A RAPID METHOD FOR DETERMINING ALPHA-AMYLASE ACTIVITY[1]

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ABSTRACT

A simple and rapid method for determination of amylase activity of flour and pulverized grain is described. The test may be completed in one or two minutes, depending on the amount of alpha-amylase present.

The principle of the method depends on heating a flour-water suspension of 5 g. flour in 25 ml. water very rapidly to determine the time required to gelatinize and, subsequently, to liquefy the flour suspension. For practical purposes the "liquefaction time" is usually sufficient for evaluating the diastatic properties. However, from the "liquefaction time" and the "gelatinization time," both of which may be determined in the same test, the "diastatic number" may be computed. The "diastatic numbers" for different flours are related linearly to the alpha-amylase activity over a broad range.

The use of variable alpha-amylase concentration to modify fermentation and dough properties and the quality of the baked products is a well-established practice. In order to measure and control alpha-amylase, numerous methods have been developed. These methods involve measurement of sugar or fermentable carbohydrate as affected by the alpha-amylase concentration, extraction of the alpha-amylase and determination of its concentration by its effect on a suitable substrate, and measurement of the effect of alpha-amylase on a starch substrate as it becomes available through gelatinization. Methods for determination of alpha-amylase effects have been reviewed by numerous investigators (1,2,3,4,5,6,7).

It is desirable in many instances to have methods for determination of the effect of alpha-amylase that are rapid, simple, nonexpensive, and, at the same time, accurate. It is convenient if the values obtained have a linear relationship to the concentration of the alpha-amylase. This is particularly important when testing sprout-damaged grain or flour. The method must be sensitive enough to measure alpha-amylase of flours or grain with low activity.

Some of the well-known methods require costly apparatus, are frequently too time-consuming, or do not give a true reflection of the enzyme concentration as related to dough performance or bread characteristics. Among methods which do not require expensive apparatus are those of Sandstedt, Kneen, and Blish (11) and Ritter (10). These methods, however, may be quite time-consuming, particularly if

only a few samples are to be tested simultaneously. These methods also have the common fault that they measure time required to cleave starch to a much greater degree than actually occurs during bread-baking. In the modified Wohlgemuth procedures (10, 11), starch cleavage proceeds until units of about 7 glucose units remain. Myrbäck and Gjörling (8) have pointed out that viscosity of the gelatinized starch drops 50% when only 0.1% of the glucosidic linkages are opened; whereas 7% of the linkages must be opened before the remaining dextrins fail to give a blue color with iodine (9). It is evident in actual baking that dextrinization and saccharification proceed much more slowly than liquefaction and that the point never is reached in bread during baking when starch is split to products not deeply colored by iodine.

It is recognized that liquefaction of a viscous gelatinized starch paste can be followed by the common viscometric procedures. Not only are the apparatus for this expensive, but they fail to give results which are related linearly to the alpha-amylase concentration over a broad range. The lack of linearity appears to be due to the fact that the temperature is raised too slowly for samples having high alpha-amylase concentration. In these cases, an appreciable decrease in the substrate concentration occurs.

The object of this research has been to develop a simple, rapid method of assay of alpha-amylase activity, which will give results that are linear over a wide range of alpha-amylase concentrations. The method is based upon rapid gelatinization of the substrate and subsequent measurement of the liquefaction time.

Description of the Method

Apparatus (1) Water bath (100°C.). The thermostatically controlled, 600-watt bath, equipped with test tube rack, approximately 20 cm. high by 15 cm. diameter (Fig. 1).
(2) Test tubes, inner diameter 21 mm., length 220 mm.
(3) Stop watch. An electric flash watch adjustable to 1 second is desirable (Fig. 1).
(4) Stirrer-viscometer. Weight 25 g. (Fig. 1). This stirrer is equipped with two stop-gages separated by 80 mm. A mechanical stirrer can be attached if desired.

Determination: Five grams of flour or finely pulverized grain and 25 ml. distilled water (20°C.) are thoroughly shaken by hand in the test tube using 20 vigorous shakes. The test tube with the stirrer-viscometer is immersed in the boiling-water bath and the stop watch started. The surface of the suspension in the test tube should be at least 5 cm. under the surface of the boiling water. Five seconds after immersion of the test tube, stirring of the suspension is started by movement of the stirrer up and down 2 times per second.

Two basic measurements based on change of viscosity of the suspension are recorded. "Gelatinization time" is defined as the time of heating required to develop a paste in which the stirrer-viscometer drops by its own weight a distance of 70 mm. in 0.25 second. Gelatinization time will vary from 25 to
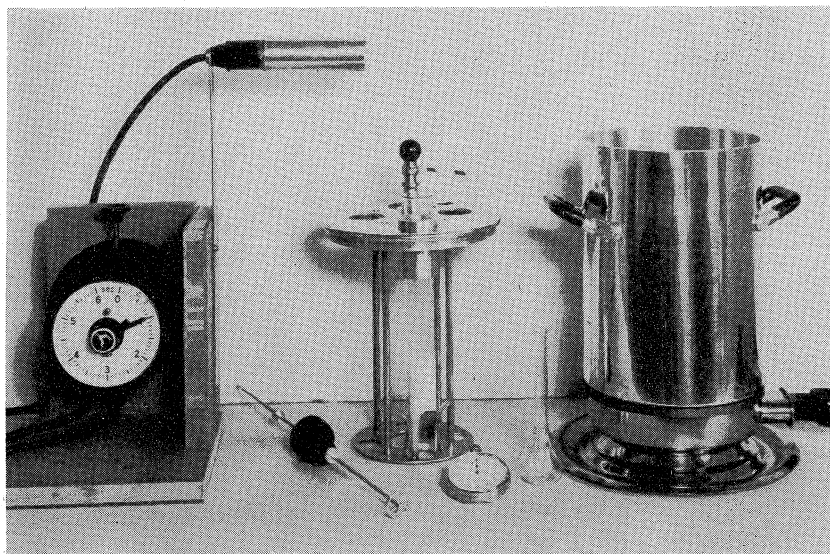
Fig. 1. An apparatus for determination of gelatinization time, liquefaction time, and diastatic number of flour. Right: water bath. Middle: test tube rack and stirrer-viscometer. Left: electrical flash watch, adjustable to 1 second.

40 seconds for wheat flour and from 20 to 35 seconds for rye flour. After the gelatinization time has passed, the paste will rapidly become thicker. The second measurement, "liquefaction time," is defined as the total time of heating required to reduce the thick paste into a thin paste in which the stirrer-viscometer will drop a distance of 70 mm. in 1 second by its own weight. Liquefaction time will vary from 30 seconds or less for wheat and rye flours with high amylase activity (e.g., flour from sprout-damaged grain) up to 300 seconds or more for flour with low amylase activity.

Calculation of the "Diastatic Number." The "diastatic number" may be calculated from gelatinization time and liquefaction time, assuming a diastatic index of 100 when 100% of the starch is liquefied in 60 seconds.

$$\text{diastatic number} = \frac{100 \times 60}{\text{liquefaction time} - \text{gelatinization time}}$$

Discussion

The method described has been found to give reliable results over a wide range of alpha-amylase concentrations. The relationships between several measurements of alpha-amylase activity and the amount of sprouted Swedish winter wheat flour present in a mixture of Manitoba wheat flour with low amylase activity are shown in Fig. 2. These data demonstrate the linear relationship between diastatic number, SKB alpha-amylase units, and the amount of alpha-amylase added to a flour. The relationship is linear over the range usually encountered.

This is in contrast to viscometric methods that depend on relatively slow heating of the suspension. The diastatic number expression will range from 20 or less to over 600 for the usual range of alpha-amylase activities of flours or wheats of commerce. This wide range of values together with the precision with which the numbers can be determined cause the method to be of value in the laboratory where a rapid, inexpensive, and sensitive method is required.

The rate of heating the suspension is important. If the method is followed as outlined, the temperature can be expected to rise as follows:

After 1 minute in boiling water — 77°C.

After 2 minutes in boiling water — 90°C.
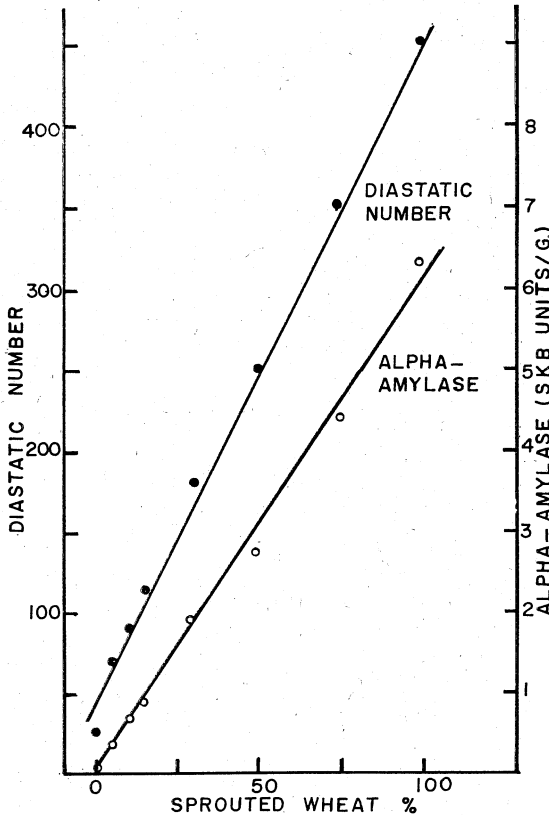
After 3 minutes in boiling water — 95°C.

Fig. 2. Relationship between diastatic number, alpha-amylase, and the amount of sprouted Swedish grain in mixture.

If a water bath of lower temperature is employed, the temperature must be high enough so that the starch is gelatinized faster than it is cleaved by the enzyme.

The ratio of flour to water may be varied. Thus, for low diastatic flours, less than 5.0 g. may be employed, and, if high diastatic flours are common, the amount of flour may be standardized at a higher level than 5.0 g. per 25 ml. of water.

For practical purposes, it is usually sufficient to simply determine the liquefaction time according to the method given.

Acknowledgment

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