A Comparison of Loss of Birefringence with the Percent Gelatinization and Viscosity on Potato, Wheat, Rice, Corn, Cow Cockle, and Several Barley Starches[1]

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ABSTRACT

A study has been made comparing the birefringence end point temperature (BEPT) with percent gelatinization and Brabender viscosities for potato, wheat, rice, corn, cow cockle, and several barley starches. In no case at the BEPT was the starch more than 80% gelatinized. The temperature of the rapid increase in Brabender viscosity agreed with BEPT only for rice and cow cockle starch. With corn and potato starch it occurred before the BEPT and with wheat and barley at a much higher temperature. The data obtained indicate no relationship between loss of crystallinity as measured by BEPT and percent gelatinization. Barley starch appears unique in that the maximum viscosity is reached only when the temperature is 20° or more above the BEPT.

The gelatinization temperature of starch has been defined as the temperature at which there is a 98% loss of birefringence when viewed microscopically (1). Although it has been recognized that loss of birefringence is the first observed change during heating (1,2) and should be used with caution, the birefringence end point temperature (BEPT) has been used in the literature interchangeably with gelatinization temperature, a stage reached only at a considerably higher temperature. Unfortunately, the instrument universally used for this determination is the Kofler electrically heated microscopic hot stage and a polarizing microscope of relatively low power which is not capable of observing loss of birefringence in small granules. It has been recently stated (3) that small granules do not have polarization crosses. This is surprising since polarization crosses have been shown in the 2 to 4 μ granules of *Silene conoidea* (4). Halick et al. (5) have demonstrated in rice that the temperature at which the loss of birefringence is observed is identical to that where the granules have swollen to the bursting point. They also indicated that when high concentrations of starch are used, the Brabender curve leaves the baseline at this same temperature. There are reports in the literature that barley starch has a low gelatinization temperature as determined by the loss of birefringence (6), whereas our pasting curves on 50 or more barley starch samples indicate this is not true.

In our opinion any reference to BEPT as gelatinization temperature is incorrect and this term should only be used to define what it actually is, namely, a 98% loss of birefringence. Gelatinization temperature should be defined as the temperature to which a starch-water slurry must be heated to obtain essentially complete susceptibility of the starch to glucamylase.

This investigation was an attempt to verify the above thesis.

MATERIALS AND METHODS

The barley starches were isolated by the usual wet-milling technique (7) from known barley varieties grown at the Montana Agricultural Experiment Station.

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The unmodified corn starch was obtained from CPC International provided through the courtesy of Dr. Keng. The wheat and rice starches were purchased from the Matheson Company Inc. of East Rutherford, N.J. Since all samples of purchased potato starch gave evidence of heat-moisture treatment, potato starch was prepared in the laboratory from Idaho Russet potatoes. The peeled potatoes were pulped in a Waring Blendor, screened on 150 mesh, and the starch allowed to settle in a beaker. The water was decanted and the dark material on the surface removed with a spatula. After washing several times and allowing to settle, the starch was dried at room temperature by a current of warm air.

The cow cockle starch was prepared by the wet-milling technique used for barley with slight modification. It is necessary to allow the purified starch to stand and decant the starch from some of the black hull which passed through the screening operation. The hull settles more rapidly than does the small granule starch. The decanted starch is then recovered by centrifugation.

Attempts to run Brabender curves at the concentrations recommended by Halick et al. (5) for rice flour were found to be impractical. After some experimentation it was observed that 12.5% concentrations were manageable and they appeared adequate to give abrupt breaks in the Brabender curve.

Samples for determination of loss of birefringence and percent of gelatinized starch were prepared by mixing dry starch for 2 min. in a Virtis mixer with 400 ml. water. The weights used were varied with the granule size to facilitate counting but in general were in the range of 0.1 to 0.2% recommended for BEPT studies. An aliquot was removed immediately for microscopic assay and the balance placed in a Brabender Viscoamylograph equipped with a United

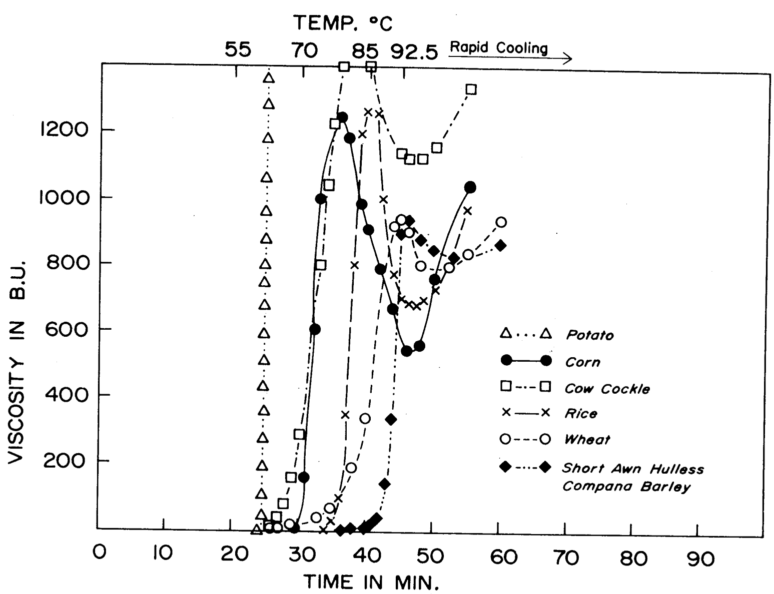

Fig. 1. Brabender curves on potato, corn, cow cockle, rice, wheat, and barley starch at the 12.5% level, dry basis, showing wide variation in pasting temperatures.

Systems Corporation digital thermometer. The samples were started at 25° C. and heated at 1.5° C. per min. until they reached the desired temperature. Aliquots were then removed for both the microscopic determination of percent loss of polarization crosses and the amount of gelatinized starch present by the use of glucamylase as described by Shetty et al. (8). Although this procedure is extremely accurate, the problems in quick cooling the samples for this application cause some error which tends to make the percent gelatinization high. As technique is developed, reasonable duplicate values are obtained.

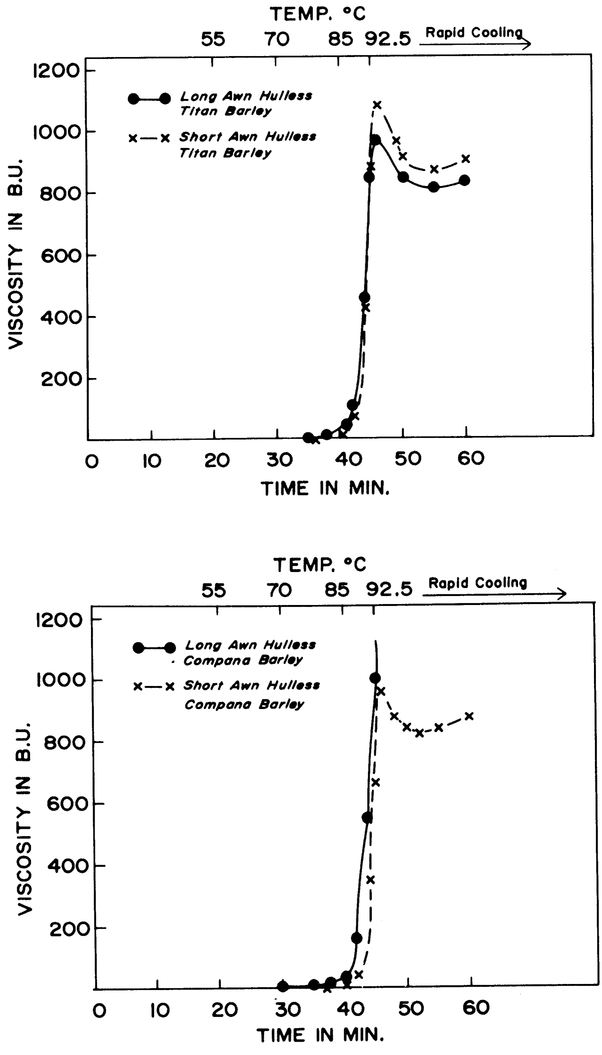

Fig. 2. Brabender curves on four barleys at the 12.5% level, dry basis, showing negligible differences in the pasting temperatures between varieties and isogenic lines.

The loss of birefringence was determined as described by Watson (1). The magnification used was from 100 to 640× depending on starch used. The higher magnifications were necessary to see the polarization crosses in the small granules (0.75 to 4 μ). In addition to the above, a sample of each starch was examined for loss of birefringence through use of the heating stage in the usual manner.

TABLE I. A COMPARISON OF LOSS OF BIREFRINGENCE WITH PERCENT OF GELATINIZED GRANULES ON CORN, RICE, WHEAT, POTATO, AND COW COCKLE STARCH

Substrate	Temp.	% Loss Birefringence	% Gelatinized
Corn	65	2	13
	70	89.6	51
	75	100	66
Rice	75	88.7	61
	80	99.3	72
	85	100	77
Wheat	55	46.8	9
	60	77.3	27
	65	100	70
	75	...	81
Potato[1]	55	0	2
	60	0	3
	65	0	36
	70	45	44
	75	100	55
	80	...	64
Cow Cockle	55	21.1	24
	60	46.7	36
	65	100	78
	75	...	91

[1]Impossible to pipet samples from Brabender amylograph; value obtained by examining under hot stage.

TABLE II. A COMPARISON OF LOSS OF BIREFRINGENCE (% LOSS B) WITH PERCENT OF GELATINIZATION (% GEL) AS MEASURED ENZYMATICALLY FOR VARIOUS BARLEY STARCHES

Sample	Temperature (°C.)					
	60	63	65	70	92.5[1]	Autoclaved
Short Awn Hull-less Titan						
% loss B	85	96	100	...	...	...
% gel	11	32	40	52	92	96
Long Awn Hull-less Titan						
% loss B	81	94	100	...	...	...
% gel	9	29	34	46	82	88
Short Awn Hull-less Compana						
% loss B	75	94	100	...	...	...
% gel	11	29	41	52	100	96
Long Awn Hull-less Compana						
% loss B	88	...	99.9	100	...	...
% gel	24	...	67	71	99	93

[1]After holding 15 min.

RESULTS AND DISCUSSION

Brabender curves on the various starches used in this study are shown in Figs. 1 and 2.

From these curves certain temperatures were selected for the determination of percent gelatinization and percent loss of birefringence. These data are presented in Tables I and II.

Table III shows a comparison of BEPT with percent gelatinization as determined by use of glucamylase together with the temperatures at which the Brabender curve leaves the baseline and temperature of first rapid increase in viscosity. The latter was the temperature at which the viscosity increased by 80 or more Brabender units (B.U.) per min.

With wheat and rice starch, the BEPT was the same whether or not the sample was heated in the Brabender. Potato starch, after being heated in the Brabender, was impossible to count because the viscosity of the paste made accurate dilutions impossible. All barley samples, corn, and cow cockle starch gave values 5° to 10° higher when temperature was determined by hot stage as compared to sample heated on the Brabender. It would appear in certain starches that agitation in the Brabender assists in the water absorption which is responsible for loss of crystallinity in the granules. It has also been observed by Miller et al. (9) that a discrepancy exists in the extent of starch gelatinization as measured by the use of the hot stage and the amylograph. They believe that hot stage methods are deficient in that the actual temperature at the site of a given granule is not that recorded by the thermocouple. Observations indicated that when heated in the Brabender amylograph the large granules lost polarization crosses first; this was not apparent in the hot stage normally used for these measurements.

In the case of rice starch the temperature at which a rapid increase in Brabender viscosity occurs is identical to the BEPT and therefore agrees with Halick's (5) observation. This is not true for any of the other starches examined. With corn starch this point is reached several degrees before the BEPT, with cow cockle starch several degrees after the BEPT, whereas for potato starch the viscosity increase is 13° before the BEPT. Corn starch reaches maximum

TABLE III. A COMPARISON OF BEPT AND PERCENT GELATINIZATION WITH BRABENDER CURVES FOR VARIOUS STARCHES

Samples	BEPT	% Gelatinization at BEPT	Brabender LBL[1]	Temperature RIV[2]
Corn	74–75	66	68.5	71.5
Rice	79–80	72	76	79
Wheat	64–65	70	64	77.5
Potato	75[3]	55	61	62
Cow cockle	65	78	62.5	67
Short Awn Hull-less Titan barley	65	40	79	88
Long Awn Hull-less Titan barley	65	34	77.5	88
Short Awn Hull-less Compana barley	65	41	79.0	88
Long Awn Hull-less Compana barley	65	67	68.5	86.5

[1]Curve leaves baseline.
[2]Rapid increase in viscosity occurs (increases at least 80 units/min.).
[3]Determined by hot stage; unable to pipet Brabender samples, 98% loss birefringence crosses.

viscosity before BEPT which is what one would expect to happen if pasting viscosity is the result of gelatinization and if 100% of the starch is gelatinized at this temperature. This could be the reason why the basic assumption was made

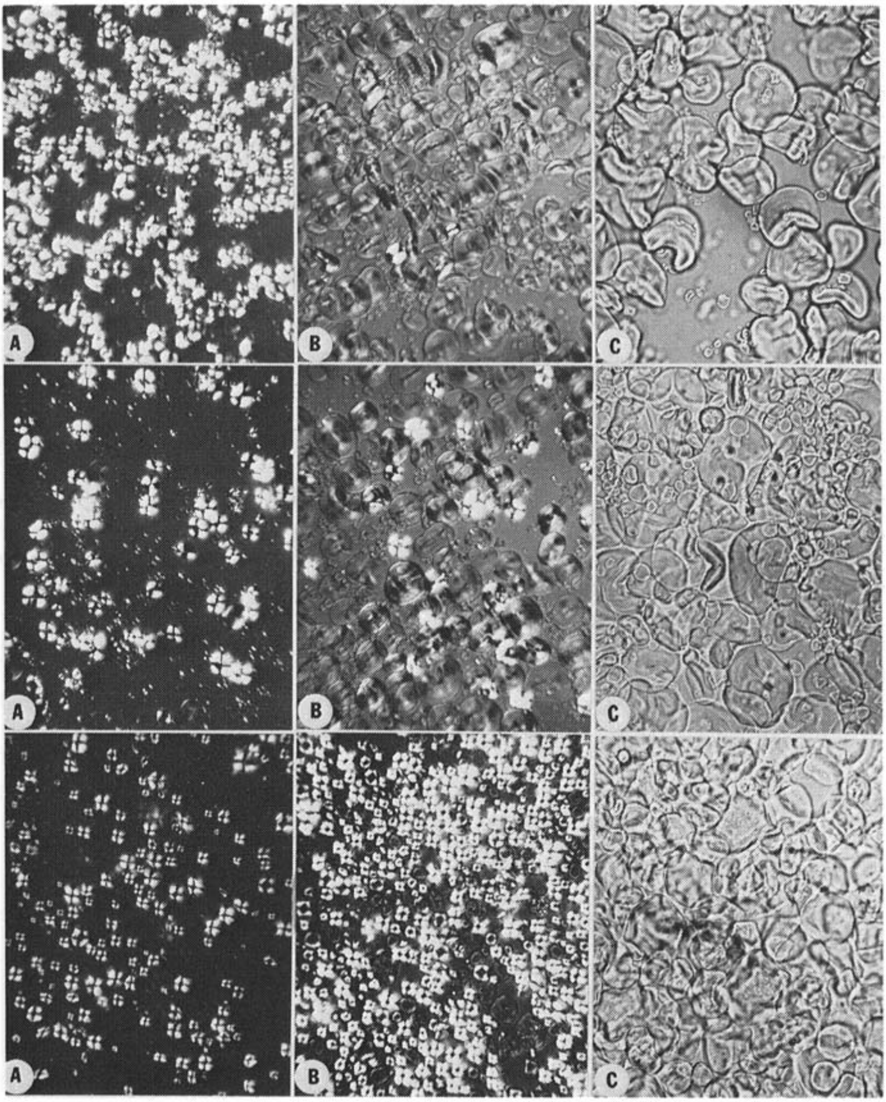

Fig. 3. Comparative heating stage photomicrographs. Top row, Long Awn Hull-less Titan; middle row, Short Awn Hull-less Compana; bottom row, corn. A) Polarized, 65°C. (250X); B) Polarized, 75°C. (250X); C) Brightfield, 90°C. (450X). Note the changes in contrast between birefringent and non-birefringent granules at 65° and 75°C. Note also that at 75°C. all of the small granules have lost polarization crosses in the LAHT (barley) and SAHC (barley), while some large and medium granules retain evidence of birefringence. Photomicrographs at 90°C. show comparative granule changes.

that the BEPT really measured percent gelatinization although at this temperature only 66% of the corn starch granules are actually gelatinized. With wheat and all the barley samples, the rapid increase in viscosity is not obtained until temperatures are increased from 12° to 23° above the BEPT.

The BEPT has been defined as the "change from dispersion to paste" (1). It has been stated that when heated in water to progressively higher temperatures, the granules first gelatinize and lose their polarization crosses and thereafter undergo a continued swelling (10). The glucamylase procedure has been demonstrated to measure the amount of gelatinized starch present in a mixture (8). The above results indicate that the BEPT does not occur when the starches are 100% gelatinized but varies from 55 to 78% gelatinization although the common starches are 55 to 70% gelatinized at this temperature. Birefringence is due to the crystallinity of the granule and is apparently the result of special packing arrangement of the molecules in the granule. It would appear that disruption of this packing (loss of birefringence) occurs appreciably before the starch granules are all gelatinized. For this reason it would appear that the BEPT should never be referred to as gelatinization temperature.

When large granules lose polarization crosses, loss of contrast is very evident and in many cases it appears that the granule is swelling and expanding in size. This is generally not the case since upon measurement by micrometer, the granule is not more than 1 or 2 μ larger following loss of the polarization crosses. As the heating stage temperature is increased, in many cases, the barley granules become misshapen and elongated or form a kidney-shaped granule displaying a continuous perimeter (top C, Fig. 3).

In this work it was also observed that when large granules lose polarization crosses, it appears as if the large granules are first to lose polarization crosses followed by the medium size and finally the small granules. Actually this is not the case and careful observation at high magnification will show that both large and small granules lose polarization crosses simultaneously (top and center A and B, Fig. 3). Since in some barley starches up to 30% of the granules are small (11), a high-power microscope is required to obtain the BEPT.

Although the barley starches used contain a normal amylose-amylopectin ratio, an extremely high temperature is obtained before the maximum increase in viscosity is observed. This could be explained if the exudate described by Miller et al. (9) is only liberated at a rather high temperature. At the higher temperature (bottom C, Fig. 3), the corn starch granules have become misshapen and in some cases appear to have disintegrated, whereas the barley starch granules are still observed as individual granules (top and center C, Fig. 3). At this temperature their size has increased slightly and the shape of the granules has changed somewhat. This raises the question of what is preventing granule disintegration. Previous work (12) has shown that barley amylose varies in size from that of commercial amylose to approximately 30% larger. The $\overline{M_n}$ (number average molecular weight) appears to be appreciably larger for barley amylopectin than was observed for corn. However, the limited numbers of samples examined to date do not allow any conclusions to be based on these data. Either there must be a difference in the structure of the amylose and amylopectin or the granule contains unique forces resisting swelling and rupture. In general, unpublished observations in our laboratory have indicated that barley starches show lower swelling and lower solubility than does corn starch.

Since the Congo red procedure for determination of gelatinization temperature is based on the fact that the dye is only absorbed by gelatinized granules, it is easy to understand why these values would be high as compared to the gelatinization temperature as measured by BEPT.

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