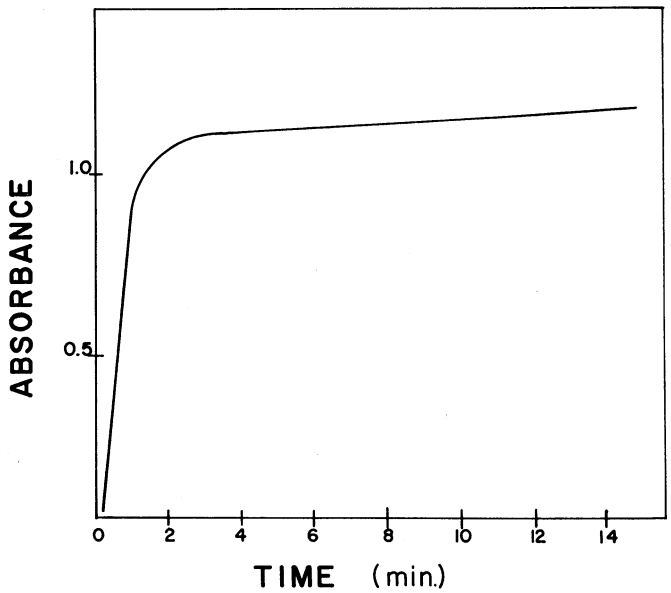

Fig. 5. Production of maltose (reducing power absorbance) as a function of reaction time with β-amylase on wheat starch (solubles and insolubles) that had been heated to 85°C.

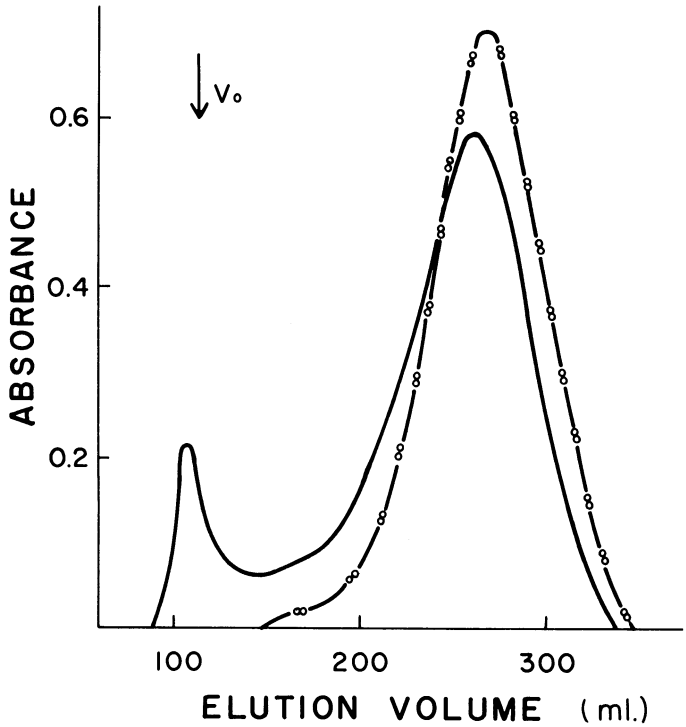

Fig. 7. Elution profiles of starch leached at 85°C (—) and its corresponding debranched sample (oo—oo—) on Sepharose 2B-CL.

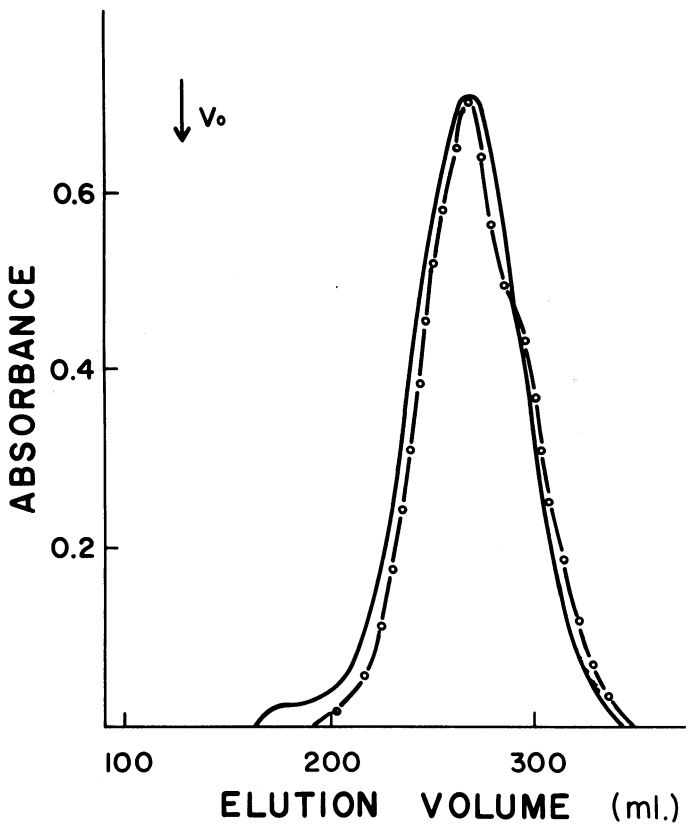

Fig. 6. Elution profiles of starch leached at 75°C (—) and its corresponding debranched sample (o—o—) on Sepharose 2B-CL.

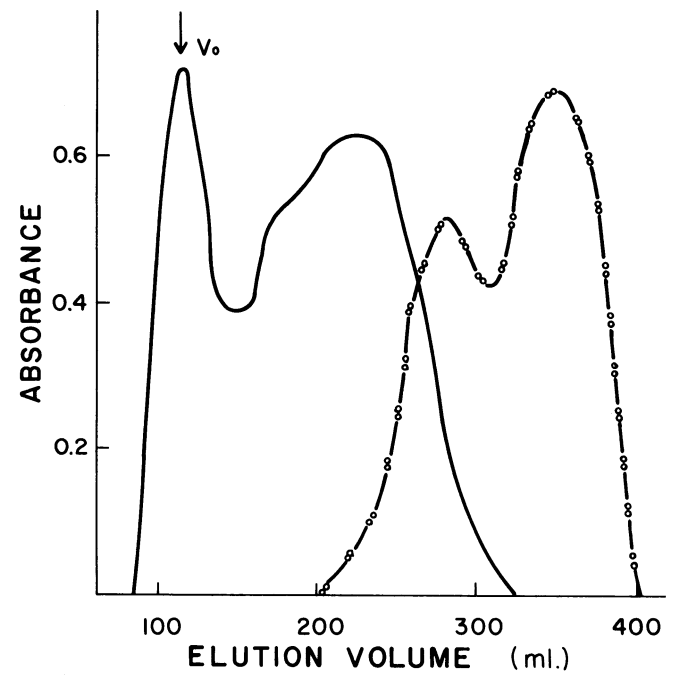

Fig. 8. Elution profiles of starch leached at 95°C (—) and its corresponding debranched sample (oo—oo—) on Sepharose 2B-CL.

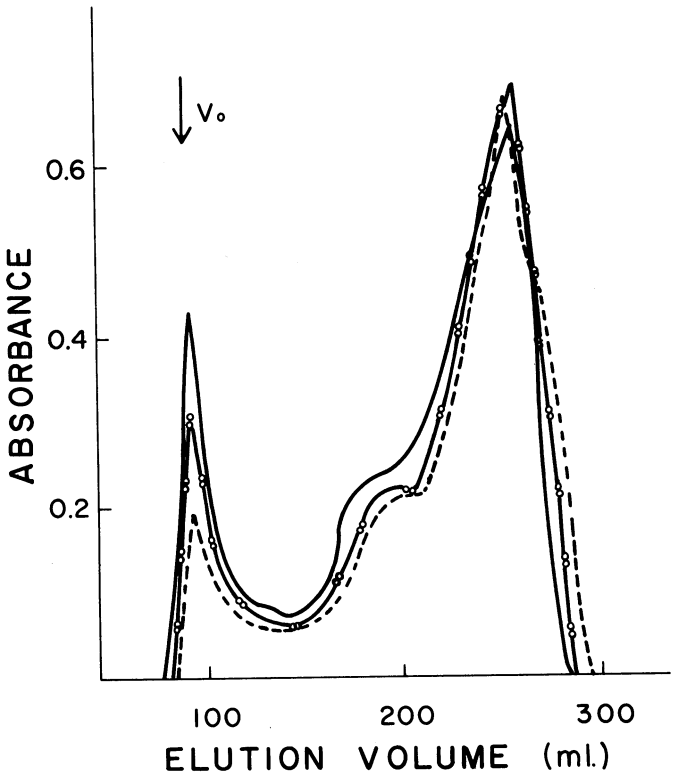

Fig. 9. Elution profiles from a Biogel P-10 column of debranched prime starch (—), debranched insoluble fraction of starch heated to 75°C (oo—oo—), and debranched insoluble fraction heated to 85°C (----).

samples, their elution profiles on Sepharose 2B-CL were similar to each other and to those of the control (Fig. 8), which indicates that MG does not change the composition of the soluble starch but only delays solubilization. The elution profiles of solubles from SSL-treated starch also were similar to those of the control (Fig. 8).

Debranching of the solubles leached from SSL-treated and MG-treated samples also were fractionated on a Biogel P-10 column. Most of the samples were eluted at the void volume of the column, with only a small quantity of a low molecular weight fraction eluted later, which indicates that most of the higher molecular weight fractions are composed of branched amylose rather than of amylopectin.

Column Chromatography of the Insoluble Fractions

After starch was heated to 75 and 85° C, the leached solubles were removed by centrifugation, and the insoluble residue fraction was debranched with pullulanase. The digest was fractionated on a Biogel P-10 column. A prime wheat starch sample was used as a control (Fig. 9). Two major peaks were obtained for each sample. The peak eluted at the void volume of the column is amylose that did not debranch with pullulanase. The second major peak is debranched amylopectin with a much lower molecular weight. The second peak's leading shoulder indicates a bimodal distribution of chain lengths, as Atwell et al (1980) reported.

The elution profiles of debranched wheat starch are similar to those of the insoluble fractions except for the size of the first peak. As expected, after heating and removing the solubles, which are mainly amylose, less material is eluted at the void volume of the column, so peak 1 contains less carbohydrate in the sample heated to 85° C than in the sample heated to 75° C, which, in turn, contains less than in unheated starch.

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