MEASUREMENT OF STARCH GELATINIZATION BY ENZYME SUSCEPTIBILITY[1]

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ABSTRACT

Gelatinization of starch in water occurs over a range of temperatures that vary with different starches. The susceptibility to beta-amylolysis of starches heated to various temperatures was compared with the loss of birefringence. A close relationship between increase in susceptibility of the starch to beta-amylolysis and the loss of birefringence was observed. The mean gelatinization temperature of starches can be defined by loss of 50% birefringence or by the production of 5 mg. of maltose during 3 hr. of beta-amylolysis. Beta-amylolysis may be used to measure the degree of gelatinization of starches in certain systems where measurement by loss of birefringence is difficult or uncertain.

Raw starch granules are relatively insoluble, nondispersible, and resistant to amylolysis (1,2). Before the starch granule can be readily hydrolyzed by the amylases, the crystalline structure must be disrupted. This can be achieved by gelatinization with chemicals or heat or by extensive mechanical treatment (1). As the crystalline organization of the starch granule is destroyed, the granules swell in the presence of water, exhibit loss of birefringence, and become dispersible in water and subject to amylolysis.

Starch gelatinization by heating in the presence of water is not an instantaneous process but occurs over a range of 8° to 10°C. The degree of gelatinization also depends on the duration of heating. Various starches are known to gelatinize at different temperatures (3,4). Likewise, various types of starches exhibit different degrees of associative bonding within the raw starch granules (5,6). This apparently accounts for differences in swelling and swelling patterns as well as relative resistance of the raw starches to amylase degradation (2).

Various means of measuring gelatinization temperatures have been used. Schoch and Maywald (4) suggested that, although starches exhibit a gelatinization range, the temperature at which 50% of the granules had lost their birefringence could be used as a measure of the gelatinization temperature of the starch. They preferred to use a range of temperatures rather than a specific temperature. Martin

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and Newton (3) followed starch gelatinization at different temperatures by measurement of the maltose produced by action of soybean beta-amylase. Novelle and Schütte (7) used sorghum malt amylase to measure the effect of heat, salts, and acids on sorghum starch gelatinization. They found good agreement between the temperature of gelatinization of sorghum starch as measured by amylolysis and that reported by Schoch and Maywald (4), who measured loss of birefringence of the starch granules.

It has been tacitly assumed that loss of birefringence and enzyme susceptibility are closely related. This work was done to show more explicitly the relationship between loss of birefringence and the increase in starch susceptibility to beta-amylase as a measure of gelatinization.

Materials and Methods

Several starches including commercial potato, arrowroot, tapioca, wheat, corn, sorghum, waxy corn, and waxy sorghum were employed. One-percent starch suspensions in distilled water were heated from 30° to 90°C. at the rate of 1.5°C. per min. in a Brabender Amylograph with the customary stirring. Samples were withdrawn at temperature increments from 55° to 90°C., cooled to room temperature, and immediately subjected to beta-amylolysis and birefringence measurements. Each starch was examined in duplicate. It was found imperative to adhere to a given time schedule, because allowing the starch gels to set for extended periods caused a gradual decrease in availability of the starch. This observation agrees with those of Jackel *et al.* (8), and Volz and Ramstad (9) who used enzyme susceptibility of the starch as a measure of retrogradation during bread staling.

The birefringence was measured on a 0.2% starch dilution of the heated sample by counting the granules exhibiting birefringence under a Bausch & Lomb microscope equipped with a polarizer.

A purified beta-amylase[3] devoid of alpha-amylase was used to measure beta-amylolysis of the heated starches. A 40-ml. aliquot of the 1% starch suspension was buffered to pH 4.65 with 5 ml. of a 1*M* acetic acid-sodium acetate buffer. After being equilibrated to 30°C., 5 ml. of the enzyme solution were added containing 4 mg. of a beta-amylase. Three-milliliter aliquots were removed immediately, and every hour thereafter for 5 hr., for measurement of the reducing sugars by the ferricyanide method (10). The initial aliquot was used as a blank to adjust subsequent aliquots. Susceptibility was expressed

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as maltose equivalents at 3 or 5 hr. of digestion, at which time beta-amylolysis reached essentially a constant rate.

Results and Discussion

The effect of time of digestion on beta-amylolysis of potato, tapioca, arrowroot, and wheat starches heated to various temperatures in water is shown in Fig. 1. Similar data representing corn, waxy corn, sorghum, and waxy sorghum starches are illustrated in Fig. 2. In general, these data are similar to those of Martin and Newton

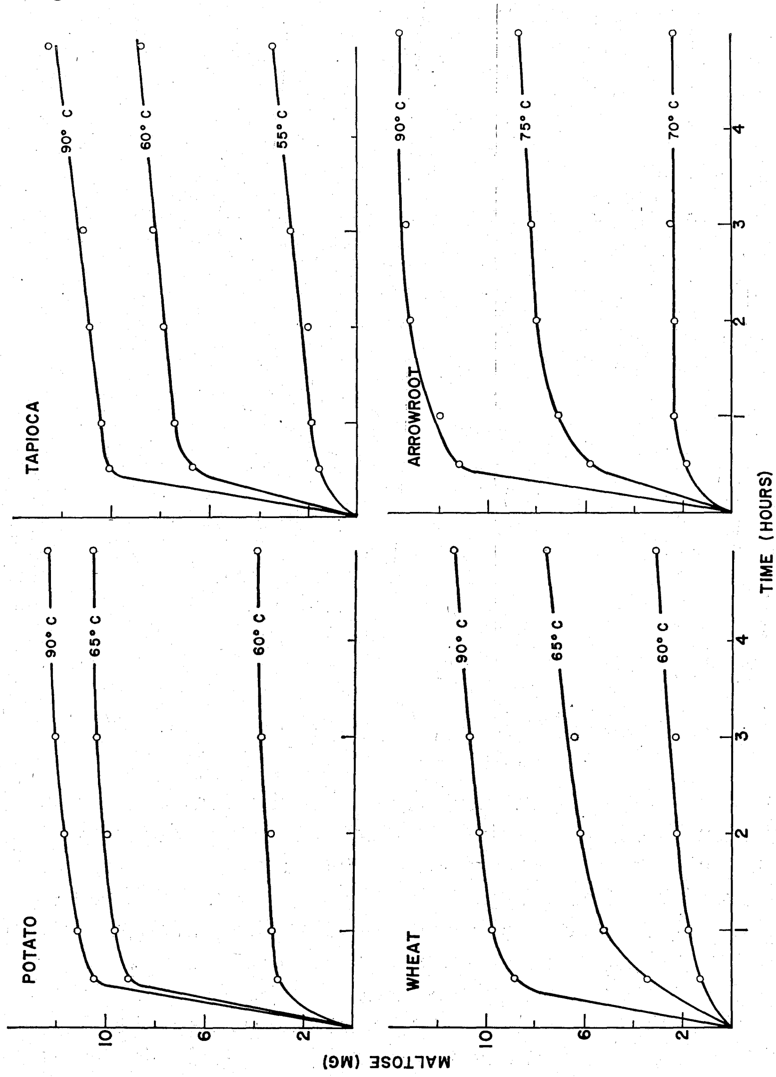

Fig. 1. Beta-amylolysis of potato, tapioca, wheat, and arrowroot starches heated in excess water to various temperatures.

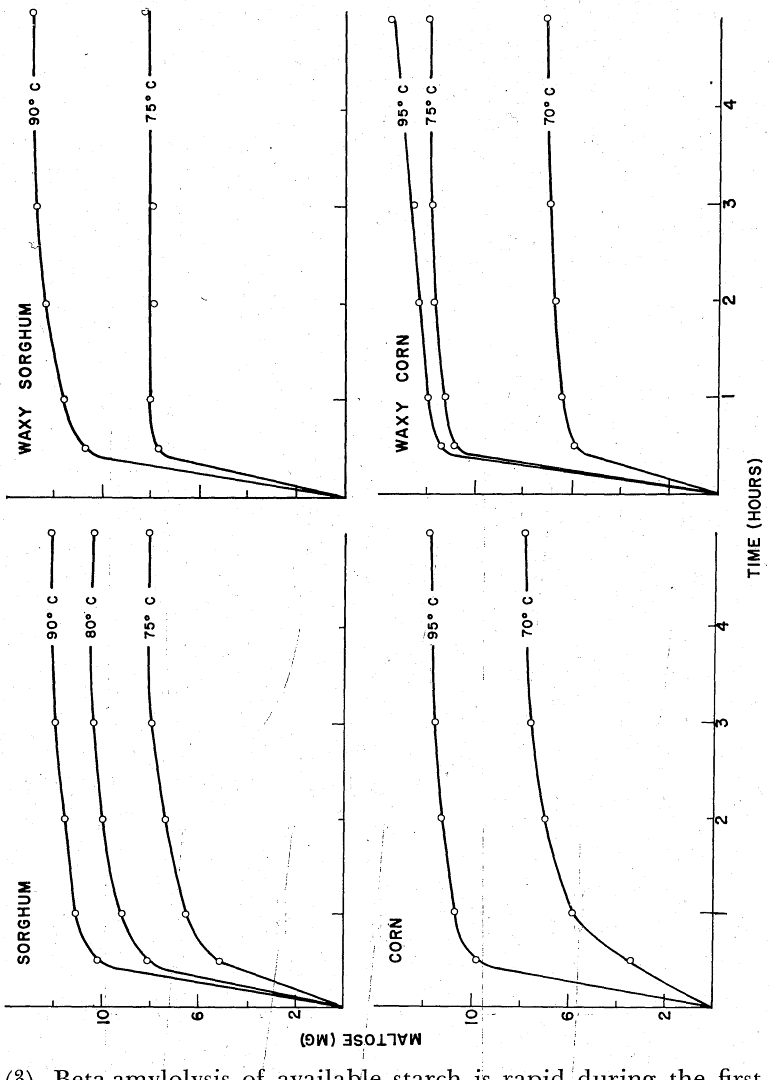

Fig. 2. Beta-amylolysis of sorghum, waxy sorghum, corn, and waxy corn starches heated in excess water to various temperatures.

(3). Beta-amylolysis of available starch is rapid during the first hour and then approaches a limiting value beyond 3 hr. of digestion.

Availability of the starches was dependent on the temperature reached during gelatinization, and the kind of starch. Differences in availability of the starch were pronounced at the lower temperature ranges, but their differences disappeared at temperatures approaching 90°C. The availability of the starches heated to 70°C. to beta-amylolysis is shown in Fig. 3. Potato and tapioca starches became slightly available at 55°C. and availability changed slightly when

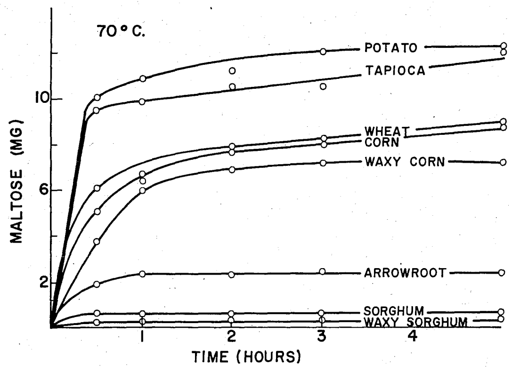

Fig. 3. Beta-amylolysis of various starches heated to 70°C. in an excess of water.

the starches were heated beyond 70°C. In contrast, sorghum and waxy sorghum starches were only slightly available at 70°C. but became increasingly available as the temperature of gelatinization approached 85° to 90°C. Wheat, corn, and waxy corn starches be-

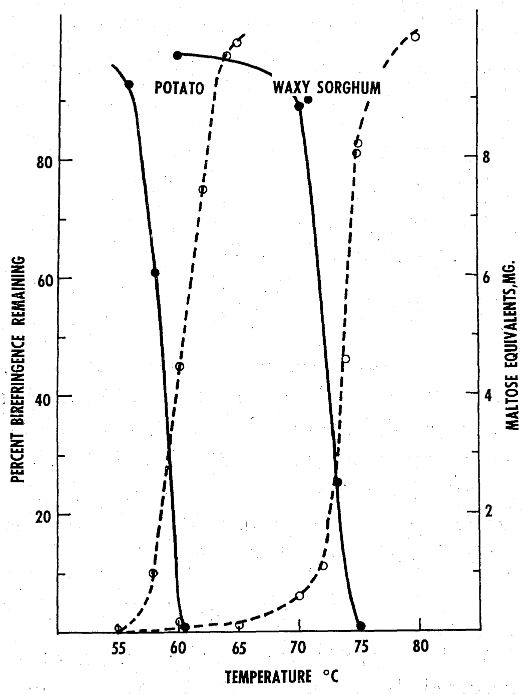

Fig. 4. Relationship between temperature, loss of birefringence, and beta-amylolysis for potato and waxy sorghum starches. Solid lines, loss of birefringence; dashed lines, beta-amylolysis.

came slightly available at 60° to 61°C. and were approximately 50% more available at 70°C. Arrowroot starch was only about 20% available to beta-amylolysis at 70°C. The differences in the availability of the starches when heated to 70°C. reflect the degree of associative bonding within the granule.

Figure 4 illustrates typical plots of loss of birefringence and beta-amylolysis for potato and waxy sorghum starches. The solid line represents maltose equivalents produced by a 3-hr. beta-amylolysis. Both loss of birefringence and increase in starch availability occurred over a relatively narrow temperature range. These data emphasize the close relationship between the two measurements. Other starches presented a similar pattern but differed in the temperature at which rapid changes occurred.

The gelatinization temperatures of several starches measured by loss of 50% birefringence and beta-amylolysis equivalent to production of 5 mg. of maltose are compared in Table I.

TABLE I

GELATINIZATION TEMPERATURES OF VARIOUS STARCHES ESTIMATED BY LOSS OF BIREFRINGENCE AND SUSCEPTIBILITY TO BETA-AMYLOLYSIS

STARCHES	LOSS OF 50% BIREFRINGENCE	BETA-AMYLOLYSIS, 5 MG. MALTOSE
	°C.	°C.
Tapioca	55	55.5
Potato	59	60.0
Wheat	60	62.0
Corn	64	66.0
Sorghum	68	70.0
Waxy corn	71	67.5
Waxy sorghum	72	74.0
Arrowroot	74	73.0

In general there is good agreement between the gelatinization temperatures estimated by the two methods. These temperatures, representing 50% gelatinization, cannot be compared directly with the temperature ranges reported by Schoch and Maywald (4) for the same kind of starch. However, it is of interest that these data, with the possible exception of tapioca starch, generally agree quite well with those reported by Schoch and Maywald (4).

Conclusion

Gelatinization of starches by heating in the presence of water can be readily measured by beta-amylolysis. While the gelatinization temperature of starches occurs over a range of 8° to 10°C., gelatinization temperature can be conveniently defined by loss of 50%

birefringence by the granules or by the production of 5 mg. of maltose through beta-amylolysis of starch for a 3-hr. period.

Beta-amylase is a particularly useful tool to measure the degree of starch gelatinization in complex natural systems where measurement of loss of birefringence is difficult and uncertain. Measurement of the degree of gelatinization by loss in birefringence is dependent on changes from the initial state of the raw starch granules. Frequently the initial state of the starch in the system being investigated is unknown. Measurement of the degree of gelatinization by beta-amylolysis is dependent only on the state of the starch at the time of sampling. Measurement of starch gelatinization by beta-amylolysis, however, suffers from the effects of starch retrogradation, which may be considerable, depending on time and conditions of storage of the starch gels.

Literature Cited

1. Blish, M. J., Sandstedt, R. M., and Kneen, E. The cereal amylases with reference to flour and malt behavior. Cereal Chem. **15:** 629–657 (1938).
2. Leach, H., and Schoch, T. J. Structure of the starch granule. II. Action of various amylases on granular starches. Cereal Chem. **38:** 34–46 (1961).
3. Martin, Vera D., and Newton, J. M. Comparative rates of amylase action on starches. Cereal Chem. **15:** 456–462 (1938).
4. Schoch, T. J., and Maywald, Eileen C. Microscopic examination of modified starches. Cereal Chem. **28:** 382–387 (1956).
5. Leach, H. W., McCowen, L. D., and Schoch, T. J. Structure of the starch granule. I. Swelling and solubility patterns of various starches. Cereal Chem. **36:** 534–544 (1959).
6. Leach, H. W., and Schoch, T. J. Structure of the starch granule. III. Solubilities of granular starches in dimethyl sulfoxide. Cereal Chem. **39:** 318–327 (1962).
7. Novelle, L., and Schütte, R. J. Kaffircorn malting and brewing studies. X. The susceptibility of sorghum starch to amylolysis. J. Sci. Food Agr. **12:** 552–559 (1961).
8. Jackel, S. S., Schultz, A. S., Schoonover, F. D., and Schaeder, W. E. The decrease in susceptibility of bread crumb starch to beta-amylase during staling. Cereal Chem. **29:** 190–199 (1952).
9. Volz, Frances E., and Ramstad, P. E. Effect of emulsifiers on the enzyme susceptibility of starch during staling of bread. Cereal Chem. **28:** 118–122 (1951).
10. American Association of Cereal Chemists. Cereal laboratory methods (7th ed.). The Association: St. Paul, Minn. (1962).