A Modified Falling-Number Method Suitable for Measuring Both Cereal and Fungal Alpha-Amylase Activity

H. PERTEN,[1] Buhler Brothers Ltd., CH-9240 Uzwil, Switzerland

Cereal Chem. 61(2):108-111

ABSTRACT

Cereal and fungal α-amylase were measured at 30°C by a modified falling-number method by replacing 50% of the flour with a pregelatinized wheat starch. Falling Number Amylase Activity units, calculated from modified falling number values, are highly correlated with the SKB values for both fungal and cereal amylases.

The kind and amount of amylases in flour are important in baking. Amylases in doughs provide fermentable sugar for yeast and increase gassing power in doughs that are deficient in sugar. Only the sugar that is in the formula or that is produced by amylolytic action at 25–45°C influences fermentation, because yeast activity ceases at 45–50°C.

Both cereal and fungal α-amylases can be used to improve fermentation of flour deficient in amylase activity. The risk of overdose is small for fungal amylase, because it is rapidly inactivated at a relatively low temperature, usually before flour starch is gelatinized. During baking, at temperatures greater than 60°C, degradation of starch to dextrins depends on the amount and thermal stability of α-amylase. Excess cereal α-amylase causes excessive dextrinization, resulting in a wet and sticky bread crumb characteristic of bread produced from sprouted wheat.

To monitor α-amylase supplementation of flours, a method that will measure both cereal and fungal amylases is needed. The falling number (FN) method, internationally standardized by ICC (method 107) (1968), AACC (method 56-81B) (1972), and ISO (method 3093) (1975), is accepted for assessing cereal α-amylase activity. Because fungal α-amylase has low thermostability, it cannot be detected by the standard FN method at 100°C. Pomeranz and Shellenberger (1962, 1963) discussed the liquefying action of fungal α-amylase on pregelatinized starch in the amylograph, and Ranum et al (1978) developed an amylograph method for routine determination of fungal α-amylase in wheat flour. Sprössler (1982) detected the addition of fungal amylase in flours by a modified falling number (MFN) method at 30°C by replacing part of the flour with pregelatinized starch.

Studies of the MFN method used by Sprössler (1982) have demonstrated the importance of standardizing the substrate and the procedure. This article describes a standardized MFN method and the calculation of α-amylase activity of cereal and fungal enzyme preparations by that method.

[1]Chairman of the study group Determination of Amylase Activity and Sprouting, International Association for Cereal Chemistry, Vienna, Austria.

MATERIALS AND METHODS

A Swedish wheat flour was analyzed for ash (0.59% d.b.), protein (10.5% d.b., N × 5.7), and FN 350, by ICC methods 104, 105, and 107, respectively.

The substrate used was made available by Falling Number AB, Stockholm, Sweden, and consisted of pregelatinized wheat starch (moisture content 6.5%), protein (0.7%, N × 5.7 d.b.), ash (0.24% d.b.), and fat (0.3%, d.b.). All particles were smaller than 500 μm, and 75% were smaller than 180 μm. Other pregelatinized starches can be used, with proper standardization.

Enzyme Preparations and Flour Samples Containing Added Enzyme

Three commercial fungal amylase preparations identified as A, B, and C, and a barley malt identified as D were analyzed for

α-amylase activity by ICC colorimetric method 108. The ICC values were converted to SKB (Sandstedt et al 1939) values using the factor 0.11 (Perten 1966). Mixtures of wheat flour and each enzyme preparation were prepared to contain approximately the same α-amylase activity, based on SKB values. The range of enzyme concentrations in the flours is shown in Table I.

Standard FN Method

α-Amylase activity of flour-enzyme preparations was determined at 100°C, using 7 g of flour and 25 ml of water.

MFN Method

The proposed MFN method determines α-amylase activity at 30°C, using 7 g of a 50:50 mixture of flour and substrate and 25 ml of distilled water tempered to 30°C.

The Falling Number 1400 apparatus with a water bath at 30 ± 0.2°C was used. Flour (3.5 ± 0.01 g) and substrate (3.5 g ± 0.01 g) were weighed separately. Twenty-five milliliters of distilled water at 30 ± 0.2°C was added to a dry falling-number tube. Flour was added and mixed with water by shaking 10–20 times. Substrate was added, and the test tube closed with a rubber stopper. A stopwatch was started and the tube shaken up and down vigorously for 5 sec. The substrate absorbed water rapidly. The upper part of the tube was scraped down with a viscometer stirrer and the test tube placed into the water bath. Exactly 30 sec after the start of mixing with the substrate, the FN apparatus was started.

The remainder of the procedure is the same as that used for the standard FN method. The total time, in seconds, from when the apparatus is started until the stirrer drops down is the MFN.

RESULTS AND DISCUSSION

The MFN method measures α-amylase activity at the temperature of dough fermentation (30°C), using pregelatinized wheat starch as substrate. Results are quite different from those obtained with the standard FN method.

Figure 1 shows the activity of the enzyme preparations determined by the standard FN method at 100°C. Fungal enzymes A and C had no significant effect on FN values. However, fungal enzyme B is apparently more heat resistant than enzymes A and C, and produced lower FN values. Addition of 0.3% malt flour, corresponding to about 1.3 SKB units per 7 g of flour, caused a drop in FN value from 350 to 235.

TABLE I
Alpha-Amylase Activity of Commercial Enzyme Preparations and Range of Enzyme Concentrations Used in Flour Samples to Obtain Equivalent Alpha-Amylase Activity

Enzyme	ICC Units	SKB Units[a]	Range of Enzyme Concentration in Flour Samples	
			Percent (w/w)	SKB Units per Gram
A (fungal)	4,760	524	0.05–0.5	0.26–2.6
B (fungal)	9,560	1,050	0.02–0.2	0.21–2.1
C (fungal)	445,000	49,000	0.0005–0.005	0.25–2.5
D (barley malt)	565	62	0.3–3.0	0.19–1.9

[a]SKB units = ICC units × 0.11 (Perten 1966).

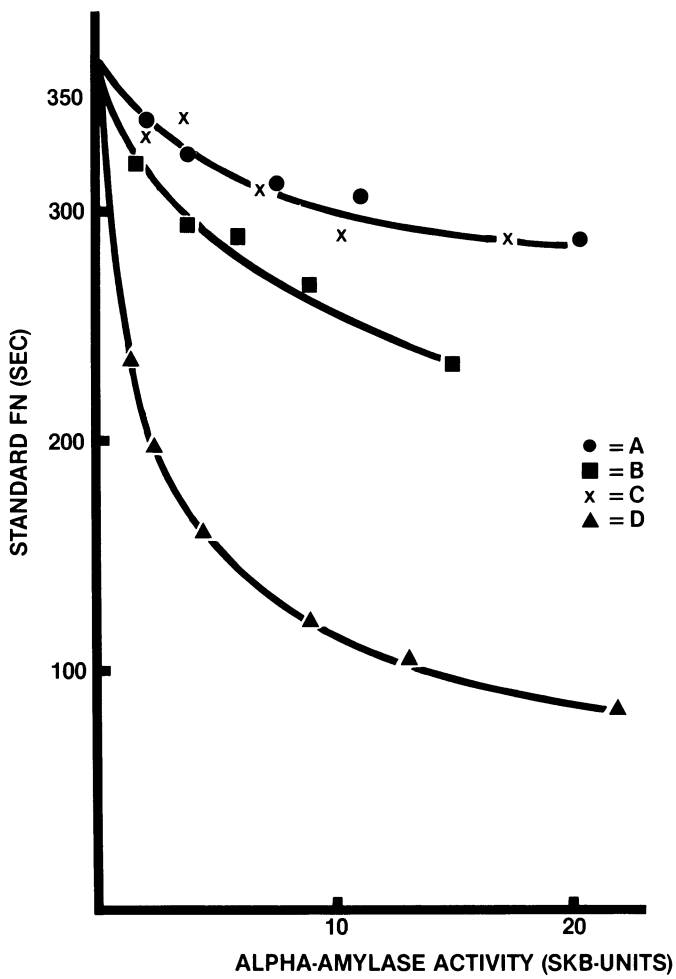

Fig. 1. Standard falling number at 100°C with 7 g flour versus α-amylase activity for enzyme preparations A–D.

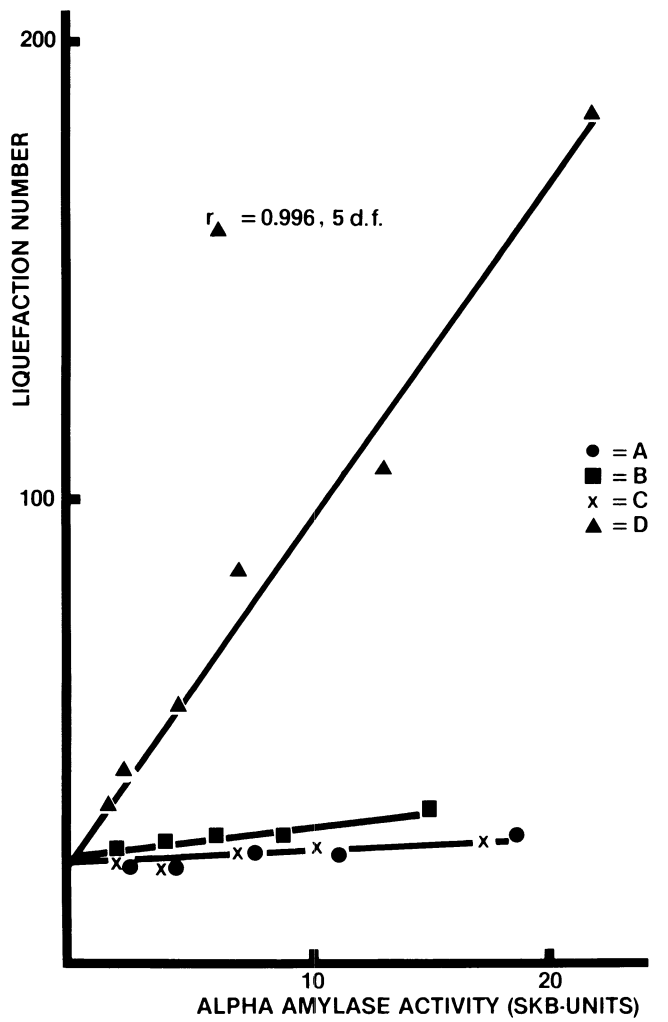

Fig. 2. Liquefaction number versus α-amylase activity for enzyme preparations A–D.

The curvilinear relationship between FN and α-amylase activity can be expressed as a straight-line function (Fig. 2) by converting the FN values to liquefaction number (LN) by the empirical formula (Perten 1964):

$$\text{Liquefaction number (LN)} = \frac{6{,}000}{\text{FN}-50}$$

In this equation, 6,000 is a constant. The number 50 is the approximate time, in seconds, required for flour starch to gelatinize enough to be made susceptible to attack by α-amylase. The LN shows, more clearly than the FN value, the large difference between cereal and fungal α-amylases measured at 100°C. The correlation coefficient between LN and α-amylase activity for cereal α-amylase (enzyme D) was highly significant (r = 0.996, 5 degrees of freedom).

TABLE II
Calculation of FNAA[a] Values for Enzyme Preparation A

Milligrams of Enzyme in 3.5 g of Flour	Modified Falling Number	Viscosity Number	FNAA[b]
0	279	35.8	...
1.75	232	43.1	4,171
3.5	206	48.5	3,629
7.0	167	59.9	3,443
10.5[c]	135	74.1	3,648
17.5	106	94.3	3,343
Mean			3,647

[a] Falling number amylase activity units.
[b] Coefficient of variation, 7.8; standard deviation, 286.
[c] Sample calculation: $\text{FNAA} = (\text{VN}_x - \text{VN}_o)/(S_x - S_o) \times 1{,}000 = (74.1 - 35.8)/(10.5 - 0) \times 1{,}000 = 3{,}648$, where VN_x value represents 10.5 mg of enzyme and VN_o value represents 0 mg of enzyme.

MFN values for all four enzyme preparations are curvilinearly related to SKB values (Fig. 3). The curvilinear relationship may be expressed as a straight line (Fig. 4) by converting MFN values to viscosity numbers (VN) by the following formula:

$$\text{VN} = \frac{10{,}000}{\text{MFN}}$$

As pregelatinized starch can be attacked immediately by α-amylase, no constant needs to be subtracted from the MFN, as is the case with calculation of LN. The correlation coefficient between VN and α-amylase activity (SKB units) was highly significant (r = 0.97, 18 d.f.).

The straight-line relationship between VN and SKB units makes it possible to calculate the α-amylase activity in terms of falling-number amylase-activity units (FNAA), when the amount of enzyme is known:

$$\text{FNAA} = \frac{\text{VN}_x - \text{VN}_o}{S_x - S_o} \times f$$

where VN_x = any VN value for a sample containing a known amount of enzyme; VN_o = VN value for a sample containing no added enzymes. Samples with a known quantity of enzymes lower than that in the sample used to obtain VN_x can be used in place of VN_o; S_x = milligrams of enzyme in sample VN_x for calculation; S_o = sample without enzyme addition, or milligrams of enzyme in sample with a smaller enzyme addition than VN_x chosen for calculation; and f = 1,000, used to express the FNAA for 1 g of enzyme.

A sample calculation of FNAA for enzyme preparation A is given in Table II.

The reliability of MFN determinations can be evaluated by plotting VN values on ordinary graph paper against the amount of enzyme added (Fig. 5).

Table III indicates a close relationship between FNAA and SKB values for all four enzyme preparations.

MFN values depend mostly on the substrate. Although other pregelatinized starches can be used, the weight of the substrate is to be adjusted so that the MFN values of low α-amylase activity flours

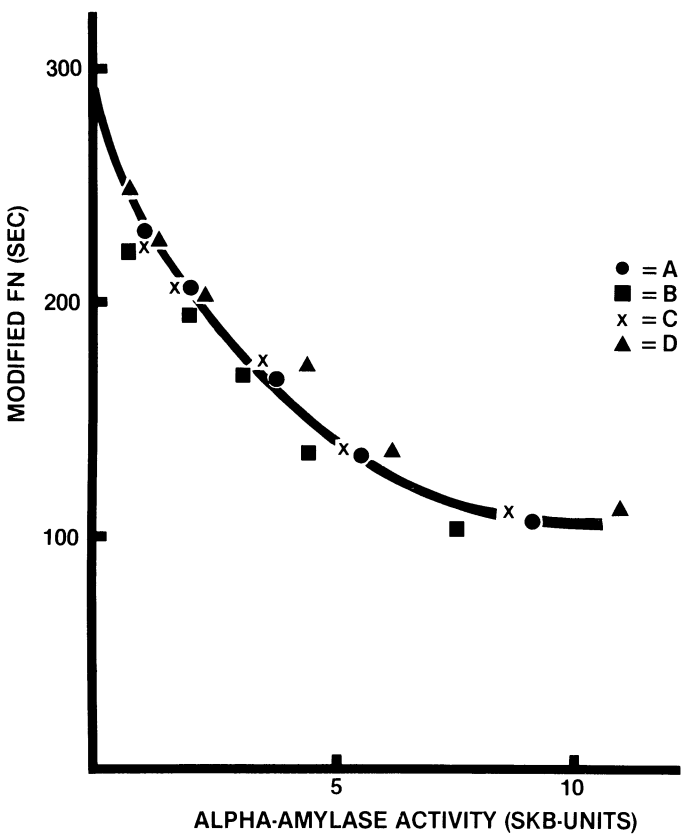

Fig. 3. Modified falling number at 30°C with 3.5 g flour versus α-amylase activity for enzyme preparations A–D.

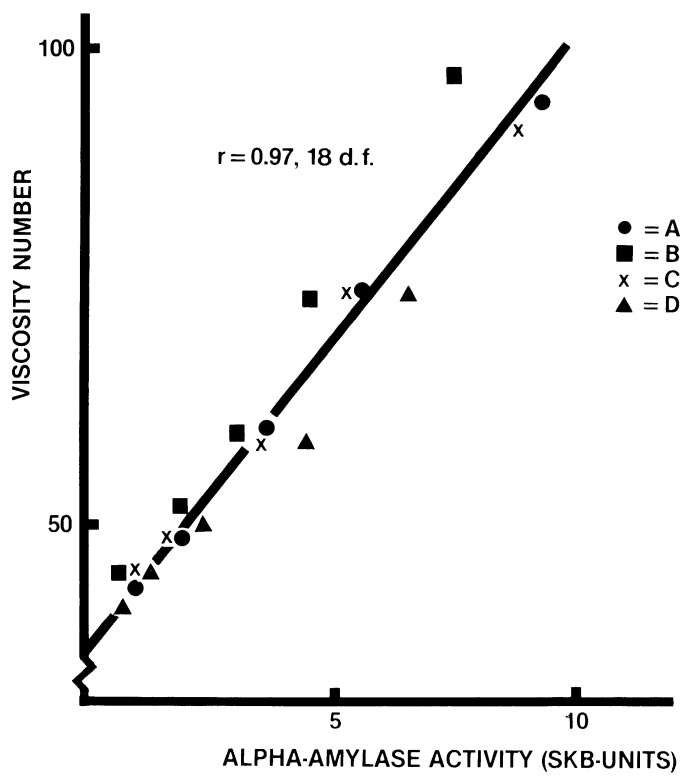

Fig. 4. Viscosity number versus α-amylase activity for enzyme preparations A–D.

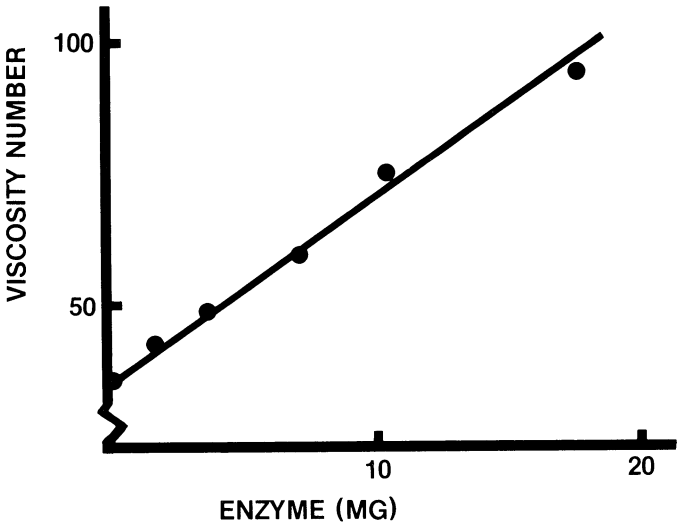

Fig. 5. Relation between viscosity number and milligrams of enzyme added to 3.5 g of flour.

TABLE III
Relationship Between FNAA[a] and SKB Values

Enzyme	Alpha-Amylase Activity		
	FNAA Value	SKB Value	FNAA/SKB
A	3,650	524	6.96
B	8,940	1,050	8.51
C	337,700	49,000	6.89
D	405	62	6.53

[a] Falling number amylase activity units.

fall in the range of 200–300. MFN values are not comparable when different types of substrates are used.

Substrate/flour/water mixing time before the start of the falling number apparatus and temperature of the reaction also significantly influence the results. Changing the starting time of the falling number apparatus after mixing the substrate with flour/water mixture, from 15 to 45 sec (standard is 30 sec) may decrease MFN values to about 25%, and changing water temperature from 30 to 20°C may increase MFN values to about 20%. The degree of starch damage in the flour has no effect on MFN values, apparently because of the short reaction time of the method.

To correctly estimate the optimum level of α-amylase in flour, the α-amylase should be measured at both fermentation and baking temperatures. Because of the high temperature employed (100°C), the standard FN method is not capable of predicting the activity of heat-sensitive fungal α-amylases, particularly their activity at fermentation temperatures. It is also questionable whether methods measuring α-amylase activity below the starch gelatinization temperature can correctly predict the effect of α-amylase under baking conditions, which depends on enzyme inactivation temperature and the resistance of gelatinized flour starch to heat.

Optimum α-amylase activity can be determined by using both the MFN method (at 30°C) and the standard FN method (100°C). Desirable standard FN values should fall between 200 and 300 for bakery flours. A range of desirable MFN values for optimum fungal amylase supplementation of flour remains to be determined by practical experience.

ACKNOWLEDGMENTS

I thank Bo Allvin and Paul Tonkin, Falling Number AB, Stockholm, Sweden, for technical assistance and Bruno Sprössler Rohm GmbH, Darmstadt, W. Germany for supplying the substrate and for technical discussions.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1972. Approved Methods of the AACC. 7th ed. Method 56-81B, approved November 1972. The Association, St. Paul, MN.

INTERNATIONAL ASSOCIATION FOR CEREAL CHEMISTRY. 1968. Standard Methods of the ICC. Method 104, approved 1960; Method 105, approved 1980; Method 107, approved 1968; and Method 108, approved 1968. Verlag Moritz Schäfer, Detmold, West Germany.

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION. 1974. International Standard ISO 3093, approved 1974. The Secretariat: Magyar Szabvanyügyi Hivatal, 1450 Budapest 9, Pf. 24, Hungary.

PERTEN, H. 1964. Application of the falling number method for evaluating alpha-amylase activity. Cereal Chem. 41:127.

PERTEN, H. 1966. A colorimetric method for the determination of alpha-amylase activity (ICC method). Cereal Chem. 43:336.

POMERANZ, Y., and SHELLENBERGER, J. A. 1962. Starch-liquefying activity of amylase. Use of pregelatinized wheat starch as substrate. Cereal Chem. 39:327.

POMERANZ, Y., and SHELLENBERGER, J. A. 1963. Effects of alpha-amylase on pregelatinized starches, as measured by the amylograph. J. Sci. Food Agric. 14:145.

RANUM, P. M., KULP, K., and AGASIE, F. R. 1978. Modified amylograph test for determining diastatic activity in flour supplemented with fungal alpha-amylase. Cereal Chem. 55:321.

SANDSTEDT, R. M., KNEEN, E., and BLISH, M. J. 1939. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. 16:712.

SPROSSLER, B. 1982. Neue analytische Methoden für Enzyme in Mehl. Die Mühle 119:425.

[Received December 7, 1982. Accepted October 10, 1983]