Variation in Enzyme Digestibility and Gelatinization Behavior of Corn Starch Granule Fractions[1]

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ABSTRACT

Dent, waxy, and Amylomaize V and VII starches that were fractionated according to granule size and found to vary in amylose content were hydrolyzed by α-amylase. The rate of hydrolysis for dent and waxy maize starches was directly proportional to the surface area of the granules. The same effect was observed for most amylomaize fractions, but those low in amylose had relatively high hydrolysis rates. Gelatinization temperature and enthalpy of amylomaizes increased with decreasing granule size.

Properties of high-amylose corn starches differ considerably from those of dent or waxy corn starch. Granules are resistant to α-amylase attack (Gallant et al 1972, Sandstedt et al 1962) and gelatinize at high temperatures and over a much wider range (Stevens and Elton 1971). Because of recent interest in using high-amylose starch in food products, these differences are important from the standpoint of functional properties, nutritional availability, and energy requirements in processing.

Recent work by Wolf, Khoo, and Inglett (1977) showed that when high-amylose corn meal was used in preparation of corn muffins, some granular structure of starch was retained throughout cooking and during ingestion by mice or humans.

This work indicated that discrete fractions of high-amylose starch may exist that have unusual resistance to heat and enzymes. The work also indicated the need for in vitro studies to isolate and characterize such fractions. Such studies would also be the basis for adopting procedures to evaluate nutritional availability of starches from experimental corn varieties.

In the first report of this study (Cluskey et al 1980), we described the differential sedimentation technique that was used to fractionate starch samples according to granule size and reported the variation in amylose content found in amylomaize starches. Apparent amylose content, as measured by iodine binding capacity, varied inversely with the size of the granules. No variation was observed in dent corn starch. Our findings regarding α-amylase digestibility and gelatinization behavior of these granule fractions are discussed in this article.

MATERIALS AND METHODS

Commercial samples of waxy maize (no amylose) dent corn (24% amylose), Amylomaize V (50% amylose), and Amylomaize VII (70% amylose) starches were obtained from American Maize-Products Company. The differential sedimentation procedure used to fractionate the granules, measurement of granule size by photomicrography, and determination of iodine-binding capacity by potentiometric titration and spectrophotometry were described previously (Cluskey et al 1980).

Enzyme digestibility studies were done using a crystalline suspension of hog pancreas α-amylase in half-saturated NaCl with $0.003\,M$ $CaCl_2$ from Worthington Biochemical Corp., with a reported activity of 1,150 units per milligram and a concentration of 34 mg/ml. One unit was defined as that activity liberating 1 μmol of maltose per min at 25° C at pH 6.9. Starch granules (50 mg) were suspended in water (25 ml). Five-milliliter aliquots were placed in a constant temperature water bath at 37° C. Water (1 ml) and $0.02\,M$ sodium phosphate buffer (6 ml) containing $0.006\,M$ NaCl (required to activate the enzyme) at a pH of 6.90 were added. The sample was stirred constantly. A 2-μl α-amylase suspension was added, and 1-ml aliquots were removed at timed intervals, pipetted into 10 ml of 50% ethanol, and centrifuged. Aliquots of the supernate were analyzed for soluble carbohydrate and measured as maltose by the phenol-sulfuric acid colorimetric method of Dubois et al (1956). Blanks were used that had been subjected to the same conditions but without enzyme. Sedimented granules were saved for microscopic study, both by light and scanning electron microscopy (SEM). For SEM, the dehydrated starch granules were dispersed in water on pieces of cover glass attached to stubs. Dried samples were coated with carbon and gold-palladium and examined in a Cambridge Stereoscan Mark VI microscope.

In addition to digestion of raw granules, amylomaize fractions were studied after partial gelatinization. Starch (10 mg) in water (5 ml) was heated 15 min at 80° C. This temperature was chosen because preliminary measurements of gelatinization by birefringence end point measurements (BEPT) indicated that samples would, on average, be 50% gelatinized by this treatment. After cooling, the digestion procedure was followed as usual. Hydrolysis of fully gelatinized granules (15 min at 100° C) was also studied.

Gelatinization studies were done by differential scanning calorimetry (DSC) using a Perkin-Elmer model DSC-2 calorimeter. For DSC studies, 3 mg of starch was placed in a pan with 10 μl of water, sealed, and equilibrated for 1 hr. Temperature was increased at a rate of 10° per min from 20 to 120° C or above. The endotherm was recorded automatically, and ΔH was calculated from the area of the endotherm, using an indium standard of 99.999% purity and a ΔH of 6.80 cal/g.

RESULTS AND DISCUSSION

Size distribution of granules, expressed as weight percent of total sample, is illustrated for all four starches in Fig. 1. A relatively narrow range was found for dent and waxy maize starches, with more than 70% of the weight of the granules being 13–15 μm in diameter. (Although granules are not perfectly spherical, particularly in the amylomaizes, they were treated as such for convenience, and the diameters reported are an average of measured length and width.) By contrast, the amylomaizes are heterogeneous, and large amounts appear at either end of the overall range. Amylomaize V separates into two major fractions, whereas Amylomaize VII is evenly distributed and contains at least three major size groups.

The variation in size distribution among the four types of starch is important and must be carefully considered in the relationships between granule size and properties or composition. Because of the limited number of small granules in dent or waxy maize starch or in the intermediate sizes in Amylomaize V, even large variations in composition or properties in these fractions have a relatively minor effect on the properties of the whole starch. In Amylomaize VII,

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however, the widespread granule size distribution indicates that changes in any fraction are important to the overall view of the properties of the starch.

Granule size is an important factor in rate of enzyme hydrolysis because of the variation in surface area. Walker and Hope (1963) showed that adsorption of amylose by starch granules is proportional to the surface area of the granules, obeying the Freundlich equation, and further demonstrated that only α-amylases that adsorbed to granules were capable of hydrolyzing raw granules. In describing the kinetics of action of α-amylases on insoluble substrates, McLaren (1963) showed that the rate of hydrolysis is proportional to the surface area of substrate in contact with solvent. Thus, at any given concentration of starch and an excess of enzyme, the rate of hydrolysis should be proportional to the surface area of the granules.

Density of starch samples was taken as 1.5 (Donovan 1979), and granules were assumed to be spherical. From the density and the fact that volume increases with one-sixth the cube of the diameter and surface area increases with the square of the diameter, surface area can be calculated by the equation: 4 × mass/diameter.

Table I shows the granule size, calculated surface area, and α-amylase digestion values attained after 1 hr and 4 hr for raw granules of all four starches and for partially gelatinized granules of the amylomaizes. Hydrolysis of dent and waxy starches varied directly with surface area. Amylomaizes were more complex: largest and smallest granules were hydrolyzed most rapidly, and intermediate sizes were most resistant. The same pattern was found for the partially gelatinized granules. Fully gelatinized granules were hydrolyzed much more rapidly, all between 70 and 90% in 1 hr.

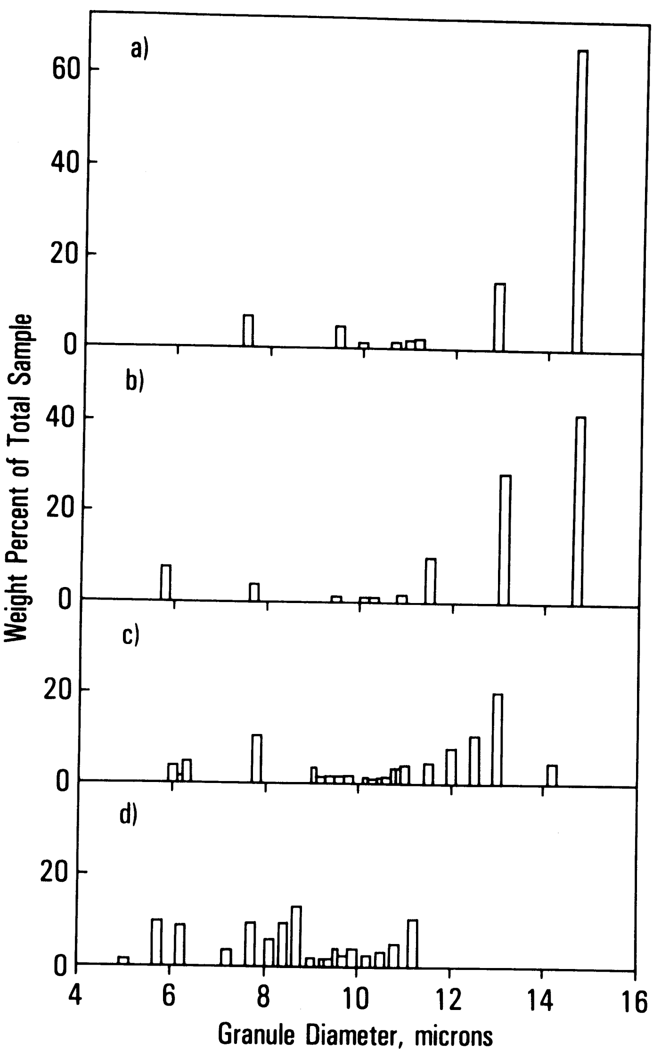

Fig. 1. Granule size distribution of starches: **a,** waxy maize; **b,** dent corn; **c,** Amylomaize V; and **d,** Amylomaize VII.

Percent digestion of samples hydrolyzed for 1 hr versus surface area was plotted in Fig. 2. One-hour hydrolysis was chosen because with the concentration of starch used in these experiments, sufficient substrate was available in all samples so that the hydrolysis rate was constant.

For dent and waxy maize granules, linear regression analysis of the points in Fig. 2 yielded lines that could be extrapolated to the origin, with correlation coefficients of >0.96. The slopes of those lines represent constants characteristic of the reactivity of the starch; these constants are 6.97 for dent starch and 13.77 for waxy maize starch. The higher reactivity for waxy maize starch agrees with the greater adsorption of α-amylase by waxy maize starch reported by Walker and Hope (1963).

Linear regression analysis of those Amylomaize VII samples that showed increasing reactivity with increased surface area gave a slope of 4.95, somewhat comparable to the dent corn starch value. This line, however, could not be extrapolated to the origin; zero reactivity was at 3.35 cm^2/mg. Partially gelatinized samples maintained essentially the same slope, but with an increased y-intercept that reflects the proportion of gelatinized starch or damaged granules. Similar behavior was observed for Amylomaize V, but the scatter of points was too great for meaningful statistical analysis.

The largest granules from the amylomaizes, those which do not fit the typical pattern of increased digestibility with increased surface area, were all fractions previously shown to contain decreased amounts of amylose (Cluskey et al 1980). Amylose contents of representative Amylomaize VII fractions are 11.2 μm, 45.6%; 9.9 μm, 56.6%; 6.2 μm, 62.1%; and 5.0 μm, 69.6%.

TABLE I
α-Amylase Digestion of Starch Fractions

Granule Size (μ)	Surface Area (cm^2/mg)	Percent Hydrolyzed at 37°C			
		Raw Starch		Partially Gelatinized (80°C)	
		1 hr	4 hr	1 hr	4 hr
Dent corn					
14.7	2.7	16.0	51.6	...	...
10.3	3.9	29.0	66.6	...	...
9.5	4.2	29.1	66.7	...	...
5.8	6.9	46.5	74.9	...	...
Waxy maize					
14.6	2.8	28.0	77.6	...	...
12.9	3.1	43.4	90.8	...	...
11.2	3.6	44.9	85.4	...	...
10.7	3.8	50.0	96.9	...	...
9.5	4.2	51.9	98.1	...	...
7.5	5.4	68.6	97.5	...	...
Amylomaize V					
14.2	2.8	5.1	21.6	84.4	89.6
13.0	3.1	...	9.6	68.1	72.9
11.0	3.6	2.2	8.7	62.4	65.5
10.5	3.8	2.5	7.4	62.0	68.9
9.6	4.2	0.7	7.3	65.9	75.2
9.1	4.4	1.6	8.1	58.2	70.0
7.8	5.1	2.8	12.9	62.8	65.9
6.0	6.7	17.0	25.2	71.3	77.3
Amylomaize VII					
11.2	3.6	9.2	23.4	55.3	60.4
9.9	4.0	4.0	15.1	37.2	49.7
9.3	4.3	4.1	10.2	34.5	51.6
8.7	4.6	5.0	12.1	40.8	53.3
8.1	4.9	8.5	16.1	42.4	56.2
7.2	5.6	10.4	18.2	40.5	49.1
6.2	6.4	15.9	30.2	46.0	61.4
5.7	7.0	20.6	32.3	51.4	65.2
5.0	8.0	21.2	35.6	54.2	70.7

Amylomaize V fractions were 14.2 μm, 40.5%; 12.9 μm, 45.9%; and 6.0 μm, 52.0%. The amount of amylose is critical to α-amylase hydrolysis; greater amounts cause the inhibition of hydrolysis. Digestibility appears to be related to the crystallinity of the granule. Robin et al (1974) and Kainuma and French (1971) showed that amylopectin is necessary to impart crystallinity to the granule.

Scanning electron micrographs of Amylomaize VII granules after 4-hr enzyme hydrolysis (Fig. 3) show that the mode of attack on amylomaize is much different than on dent corn starch. Instead of the typical cratering effect in dent corn starch, only scaling and roughening of the surface give evidence of granule deterioration.

Results of gelatinization studies using DSC are shown in Fig. 4.

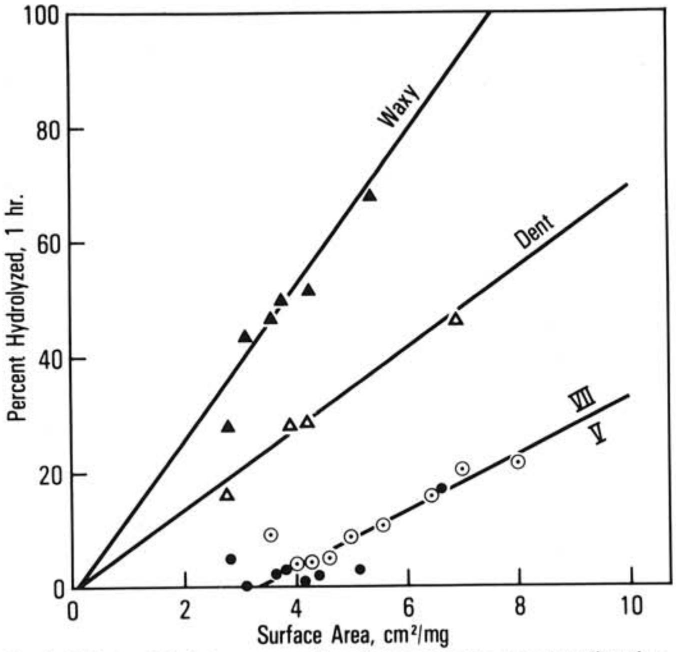

Fig. 2. Relationship between granule surface area and α-amylase digestion. Raw granules, 1 hr at 37° C.

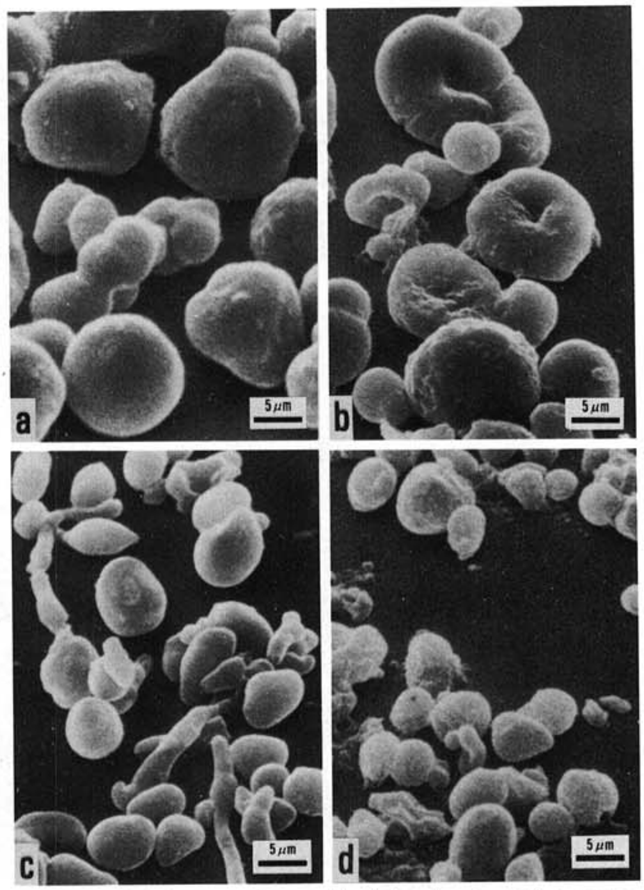

Fig. 3. Scanning electron micrograph of Amylomaize VII granules. **a,** 11-μm Granules before α-amylase hydrolysis; **b,** 11-μm granules after 4 hr of hydrolysis; **c,** 5-μm granules before hydrolysis; **d,** 5-μm granules after 4 hr of hydrolysis.

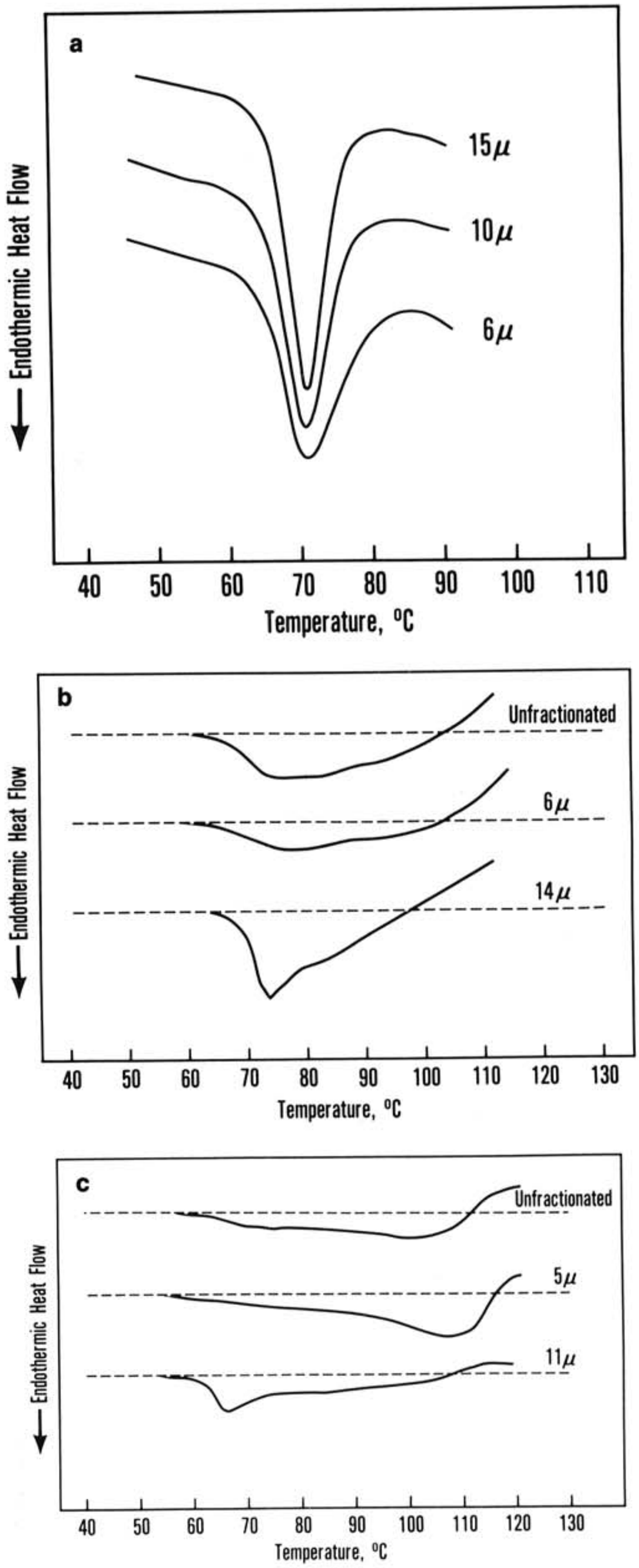

Fig. 4. Differential scanning calorimetry of starch granules. **a,** Dent corn starch; **b,** Amylomaize V; and **c,** Amylomaize VII.

Endotherms and enthalpies of unfractionated samples are comparable to those reported by Stevens and Elton (1971) for maize starches. Fractions of dent or waxy maize starch showed no change, except for slight broadening and flattening of the endotherms of the smaller granules. This is expected because the smaller fractions contain more particles per sample. Enthalpy of all dent starch fractions was 3.2 cal/g.

Amylomaize starches had unusual endotherms, broad and flat in comparison to the normal pattern for dent corn starch. Fractions showed great variation in endotherm shape and in temperature of the maximum peak. The Amylomaize VII fractions also exhibit a considerable change in ΔH. Data for ΔH and T_{max} are given in Table II for large, small, and intermediate size granules for both Amylomaize V and VII. The fact that T_{max} shifts so greatly, but that the endotherms are broad compared to normal dent starch, indicates that the fractions are still somewhat heterogeneous. Because microscopic examination of fractions indicates that granule size is fairly homogeneous in each fraction, the heterogeneity of the gelatinization, as defined by DSC data, appears to involve something other than size alone, such as irregularity of granule shape. The endotherms of the fractions do, however, reveal that gelatinization of amylomaize starches is extremely variable, and processing temperatures are a significant factor in the gelatinization of these starches.

The ΔH values of the amylomaizes are interesting in comparison with those of normal starch. Amylomaize V shows essentially constant ΔH in all fractions, and this value is lower than that obtained for dent corn starch even though the temperature required for gelatinization is higher. Amylomaize VII fractions show variation in ΔH; but even the small granules, which require the highest temperature of all for complete gelatinization, have a ΔH only slightly higher than that for dent corn starch. This seems to indicate that the gelatinization behavior is not caused by variation in organization or crystal structure within the granule, but rather to the impermeability of the surface and the internal pressure within the granule required to disrupt that surface. Our DSC data were all obtained in the presence of excess water. Donovan (1979) showed that changes in endotherms occur when water content is limited. Studies are in progress on the effect of water content on the DSC of these fractions.

TABLE II
Variation in Gelatinization Behavior of Amylomaize Granule Fractions as Measured by Differential Scanning Calorimetry

	Granule Size (μ)	T_{max} (°C)	ΔH (cal/g)
Amylomaize V	14.2	73	2.5
	10.5	83	2.7
	6.0	76	2.5
Amylomaize VII	11.2	70	2.2
	9.9	84	2.6
	5.0	99	3.5

CONCLUSIONS

The relationship between composition and properties of amylomaize starches was investigated using samples that were fractionated according to granule size. The apparent amylose content of these fractions previously was found to vary inversely with the diameter of the granule (Cluskey et al 1980).

Rate of hydrolysis of raw starch with α-amylase is directly affected by size of the granules because of the interrelationships between surface area and enzyme adsorption. In amylomaize, this situation is complicated by the fact that the rate of hydrolysis decreases with increased amylose content, and the greatest amylose content is found in the smallest granules, which have the greatest surface area.

Temperature and enthalpy of gelatinization of amylomaize granules increase with decreasing granule size. The effect of amylose content could not be ascertained.

Amylomaizes are known to contain an intermediate material in addition to amylose and amylopectin (Banks et al 1974). More extensive compositional study of these granule fractions is necessary to properly evaluate composition-property relationships. The crystalline nature of these fractions and the effect of granule lipids on properties also need to be examined.

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