A Comparison of the Properties of Large- and Small-Granule Starch Isolated from Several Isogenic Lines of Barley[1]

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ABSTRACT

Small and large starch granules were isolated from six different barleys including high-amylose, waxy, and high-lysine varieties along with their normal counterparts. Pasting temperatures, Brabender cooking curves, solubility, swelling power, β-limit, and amylose content were compared. The only consistent differences observed were in the β-limit level where the small granules had higher limits and slightly higher values for the birefringence end point temperature of the small granules. The amylose content of the small granules was either equal to or less than that found for the large granules.

Recently Bathgate and Palmer (1) reported that small-granule barley starch had an amylose content of 41.3%, whereas the large granules from the same source had a normal amylose content of 24.9%. In addition they reported a difference of 35° between the gelatinization point of the large and small granules. As a result of these observations they have proposed that the starch synthesis in the small granules of barley is under different genetic control than that in the larger granules. Since our previous work on small-granule starches (2–5) has shown them to have a normal amylose content and a low-to-normal pasting temperature, the question was raised whether Bathgate and Palmer had accidently examined a very unusual case or whether or not their observation was generally true. To examine this thesis three barleys differing widely in their properties and their isogenic counterparts were selected for study.

MATERIALS AND METHODS

Preparation of Starches

The barley selections used were all of known history grown on plots at the Montana Agricultural Experiment Station. The barley and cow cockle starches were separated by wet milling and the starch fractionation conducted according to the procedure described by Goering et al. (6).

Determinations

Protein content was determined by the Kjeldahl method (7, p. 16) (conversion factor 6.25). The total free fat was determined by ether extraction (7, p. 128). Brabender viscosity was determined with the procedure described by Mazurs et al. (8). The pasting temperature range was determined by amylograms in the presence of carboxymethyl cellulose (CMC) as described by Crossland and Favor (9) and modified by Sandstedt and Abbott (10). Solubility and swelling power were determined according to Leach et al. (11). Iodine affinity was determined by the technique of Lansky et al. (12) modified by the use of dimethyl sulfoxide (DMSO) as a solvent.

The β-limit was determined using the procedure described by Whelan (13).

The birefringence end point temperature (BEPT) was determined as described by Watson (14).

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TABLE I. CHEMICAL COMPOSITION

	Protein %	Ether Extractables %
Compana		
Small granule	0.66	0.10
Large granule	0.06	0.10
Waxy Compana		
Small granule	0.17	0.19
Large granule	0.11	0.16
Glacier		
Small granule	0.39	0.20
Large granule	0.39	0.24
High-Amylose Glacier		
Small granule	0.39	0.23
Large granule	0.11	0.17
Hiproly Normal		
Small granule	0.33	0.24
Large granule	0.16	0.23
Hiproly High Lysine		
Small granule	0.39	0.07
Large granule	0.11	0.11

RESULTS AND DISCUSSION

Photomicrographs indicating the quality of separation of small and large granules by this technique have been published previously (6), and although the separation is not perfect, it does separate large and small granules. A few intermediate-size granules appear with the small-granule fraction. Since we have determined the mass of large and small granules for four of the varieties studied (6) these interfering granules do not make up a sufficient part of the weight to cause any measurable effect. One sample, Hiproly Normal, was found to be far more difficult to fractionate than any of the others.

The protein and fat contents of these starches are given in Table I.

These data indicate that there is no substantial difference in either fat or protein content of the large and small granules. The slightly higher protein content of the small granules would be expected since the starch-synthesizing enzymes are probably attached to the surface and since small granules have more surface area per gram. Only in the case of Compana was the difference significant and in no case did we find the high values reported by Bathgate and Palmer (1).

Table II shows data on solubility, swelling power, iodine affinity, β-limit, and BEPT.

No consistent difference seems to exist between the small and large granules in regard to percent solubility, swelling power, or iodine affinity. In Waxy Compana and in Hiproly High Lysine the small granules did show an increase in both solubility and swelling power. With High-Amylose Glacier the larger granules appear to be more soluble. In terms of iodine affinity in general the small granules possess a value either equal to or less than that observed for the large granules which is quite different than the results reported by Bathgate (1). We did separate approximately 40 lb. of High-Amylose Glacier starch without fractionating it. The iodine affinity of the sample was determined to be 8.5. This was then fractionated into large and small granules with iodine affinities as

TABLE II. PROPERTIES OF SMALL- AND LARGE-GRANULE STARCH

Barley Variety	at 90°C. % soluble	S.P.[1]	I.A.[2] %	β-limit %	(98% loss) BEPT[3]
Waxy Compana					
Large granule	13.1	33.2	0.0	48	72
Small granule	17.5	52.7	0.1	48	74
Compana					
Large granule	4.0	7.2	6.1	54	71
Small granule	3.8	6.7	5.7	61	74
High-Amylose Glacier					
Large granule	7.1	5.1	8.9	44.5	77
Small granule	4.3	5.6	7.9	53.5	81
Glacier					
Large granule	3.8	6.3	5.7	53	74
Small granule	5.4	6.1	5.7	65	73
Hiproly High Lysine					
Large granule	3.9	7.2	5.4	53	76
Small granule	7.2	9.3	4.3	59	78
Hiproly Normal					
Large granule	5.1	7.0	6.0	39	74
Small granule	4.6	7.0	5.1	43	75

[1]Swelling power.
[2]Iodine affinity.
[3]BEPT = birefringence end point temperature.

reported in Table II. Using values for percent by weight of small and large granules as determined previously (6) a calculation of iodine affinity on the basis of value obtained for large and small granules gave a value in substantial agreement with original value.

The β-limits for all the small-granule starches with the exception of Waxy Compana were appreciably higher than those of the large granules. This would suggest that as granule size increases the molecular size may also increase, perhaps making some A-chains inaccessible and therefore not available to β-amylase. The very low values for Hiproly Normal suggest that it contains unusual starch fractions.

Note that the BEPT are slightly higher for the small granules in all but one case. However, the differences are small and far below the value Bathgate and Palmer (1) found for the small-granule barley starch. However, they used the Congo red[2] procedure which is extremely questionable for this determination (15) and could be partially responsible for the extremely high value obtained by the above authors. One would not expect any substantial difference in BEPT of small and large granules because with significant magnification one can observe small granules and large granules losing polarization crosses at the same temperature.

It is interesting that Waxy Compana and Compana have similar BEPT, since it was shown previously (16) that the pasting temperature of both Waxy Compana and Waxy Oderbrucker were 20° below that of the parent barleys. This suggests that the amylose is not involved in the crystallinity of the granule, but may contribute to the higher pasting temperatures.

[2]Congo red stains gelatinized granules but does not stain ungelatinized granules.

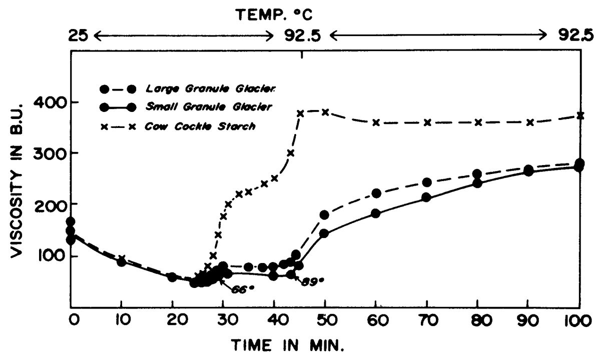

Fig. 1. Pasting of small- and large-granule Glacier barley starch and cow cockle starch; 5.5% starch plus 0.8% carboxymethyl cellulose.

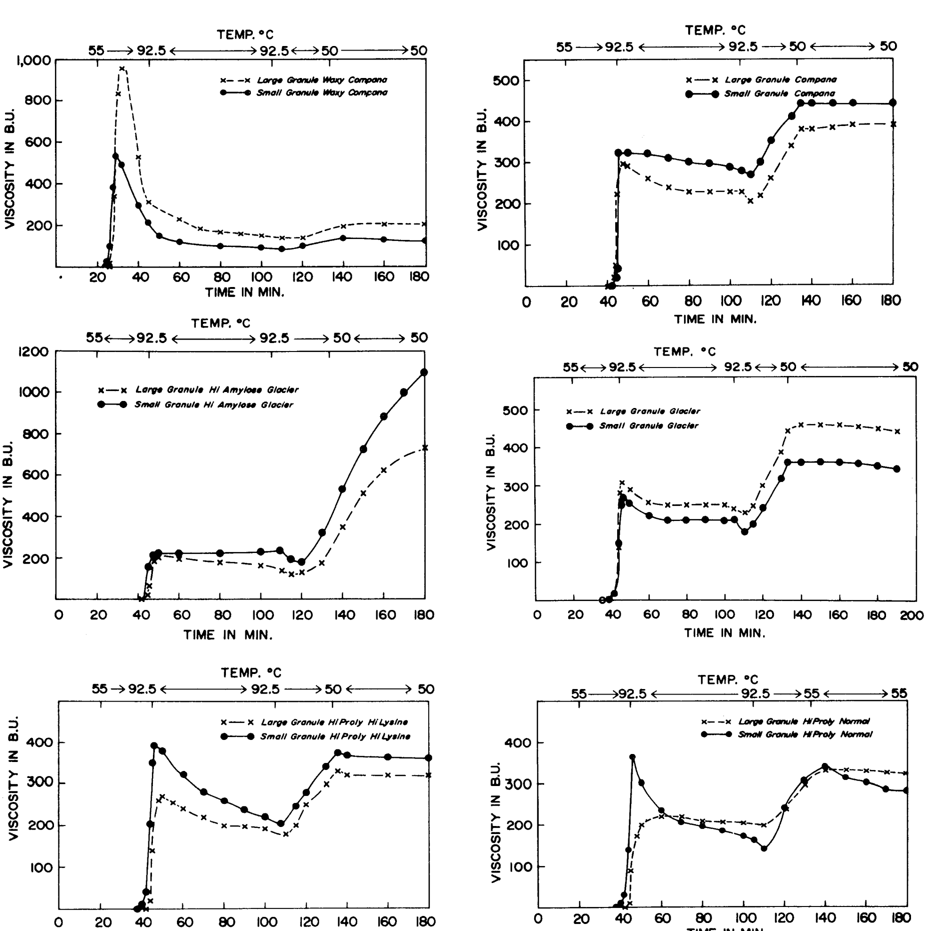

Fig. 2. Brabender amylograms at 8% level for small and large starch granules from six different varieties of barley.

Due to the difficulty in obtaining adequate amounts of small-granule starch, the pasting temperature was run on only one variety. In Fig. 1 a comparison has been made on the small and large granules of Glacier with cow cockle starch. Since the viscosity of CMC decreases with increasing temperature its use results in obtaining a sharper break in the Brabender curve.

The difference in behavior of the small and large starch granules from Glacier appears insignificant. This barley appears to give a two-stage pasting curve, the first break at the temperature where the large granules are rapidly losing birefringence. However, the major change occurs long after the loss of all birefringence. It is apparent that the cow cockle starch pasting curve is quite different from the small-granule Glacier. This is additional proof that something other than small granule size is responsible for the pasting characteristics of cow cockle starch.

The Brabender cooking viscosities are shown in Fig. 2. There is no consistent difference in the curves for large and small granules. The greatest deviation occurs with Waxy Compana, Hiproly High Lysine, and Hiproly Normal, and this is primarily associated with cooking peak.

Note that with the Hiproly both isogenes indicate the small granules are more sensitive to disruption during cooking. This observation is contrary to what one usually finds in small-granule starch, namely, that it is very stable during cooking (2–5). The cooking curve of the small-granule starch from Hiproly Normal deviated more from that of the large-granule starch than was found in any of the other samples examined.

The above data certainly suggest that there is no substantial difference in the properties of the large- and small-granule starch separated from mature barley.

This observation confirms the assertion of Banks and Greenwood (17) that small granules observed in mature barley starch are a second discrete population and not immature granules.

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