CEREAL CHEMISTRY

VOL. 41 MAY, 1964 NO. 3

APPLICATION OF THE FALLING NUMBER METHOD FOR EVALUATING ALPHA-AMYLASE ACTIVITY[1]

HARALD PERTEN[2]

ABSTRACT

Determination of the alpha-amylase activity in finely ground grains and flours is described. The results of the falling number method are compared with those of other methods for measuring alpha-amylase activity. Certain applications of the method are also described. These include studies on the influence of wheat and rye alpha-amylases on different kinds of starches, computation of flour mixtures with desired viscosity characteristics, predicting the malt supplement required to produce a given viscosity, and the effect of alpha- and beta-amylases on the quality of bread crumb.

Millers and bakers are greatly interested in reliable methods for the determination of alpha-amylase activity in grain and flour. These methods permit alpha-amylase activity in flour to be adjusted to a desired level either by the addition of malt or by blending flours of different alpha-amylase activities. A number of methods have been described. They involve measurement of sugar (1–3), viscosity (4–11), and starch-iodine color (12–21).

Rotsch (22) showed remarkable differences among the baking properties of starches isolated from the same cereal. Still greater differences prevail between starches from different kinds of grains. Since the effect of alpha-amylase activity depends not only on the properties of the enzyme but also on the properties of the starch, an autolytic method for the determination of alpha-amylase activity is suggested. Methods utilizing the starch-iodine color reaction do not provide information on the properties of the starch during baking. Viscometric methods have proved to be the most valuable.

Recently, a simple and reliable viscometric method known as the "falling number" method has been developed by Hagberg (23,24) and Perten. This method determines alpha-amylase activity using flour as the

[1]Manuscript received March 9, 1962. Presented in part at the 48th annual meeting, Minneapolis, Minn., April 1963.

[2]Cereal Laboratory, Statens Institut for Hantverk och Industri, Stockholm 4, Sweden. Chairman of the Study Group, Determination of Amylase Activity and Sprouting, of the International Association for Cereal Chemistry.

native substrate. The method is based upon rapid gelatinization of a flour suspension and subsequent measurement of the degradation of the starch paste by alpha-amylase, under conditions similar to those encountered during baking. The falling number method has been tested by the International Association for Cereal Chemistry (I.C.C.) and has proved to have good reproducibility (25).

The purpose of the present work is to describe the application of the falling number method to various cereals and to compare various methods for measuring alpha-amylase activity. These include the maltose determination, amylograph, and SKB methods. The falling number method is also discussed as a means for predicting the malt supplement required, as well as the effect of alpha- and beta-amylases on the quality of bread crumb.

Materials and Methods

Sixty-five samples of wheat and 30 of rye were tested. The wheats included Manitoba, Australian, Irish, Russian, French, Swedish winter, and Swedish spring wheat. The wheat and rye samples used to compare starches and amylases were grown in Sweden. They had protein contents of 12.0 and 8.0% and ash contents of 1.56 and 1.45%, respectively. All samples were finely ground in a hammer-type laboratory mill, to pass through a 0.8-mm. sieve.

Barley malt with an alpha-amylase activity of 140 H-units (59 SKB units) was used as a source of cereal amylolytic activity.

Several methods for determining alpha-amylase were employed. Maltose values were determined according to the AACC method (1). In the amylograph, 90 g. of finely ground grain (14% m.b.) and 450 ml. distilled water were used for testing both wheat and rye samples. The alpha-amylase activity was also determined by the colorimetric method of Hagberg (12). This method is based on the Wohlgemuth principle (21) and is similar to the SKB method (19). The values obtained are called H-units (period of half-life) and may also be expressed in terms of the well-known SKB units.

The falling number method (23,24) was employed to compare different starches and amylases. On alpha-amylase-free starch, 5 g. of starch and 25 ml. of a 10% aqueous enzyme extract (v/w) of finely ground wheat and rye were used. For determining the influence of proteins on the falling number results, an addition of 15% of a commercial dry wheat gluten (0.75 g.) to 5.0 g. of wheat starch was used.

Results and Discussion

Time-Temperature Relationship in the Falling Number Method *and in Baking.* The influence of the enzymes on undamaged starch granules at normal fermentation temperature is very small. After gelatinization, the starch is more easily attacked by the amylases and may be hydrolyzed rapidly. The interval between the gelatinization of starch (55°–65°C.) and the inactivation of the enzymes during baking (75°–80°C.) is a decisive factor in determining bread crumb quality. In the falling number test, it takes about 30 sec. to pass the critical temperature range for amylase activity in bread crumb. During baking, using 70 g. dough at oven temperature 250°C., it takes about 40 sec. to pass the same range. (By baking of larger doughs, e.g., using 800 g. dough, this time will be about 140 sec.) Thus, in the falling number method, the starch passes the critical temperature zone for alpha-amylase activity in nearly the same period of time as during baking. This relationship between time and temperature is shown in Fig. 1.

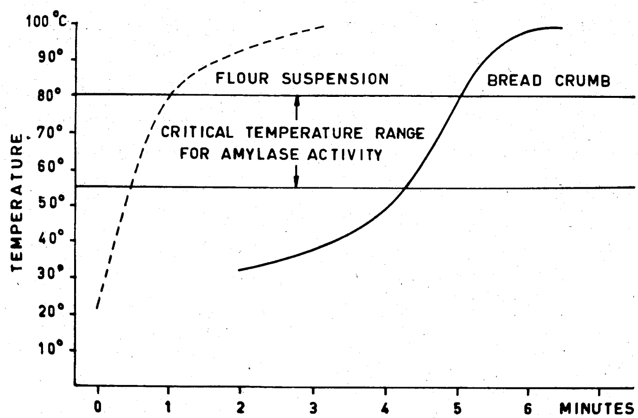

Fig. 1. Increase of temperature in flour suspension according to "falling number" method (7 g. flour, 25 ml. water — 100°C.) and in bread crumb, during baking (70 g. dough, oven temp. 250°C.).

Comparison of Results by Different Methods for Determining Alpha-Amylase Activity. The relation between falling numbers and maltose values is presented in Fig. 2. The correlation coefficient for results obtained by the two methods for wheat is −0.796; and for rye, −0.765. Since Sandstedt and Mattern (26) as well as Sullivan *et al.* (27) have proved that the maltose value of flour depends to a very high degree on the amount of starch damaged by grinding, the relatively good correlations in Fig. 2 may result from the uniform grinding technique.

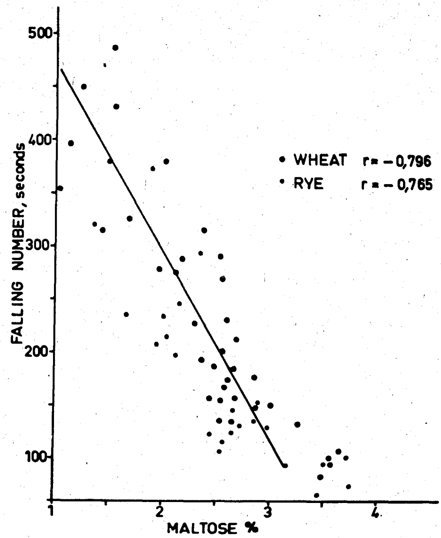

Fig. 2. Comparison between maltose values and "falling numbers" of wheat and rye.

Figure 3 shows the falling numbers and the amylograph values of wheat and rye for comparison. Although there is correlation between these values, the curves for wheat and rye are quite different. This is due to a difference in substrate rather than a difference in the amylase

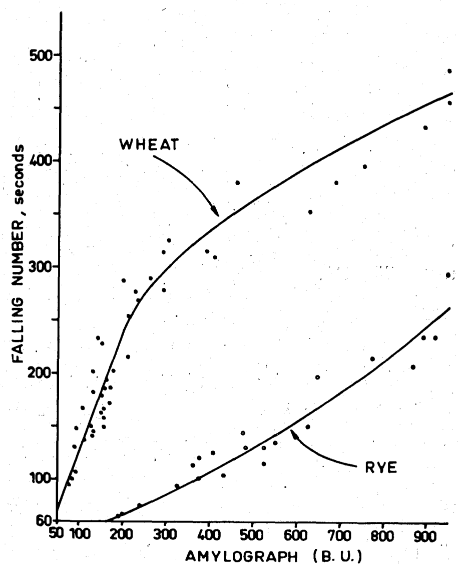

Fig. 3. Comparison between amylograph values and "falling numbers" of wheat and rye.

activity. The amylograph and the falling number methods measure the viscosity in different ways. The amylogram units designate the maximum peak height viscosity after gradual heating of the starch suspension (1.5°C. per min.). The falling number method indicates the viscosity of the gelatinized starch at nearly 100°C., after enzymatic decomposition during a relatively short period of heating.

The relation between the falling numbers and the alpha-amylase activity is evident in Fig. 4. The falling numbers for wheat and rye are related curvilinearly with alpha-amylase activity. The curves have similar characteristics but are distinctly separated from each other. Rye has a lower falling number than wheat at equal alpha-amylase activities. Normally, bakery-type wheat flour has a falling number between 200 and 250. When the falling number lies below 150, there is great danger that the bread crumb will be sticky. When the falling number is greater than 350, bread volume is diminished and a dry crumb results, unless the defect is balanced by the addition of malt.

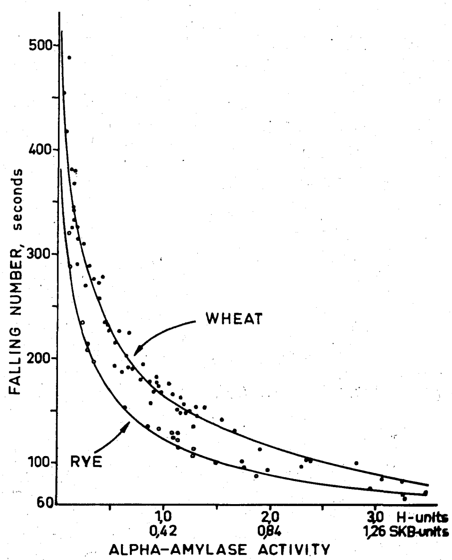

Fig. 4. Comparison between alpha-amylase activities (H-units and SKB-units) and "falling numbers" of wheat and rye.

The curvilinear relationship between the falling number and the alpha-amylase activity (Fig. 4) may be expressed as a straight-line function by converting the falling number into a liquefaction number (LN) as indicated by the empirical formula below:

$$\text{Liquefaction Number} = \frac{6{,}000}{\text{falling number} - 50}$$

In this equation, 6,000 is a constant. The number 50 corresponds approximately to the time in sec. required for the flour starch to gelatinize sufficiently so that it will be available for attack by the enzymes. The data in Fig. 5 show that the LN is proportional to the alpha-amylase activity over the range encountered in commercial flours. This range corresponds to falling numbers between 80 and 500. The correlation coefficient between liquefaction number and alpha-amylase activity for wheat is 0.975, and for rye 0.919.

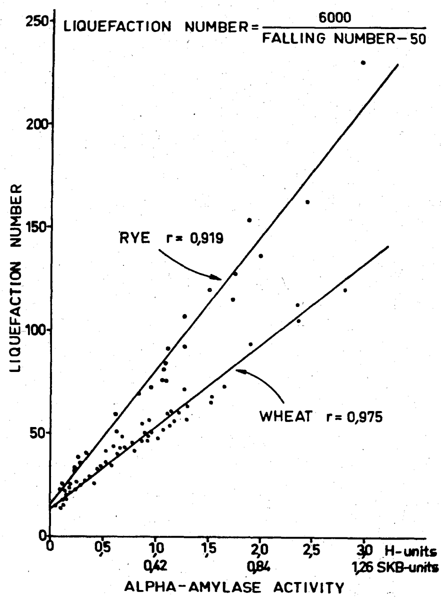

Fig. 5. Comparison between H-units (SKB) and "liquefaction numbers" for wheat and rye.

Figure 5 shows, somewhat more distinctly than Fig. 4, that rye starch was degraded to a greater extent than wheat starch at the same alpha-amylase activity. This observation led the author to the supposition that rye alpha-amylases have higher liquefaction than dextrinization activity compared to wheat alpha-amylases. It is also possible that wheat starch is more resistant than rye starch to alpha-amylase attack. Using the falling number method, wheat and rye enzymes, as well as wheat and rye starches, were compared under conditions similar to those encountered during baking. Analyses of the grains used for enzyme extraction are given in Table I. These data show that the relative activity of the alpha-amylases in wheat and rye is quite dependent on the method used for analysis.

TABLE I
ANALYTICAL DATA FOR WHEAT AND RYE GRAIN

	Ash	Protein	Alpha-Amylase Activity		Falling Numbers	Amylo-graph	Maltose Value
	%	%	*H-Units*	*SKB-Units*	*sec.*	*B.U.*	%
Wheat	1.56	12.0	2.17	0.87	131	55	3.0
Rye	1.45	8.0	2.07	0.83	103	190	3.0

As is evident from Fig. 6, there is no difference between the activities of wheat and rye amylases as determined by the falling number method. The dextrinization and liquefaction power of these amylases are the same. However, great differences exist among the various kinds of starches. Corn and wheat starches have considerably higher resistance to enzyme activity than do rye and potato starches. The falling number of rye starch is lower than that of wheat at equal amylase activities. This is caused by the lower resistance of rye starch to enzyme digestion and the fact that rye starch gelatinizes at lower temperatures than wheat starch. Consequently, the enzymes attack the gelatinized rye starch for a longer period of time before they are inactivated. These

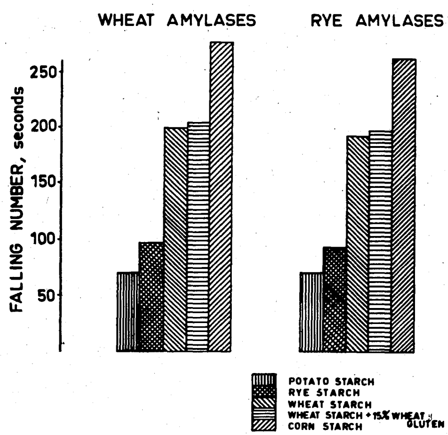

Fig. 6. Degradation effects of wheat and rye amylases on different starches according to "falling number" values.

data indicate the importance of determining simultaneously the alpha-amylase activity together with the enzymatic susceptibility of the flour starches. The experiment suggests also that gluten may not exert as large an influence on the starch paste as has often been assumed.

Preparation of Flour Mixtures with Desired Falling Number. The linear relation between the liquefaction number and alpha-amylase activity makes it possible to calculate arithmetically or graphically the composition of flour mixtures with a desired falling number.

Example. Grain No. 1 has a falling number of 80; therefore,

$$\mathrm{LN} = \frac{6{,}000}{80 - 50} = 200$$

Grain No. 2 has a falling number of 300; therefore,

$$\mathrm{LN} = \frac{6{,}000}{300 - 50} = 24$$

If the desired falling number of the mixture is assumed to be 150, the corresponding

$$\mathrm{LN} = \frac{6{,}000}{150 - 50} = 60$$

The percentage (x) of Grain No. 1 in the final mixture is calculated as follows:

$$\frac{x\,(200)}{100} = \text{LN contributed by Grain No. 1} \qquad (1)$$

$$\frac{100 - x\,(24)}{100} = \text{LN contributed by Grain No. 2} \qquad (2)$$

$$\frac{x\,(200)}{100} + \frac{100 - x\,(24)}{100} = 60 \qquad (3)$$

$$x = \frac{100\,(60 - 24)}{200 - 24} = \frac{3{,}600}{176} = 20.5\% \qquad (4)$$

Flour blends may be mixtures of wheat, or rye, or of wheat and rye. To assure correct computation, however, the components of each mixture must be ground equally fine. The data in Table II show the effect of mixing wheat and rye according to the liquefaction number. The linear relation between the liquefaction number and the composition

TABLE II

ENZYME ANALYSES FOR MIXTURES OF WHEAT AND RYE FLOURS

Wheat Flour	Rye Flour	Ash	Protein	Falling Number	Liquefaction Number	Amylograph	SKB Units
%	%	%	%			*B.U.*	
100	..	0.55	10.9	205	39	125	0.16
75	25			133	72	70	0.57
50	50			98	125	50	0.99
25	75			89	154	75	1.42
..	100	0.76	7.2	80	200	135	1.86

of the flour mixture for the data in Table II is evident from Fig. 7. The linear relation between the "liquefaction number" and malt addition, as is evident from Fig. 8, makes possible a graphical determination of the amount of malt supplement required for balancing the alpha-amylase activity in flour.

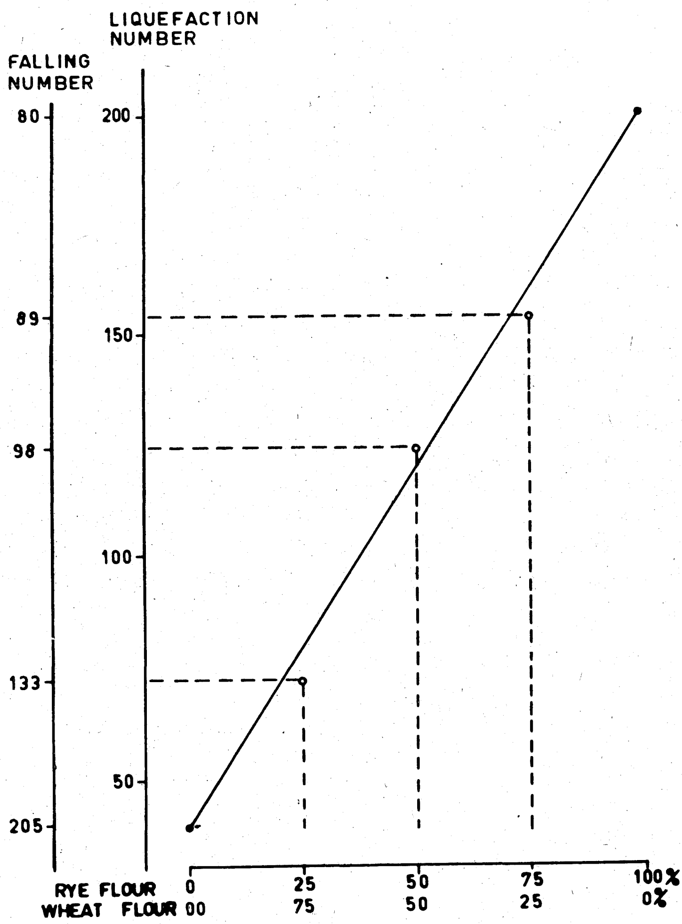

Fig. 7. Wheat and rye flour mixture relations based on liquefaction number.

The data show that wheat and rye starches are different with respect to their susceptibility to enzyme digestion. The resistance of the flour starch varies not only for different grains such as for wheat and rye, but also for one grain type (Fig. 9). Because of this difference it is necessary to determine the resistance of the flour starch against the malt used by two or more falling number determinations. Thereafter,

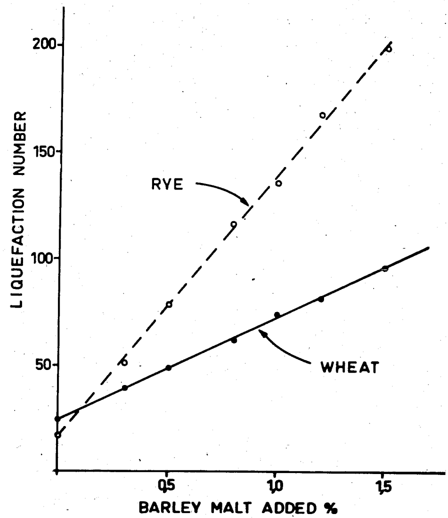

Fig. 8. Relation between liquefaction numbers and amount of malt supplement added to wheat and rye.

it is possible to determine graphically the amount of supplement required. To balance the amylase activity to the same falling number level (e.g., corresponding to liquefaction number 40), the amount of malt that needs to be added can be determined from the diagram. Australian wheat requires about 0.2% of a certain malt (Fig. 9), Swedish wheat about 0.3%, and Manitoba 0.4% in order to attain the

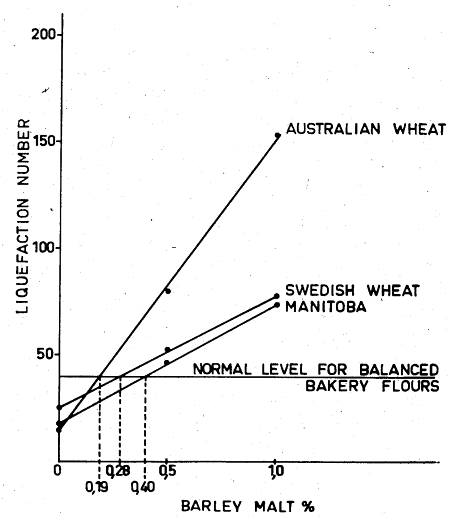

Fig. 9. Calculation of the desired amount of malt supplement based on liquefaction number.

same falling number. Among the wheats tested, the Australian wheat exhibited the lowest resistance to malt. All other wheat samples had resistances similar to those of Manitoba and Swedish wheat.

Choice of Methods for Determining Alpha-Amylase Activity. The method chosen for measuring alpha-amylase activity in grain products is very important since the fermentability of the dough (in formulas containing little sugar), as well as the quality of the bread crumb, depends upon alpha-amylase activity. The amylases convert flour starches into fermentable sugar and maltose, and the fermentation of the dough depends on the formation of sugar. Only that amount of sugar which is formed at temperatures between about 25° and 40°C. influences the fermentation process; therefore, the formation of maltose, which depends on the co-activity of alpha- and beta-amylases but which is mainly specific for the beta-amylase activity, must be measured at these temperatures. The quality of the bread crumb depends on the enzyme activity at temperatures between 55° and 80°C. (i.e., between the gelatinization of the starch and the inactivation of the enzymes). In this temperature range, the starch is hydrolyzed mainly into dextrins by alpha-amylase. The extent of the starch hydrolysis should be measured in the range above the gelatinization temperature, because gelatinized starch is more susceptible to attack by the enzymes.

Results obtained by different methods for determination of amylase

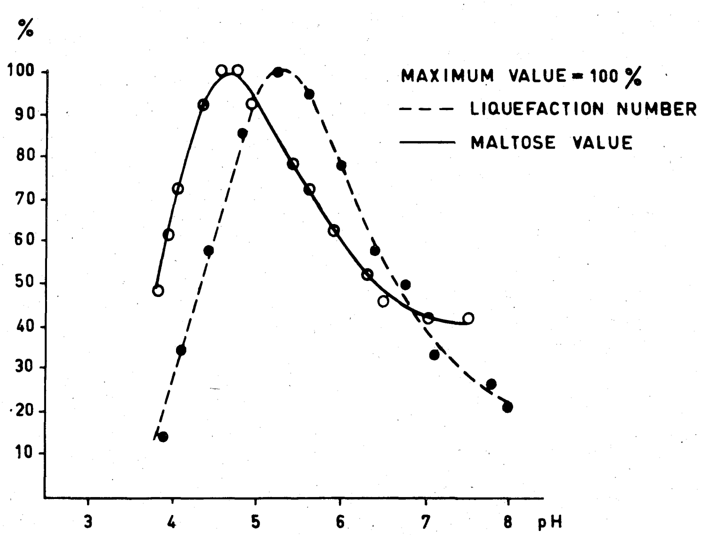

Fig. 10. Amylase activity in rye flour at different pH values according to liquefaction number (calculated by "falling number") and maltose value.

activity may vary, depending upon the nature and conditions of the measurements of alpha- or beta-amylase activity. For instance, the pH optimum for alpha-amylase activity lies within different ranges depending on the determination method. To illustrate the importance of pH, aqueous suspensions of rye flour (0.76% ash, 14% m.b.) were prepared and their pH values were adjusted to different values with lactic acid or sodium hydroxide. The falling numbers were determined as well as the maltose values at 27°C. according to the method of Berliner (2). The curves (Fig. 10) show that the pH optimum for alpha-amylase activity, according to the falling number method and maltose method, agrees with the alpha- and beta-amylase activities, respectively, as determined by Ohlsson and Uddenberg (28).

Conclusions

The influence of beta-amylase in baking is of minor importance to the quality of the bread crumb, since beta-amylase cannot attack undamaged or nongelatinized starch. The sugar formation by this enzyme at fermentation temperatures is substantially dependent on the amount of starch which was damaged by grinding. Furthermore, the enzyme is inactivated at relatively low temperature, often simultaneously with the occurrence of starch gelatinization. Beta-amylase affects only slightly the viscosity of the starch paste and cannot be measured by the falling number method.

The influence of alpha-amylase activity is of major importance in determining bread crumb quality. Alpha-amylase rapidly decreases the viscosity of the starch paste and hydrolyzes the starch to water-soluble dextrins. Alpha-amylase can be measured by the falling number method. Although the alpha-amylase influences sugar formation by preparing additional accessible points of attack for attack by beta-amylase, the amount of maltose which is produced by alpha-amylase itself may be considered negligible. Therefore, the alpha-amylase activity at temperatures higher than the gelatinization temperature cannot be predicted reliably from the amount of sugar formed at lower temperatures.

The formation of sugar is greatest in the acid range of pH where alpha-amylase is less active. Because alpha-amylase is highly sensitive to low pH, its activity may be easily decreased by addition of acid. The optimal pH range for alpha-amylase of cereal type is 5.2 to 5.4. In Sweden, where rye bread is baked mainly without leavening and often with rye flours having high alpha-amylase activity, a distinct improvement of the crumb quality is observed when lactic acid is added to adjust the pH to about 5.0.

Acknowledgment

The author gratefully acknowledges the technical assistance of B. Belenius, Mrs. K. Dalenius, Mrs. J. Lunin, and Mrs. M. Salaj. The author also expresses his appreciation to Kenneth A. Gilles and Byron S. Miller for their interest and assistance in the preparation of this report.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. Eckardt, O. Getreide und Mehluntersuchung, Kolorimetrische Maltosebestimmung nach Berliner, p. 58. Verlag M. Schäfer: Detmold (1957).
3. Rumsey, L. A. The diastatic enzymes of wheat flour and their relation to flour strength. Am. Inst. Baking. Bull. No. 8 (1922).
4. Biechy, T. Einfluss des Mehlalters auf den Verkleisterungsverlauf beim Roggenmehl. Mühlenlaboratorium **5:** 129–140 (1935).
5. Belderok, B. Een viscosimetrische micromethode tar bepaling van het gehalte aan alpha-amylase in graan on maalproducten. Chemisch Weekblad **52:** 649–654 (1956).
6. Blom, J., and Bak, A. Ueber die Bestimmung der Amylasen durch Verflüssigung von Stärkekleister. Z. Physiol. Chem. **256:** 197–207 (1938).
7. Brabender, C. W. Beiträge zur Strukturforschung von Weizen- und Roggenteigen. Bericht vom Nordisk Cerealkemikerföreningskongress, Oslo, pp. 52–70 (1937).
8. Anker, C. A., and Geddes, W. F. Gelatinization studies upon wheat and other starches with the amylograph. Cereal Chem. **21:** 335–360 (1944).
9. Hagberg, S. Some methods for determining the amylase activity of cereals and their mill products. Trans. Am. Assoc. Cereal Chemists **9:** 53–64 (1951).
10. Jozsa, S., and Johnston, W. R. Determination of alpha-amylase. Ind. Eng. Chem. (Anal. Ed.) **7:** 143–146 (1935).
11. Landis, Q., and Redfern, S. Methods for determination of alpha-amylase. III. Improved starch liquefying method. Cereal Chem. **24:** 157–168 (1947).
12. Hagberg, S. Modified Wohlgemuth methods for alpha-amylase activity of wheat and rye. Cereal Chem. **38:** 241–250 (1961).
13. Hoskam, E. G. Photometric determination of alpha- and beta-amylase in flour. Biochim. Biophys. Acta **1:** 419–427 (1947).
14. Jongh, G. Colorimetrische bepaling van alfa-amylase. Chem. Weekblad **53:** 597–602 (1957).
15. Bawden, R. F., and Artis, W. G. Use of a photoelectric photometer to obtain the end point in the alpha-amylase determination. Am. Soc. Brewing Chemists, Proc. **1951**, pp. 41–44.
16. Kneen, E., Sandstedt, R. M., and Hollenbeck, C. M. The differential stability of the malt amylases — separation of the alpha and beta components. Cereal Chem. **20:** 399–423 (1943).
17. Olerod, R. Eine kinetische Methode zur Bestimmung der Alpha-Amylaseaktivität in Brotgetreide. Getreide Mehl **9:** 49–52 (1959).
18. Redfern, S. Methods for determination of alpha-amylase. IV. A glass end point color standard for use in the dextrinizing method; effect of temperature and starch lot on this method. Cereal Chem. **24:** 259–267 (1947).
19. Sandstedt, R. M., Kneen, E., and Blish, M. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16:** 712–723 (1939).
20. Stone, I. A. A sensitive method for the determination of alpha-amylase activity of flour. Cereal Chem. **29:** 228–234 (1952).
21. Wohlgemuth, J. Ueber eine neue Methode zur quantitativen Bestimmung des diastatischen Ferments. Biochem. Z. **9:** 1–9 (1908).
22. Rotsch, A. Ueber die Bedeutung der Stärke für die Krumenbildung. Brot Gebaeck **7:** 121–125 (1953).
23. Hagberg, S. A rapid method for determining alpha-amylase activity. Cereal Chem. **37:** 218–222 (1960).
24. Hagberg, S. Note on a simplified rapid method for determining alpha-amylase activity. Cereal Chem. **38:** 202–203 (1961).

25. PERTEN, H. Bericht ueber die Resultate der ICC's Vergleichsuntersuchungen für Amylase-Aktivitätsbestimmungen. April-Maj 1961. Die Müllerei **16:** 95–97 (1963).
26. SANDSTEDT, R. M., and MATTERN, P. J. Damaged starch. Quantitative determination in flour. Cereal Chem. **37:** 379–390 (1960).
27. SULLIVAN, BETTY, ANDERSON, M. L., and GOLDSTEIN, A. M. The determination of starch damage of flour. Cereal Chem. **39:** 155–167 (1962).
28. OHLSSON, E., and UDDENBERG, C. E. Amylasen in ruhenden und keimenden Samen. II. Roggen. Hoppe-Seylers Zeitschr. f. Phys. Chemie **221:** H. 3 u. 4 (1933).