Wheat Starches. II. Effect of Polar and Nonpolar Lipid Fractions on Pasting Characteristics[1]

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ABSTRACT

Wheat starch was extracted with solvents of varying polarity. Pasting properties of the extracted starch were determined by the carboxymethyl cellulose-amylograph technique. Differences in the pasting characteristics of samples extracted with different solvents compared to the control were relatively small. However, the data suggested that polar and nonpolar lipids exhibited different effects on the pasting properties of starch. This was confirmed by reimpregnating defatted starch with polar and nonpolar lipid fractions. The pasting properties of defatted starch reimpregnated with polar lipids showed reduced viscosity in both steps of the pasting curve. Defatted starch reimpregnated with nonpolar lipids showed relatively little change in the first step in the pasting curve, but a significant increase in maximum peak height.

Cereal starches contain a small but significant amount of lipid material (1). For wheat starch, this amount is generally 0.6–0.8%, as determined after acid hydrolysis.

The lipids of starch are difficult to remove by solvent extraction (2). Normal "fat solvents" remove very little of the total starch lipids. Better extraction is achieved with polar solvents such as methanol or aqueous dioxane (3). Even with these solvents, long extraction times are necessary to reduce the lipid content below 0.1%. Early work established that this lipid material is distributed throughout the starch granule (4). Undoubtedly, the polar lipids form a complex with the linear starch fraction (5).

It is known that the gelatinization characteristics of defatted starches differ from those of raw starches (6,7). Considerable work has been reported on the addition of various lipid materials to starch suspensions and the effect of these lipids on the pasting properties of such systems (8–10).

The effect on pasting properties of lipids present in the starch granule prior to swelling has not been studied in detail. In this work, the effect of polar and nonpolar lipid fractions present within the granule on the two-step pasting characteristics of wheat starch has been investigated.

MATERIALS AND METHODS

Starch

Commercial wheat starch ("Starbake") was obtained from the Hercules Powder Co., Harbor Beach, Michigan. Wheat starch was also isolated in the laboratory from HRS and durum wheat flours.

Pasting Characteristics

Starch pasting curves were obtained with the use of the carboxymethyl cellulose(CMC)-amylograph technique (11) as described previously (12).

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The temperature was raised from 25° to 95°C., and then held at 95°C. for 15 min. Values for a blank curve with the use of only an aqueous suspension of CMC were subtracted from the starch-CMC curves to give the corrected starch curves reported in this paper.

Two values were obtained from these curves and used to summarize pasting characteristics. The 76° height was the viscosity of the sample when the temperature reached 76°C. At about this temperature (see Fig. 1), the first step in the viscosity curve leveled off. The 76° height was chosen therefore as a measure of the viscosity reached during the first step in the pasting curve. Peak height was the maximum viscosity obtained; in all cases this occurred shortly after the temperature reached 95°C.

Solvents

All solvents were reagent grade and were used without purification. Solvents used were petroleum ether (b.p. 30°–60°C.), ethyl ether, acetone, and methanol.

Extraction

Starch samples (100 g.) were extracted overnight in a Soxhlet extraction apparatus. After removal of the solvent by vacuum filtration, the starch was air-dried for 12 hr. It then was rehydrated in an 80% r.h. cabinet for 12 hr. to remove last traces of solvent, and again air-dried for 12 hr. Moisture contents ranged from 9.0 to 11.0%. At least duplicate extractions were performed for each solvent.

The lipids were concentrated to dryness under reduced pressure and the residue was weighed.

Starch lipids used for reimpregnation experiments were extracted from wheat starch with the use of boiling methanol as described by Schoch (13). Flour lipids were extracted from a HRS wheat flour with water-saturated 1-butanol. Portions of the above flour and starch lipids were separated on a large silicic acid column into polar and nonpolar fractions; the solvents used are described below.

Separation into Polar and Nonpolar Fractions

The extracted lipids were separated into polar and nonpolar fractions by silicic acid column chromatography. The lipid residue extracted from 100 g. of starch was taken up in a small amount of chloroform and transferred to the top of a 15 cm. × 1 cm. glass column containing 100-mesh silicic acid. Anhydrous ethyl ether (75 ml.) was used to elute the nonpolar fraction, followed by 75 ml. of anhydrous methanol to elute the polar fraction. Solvents were removed under reduced pressure in tared flasks and the amount of each lipid fraction was determined by weighing. Column yields ranged from 90 to 100%.

Anhydrous ethyl ether eluted the lipid components having a polarity equal to or less than that of the monoglycerides. An analysis of the components present in starch lipids will be presented in a subsequent paper (14).

Acid Hydrolysis

The lipid content of the various starches was determined after acid hydrolysis by the procedure of Rogols (15).

Reimpregnation of Defatted Starch

Wheat starch was extracted three times with boiling methanol according to the procedure described by Schoch (13). Thirty-gram samples of this defatted starch then were suspended in 100 ml. of solvent. The lipid fraction to be used for reimpregnation was dissolved in the solvent prior to the starch addition. Solvents used were methanol and petroleum ether. In some cases, the polar lipids were not completely soluble in petroleum ether and the nonpolar lipids were not completely soluble in methanol. In these cases, a sufficient quantity (usually 5–15 ml.) of either methanol or petroleum ether was added to obtain complete solution. The starch-solvent system was stirred constantly to keep the starch in suspension, and heated under reflux for 5 hr. The solvent was removed by vacuum filtration; the starch was washed with a small quantity of fresh solvent, and then air-dried and rehydrated as described above. Each reimpregnation experiment was done in duplicate. Amylograph results between duplicate experiments agreed within ± 20 B.U.

The fatty acid mixture used in these experiments consisted primarily of linoleic (74%), oleic (15%), and palmitic (7%) acids. Crude lecithin (animal source), obtained from Nutritional Biochemical Corporation, was used.

RESULTS AND DISCUSSION

Extraction of the lipids from starch is known to affect gelatinization properties. In this work, an attempt was made to extract starch with solvents of varying polarity and to relate the pasting characteristics of the extracted starch to the amount and type of lipid removed. The CMC-amylograph technique was used in order to detect the two-step pasting curve of wheat starch. Typical results are shown in Table I. All extractions were performed in duplicate on commercial wheat starch. When duplicates varied by more than 20 B.U., the experiment was repeated. In addition to the data in Table I, extractions were made on laboratory-isolated HRS and durum wheat starches. The results were similar for the three starches, and only the data for commercial starch are included. Several additional solvents also were used.

TABLE I
EXTRACTION DATA AND PASTING PROPERTIES

SOLVENT	AMOUNT OF LIPID EXTRACTED	RATIO, NONPOLAR: POLAR	76° HEIGHT[a]	PEAK[b] HEIGHT
	g./100 g.		*B.U.*	*B.U.*
None	–	–	180	485
Petroleum ether	0.02	1:0.44	180	475
	0.02	1:0.46	180	480
Ethyl ether	0.03	1:0.21	175	465
	0.03	1:0.26	180	475
Acetone	0.04	1:0.66	175	470
	0.04	1:0.54	185	480
Methanol	0.53	1:4.8	275	515
	0.59	1:4.8	285	520

[a] Viscosity of the sample when the temperature reached 76°C. Chosen as a measure of the viscosity during the initial step in the pasting curve.
[b] Maximum viscosity obtained.

While much of the amylograph data on extracted starches was within, or nearly within, the experimental limits of the method (± 20 units in our laboratory), some general trends could be observed in the extraction data from all three starches. As expected, the nonpolar solvents extracted relatively little lipid as compared to the polar solvents. In addition, the relative amount of nonpolar lipid was much greater in the material extracted with nonpolar solvents. Conversely, the relative amount of polar lipid was much higher in the material extracted with polar solvents. In general, extraction with methanol, a polar solvent, resulted in a considerable increase in viscosity during the initial step in the pasting curve (higher 76° height) compared to the raw starch control. Peak height showed a slight increase in most cases. Extraction with petroleum ether, a nonpolar solvent, generally resulted in little change in the first step compared to the control and a slight decrease in peak height.

The trends observed in the extraction data suggested that polar and nonpolar lipids present within the starch granule may exert different effects on the pasting characteristics of the starch. To confirm these observations with definitive experiments, defatted starch was reimpregnated with several different polar and nonpolar lipid fractions, and the pasting properties of these starches were examined.

Starch and flour lipids were separated into polar and nonpolar fractions as described above. These fractions were similar, but certainly not identical, in composition (14). The fatty acid mixture and crude animal lecithin provided a third source of nonpolar and polar fractions, respectively. Methanol

TABLE II

SUMMARY OF DATA ON LIPID CONTENT AND PASTING PROPERTIES OF DEFATTED STARCH REIMPREGNATED WITH VARIOUS LIPIDS

LIPID ADDED	SOLVENT	QUANTITY OF LIPID ADDED[a]	LIPID CONTENT OF REIMPREGNATED STARCH[b]	AMYLOGRAPH DATA	
				76° Height[c]	Peak Height[d]
		g./30 g. starch	%	*B.U.*	*B.U.*
Raw starch control	....	....	0.7	185	485
Defatted starch control	....	....	0.1	275	500
Starch lipid, whole	Methanol	1.90	0.6	235	465
polar	Methanol	2.32	0.7	225	455
nonpolar	Methanol	1.23	0.3	250	545
Flour lipid, polar	Methanol	1.25	0.4	230	440
nonpolar	Methanol	1.23	0.4	255	560
polar	Pet. ether[e]	1.83	0.6	220	475
nonpolar	Pet. ether[e]	1.27	0.3	270	590
Lecithin	Methanol	5.00	....	230	460
Fatty acids	Methanol	5.00	....	255	590

[a] Amount of lipid dissolved in 100 ml. of solvent followed by 5 hr. refluxing of starch-solvent suspension.

[b] Determined after acid hydrolysis.

[c] Viscosity of the sample when the temperature reached 76°C. Chosen as a measure of the viscosity during the initial step in the pasting curve.

[d] Maximum viscosity obtained.

[e] Petroleum ether (b.p. 30°–60°C.).

was used as the solvent for reimpregnation except in the case of the flour lipid fractions, where both methanol and petroleum ether were used. A summary of the data on reimpregnated starch is shown in Table II.

Reimpregnation was difficult to achieve because the conditions used were essentially the same as those used for defatting. Thus, a relatively large excess of lipid was required in the solvent to obtain a significant increase in the lipid content of the reimpregnated starch.

Reimpregnation with whole and polar starch lipids resulted in a significant decrease both in the viscosity during the first step in the pasting curve (lower 76° height) and in final peak height. The nonpolar fraction gave a relatively small decrease in the first step compared to the defatted starch control, but markedly increased maximum peak height. These results are shown graphically in Fig. 1.

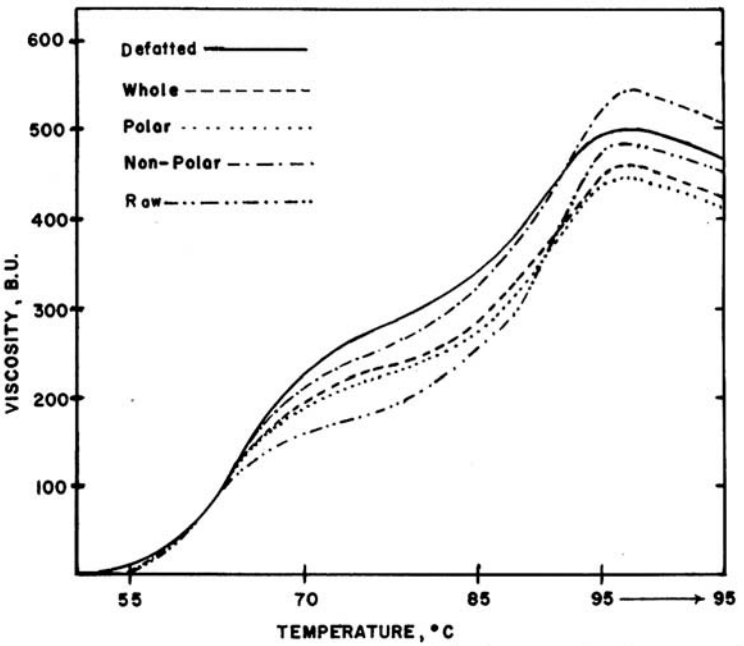

Fig. 1. Pasting curves of defatted commercial wheat starch reimpregnated with starch lipid fractions.

Results (Table II) were similar for starch reimpregnated with flour lipid fractions and with the fatty acid mixture and lecithin. In the case of the flour lipid fractions, use of petroleum ether as the solvent for reimpregnation gave results similar to those obtained with methanol.

In a separate series of experiments, defatted HRS wheat starch was reimpregnated with various amounts of polar and nonpolar flour lipid fractions. The effect of various quantities of polar and nonpolar lipids, added to the starch prior to gelatinization, on 76° height and peak height is shown in Fig. 2.

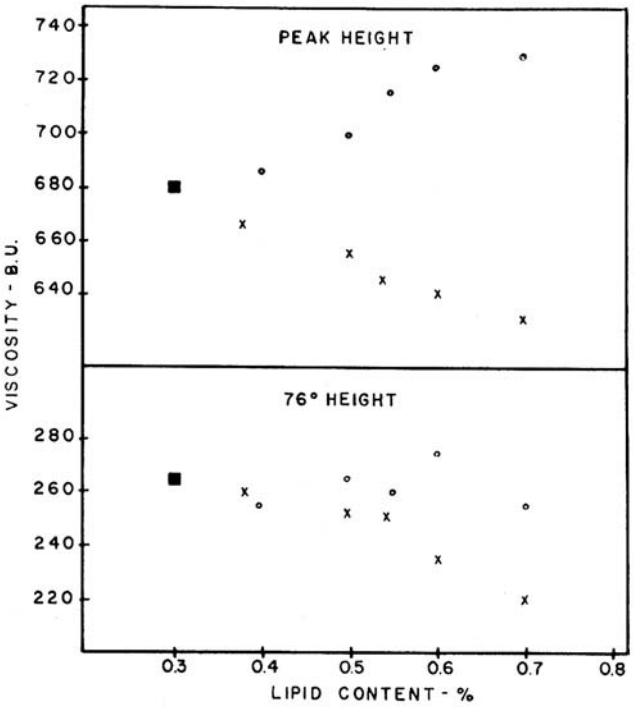

Fig. 2. Amylograph peak heights and 76° heights of defatted HRS wheat starch reimpregnated with various amounts of polar and nonpolar flour lipid fractions. Solid square, defatted control; x, reimpregnated with polar lipid; o, reimpregnated with nonpolar lipid.

Increasing quantities of nonpolar flour lipids had little effect on 76° height. Increasing quantities of polar flour lipids progressively lowered the 76° height compared to the defatted starch control. Increasing quantities of nonpolar flour lipids resulted in progressively higher peak heights, whereas increasing quantities of polar flour lipids gave progressively lower peak heights, compared to the defatted starch control.

These results clearly supported the trends observed in the pasting properties of starch extracted with various solvents. The results indicated that polar and nonpolar lipid fractions, when present within the starch granule prior to pasting, exhibited different effects on pasting properties. Polar lipid fractions lowered the observed viscosity in both steps of the pasting curve; nonpolar lipid fractions had relatively little effect on the initial step in the pasting curve, but resulted in higher maximum peaks compared to the defatted starch control.

These data may be rationalized by considering the possible role of the various portions of the starch granule during swelling. The initial step in the pasting curve is attributed to hydration of the amorphous, more readily accessible areas of the granule (16). The polar fraction may form a complex with molecules of starch in this region rather easily, since they are not closely

packed. This complex would tend to reduce or retard the rate of hydration of the starch molecules in this area of the granule. Thus, addition of polar lipids should result in a decrease in viscosity during the first step in the pasting curve. Nonpolar lipids would not be expected to form such a complex, to any extent. The reduction in hydration caused by presence of the polar lipids also would tend to lower the maximum peak height.

Peak height depends upon the relative ease of penetration of the more crystalline, or micellular, regions of the starch granule (6). During pasting, viscosity continues to increase until these areas are penetrated. Once the micellular regions are hydrated, the granules lose their integrity and the viscosity decreases.

Addition of nonpolar lipid resulted in greater maximum viscosities. The primary effect of this fraction thus appears to be prevention of hydration of the micellular regions of the granule. This is in contrast to the polar fraction, whose primary effect appears to be prevention of hydration of the amorphous regions of the granule.

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