MODIFIED AMYLOGRAPH TEST FOR DETERMINING DIASTATIC ACTIVITY IN FLOUR SUPPLEMENTED WITH FUNGAL α-AMYLASE

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ABSTRACT

A modified amylograph test, which uses a slurry of wheat flour and pregelatinized wheat starch as the substrate, was used to determine the diastatic activity of flours supplemented with fungal α-amylase. The difference in amylograph curves permitted distinction between fungal and cereal sources of the enzyme. The modified amylogram of flours with fungal α-amylase showed a minimum viscosity, which was inversely proportional to the amount of added enzyme. Modified amylograms of flours supplemented with cereal α-amylase (barley malt) exhibited an equivalent minimum viscosity, which was directly related to the peak viscosity determined by the conventional amylograph procedure. The present method is also suitable for testing α-amylase activity in wheat, malt, and enzyme preparations. Among the factors affecting the procedure are the type and amount of the pregelatinized wheat starch used, the level of starch damage of the flour, and the pH of the slurry.

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Fungal α-amylase preparations derived from the mold *Aspergillus oryzae* are becoming increasingly popular for supplementing the diastatic activity of flour. One difficulty in using this enzyme has been the lack of a convenient assay method for estimating its level in flour.

Viscosimetric procedures, such as the amylograph and falling number test, are often the methods of choice in the milling industry for measuring diastatic activity. These procedures are autolytic in the sense that the flour contains both the enzyme and the substrate. The latter is gelatinized wheat starch produced by heating native starch granules present in the flour slurry. Cereal amylases are more heat stable than those of fungal origin. Consequently, they retain activity above the starch gelatinization range and can use the starch of flour as substrate in these assays. On the other hand, fungal α-amylase loses activity before the starch gelatinizes and becomes susceptible to amylolysis.

Pomeranz and Shellenberger (1,2) showed that fungal α-amylase will produce a liquefying action on pregelatinized starch in the amylograph, and suggested this as a basis for estimating α-amylase activity in various fungal enzyme preparations. This approach was used in the present study, the objective of which was to develop a procedure suitable for routine determination of fungal α-amylase in wheat flour.

MATERIALS AND METHODS

Flour and Enzymes

A commercially milled untreated baker's flour (protein, 12.0% [N × 5.7]; ash, 0.47%) was supplemented with varied levels of either barley malt (Pillsbury

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Company) or fungal α-amylase (Doh-Tone, Pennwalt Corporation). The malt and fungal α-amylase preparation were added to flour at levels of 25, 50, 75, 100, and 200 SKB units/lb (3). Other types of wheat flour used in the study are identified in the text.

Pregelatinized Wheat Starch

The pregelatinized wheat starches used were Nesco Gel Starch (New Era Milling Company, Arkansas City, KS) and CWS-1600 Starch (Midwest Solvents, Hutchinson, KS). Both products gave similar results. Pregelatinized wheat starch for this procedure is available from Pennwalt Corporation, Broadview, IL.

Diastatic Activity Tests

Conventional amylograph, falling number, maltose value, and gassing power tests were done according to AACC approved methods (4).

Modified Amylograph Procedure

A mixture of 70.00 g of pregelatinized wheat starch (8% moisture) and 30.0 g of flour to be tested were gradually added over a period of 1.5 min, to 460 ml of the dilute pH 4.3 amylograph buffer (4) while mixing with a three-speed portable egg beater. This was done in a 1-L stainless steel beaker. Mixing was performed at low speed for the first minute, at medium speed for the next half minute, and at high speed for the final minute, giving a total mixing time of 2.5 min. The slurry

TABLE I
Diastatic Activity Test Results on Hard Wheat Baker's Flour Having Various Levels of Added α-Amylase Activity From Either Barley Malt or Fungal Sources

α-Amylase Added (SKB/lb)	Enzyme Source	Falling Number (sec)	Maltose Value (mg/10 g)	Gassing Power 5th Hr (mm Hg)	Regular Amylograph PV[a] (BU)	Modified Amylograph MV or EMV[b] (BU)
0		410	282	412	1,320	650
25	Malt	338		477	660	300
	Fungal	370		458		300
50	Malt	277	373	496	480	195
	Fungal	388	334	464	1,210	195
75	Malt	262		504	395	140
	Fungal	373		472		130
100	Malt	218	438	516	320	95
	Fungal	383	353	490	1,230	100
200	Malt	182	512	544	220	50
	Fungal	396	353	509	1,235	65

[a]PV = peak viscosity.
[b]MV = minimum viscosity, EMV = equivalent minimum viscosity.

was transferred to the amylograph bowl with the help of a rubber spatula. The amylograph, with a 700 cm g sensitivity cartridge, was set at 25°C starting temperature, the pen adjusted to a zero time line, and the clutch set at the temperature increase position. The amylograph was started 5 min after the start of mixing the slurry. The amylograph pen was held by hand and eased over when starting the amylograph to prevent damage to the machine and ink from splattering. The timing of operations was controlled to assure a uniform reaction time.

Enzyme Extraction

A fungal α-amylase preparation (100 mg) or barley malt (300 mg) were extracted at 30° C with 100 ml of a solution containing 0.5% NaCl and 0.02% $CaCl_2$. The solution was then filtered and diluted to contain 0.5 SKB units/ml. Aliquots of the extract were added to the concentrated buffer (46 ml), which was then diluted with water to a volume of 460 ml.

RESULTS AND DISCUSSION

Diastatic Activity Tests

As is evident from the results given in Table I, both the conventional amylograph and falling number tests fail to respond to increasing levels of added

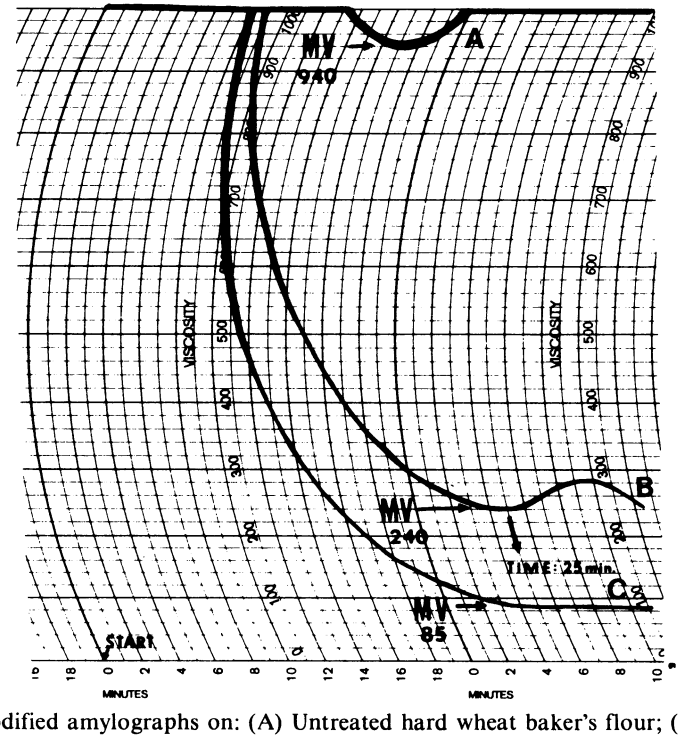

Fig. 1. Modified amylographs on: (A) Untreated hard wheat baker's flour; (B) normal fungal α-amylase–treated flour (63 SKB units/lb); (C) high fungal α-amylase–treated flour (175 SKB units/lb). MV = minimum viscosity.

fungal α-amylase. The maltose values show a slight response, but the trend is of an insufficient magnitude to be useful for mill control purposes. The values of fifth-hour gassing power are lower for fungal α-amylase than for a comparable malt amylase activity. Of these tests, only the gassing power would be useful as an index for controlling the addition of fungal α-amylase to flour.

Modified Amylograph Test

Fungal amylases. A typical modified amylogram of a flour supplemented with fungal enzyme is illustrated in Fig. 1. The high initial viscosity decreases rapidly during heating by the liquefying action of α-amylase on the pregelatinized wheat starch and partly by the viscosity-reducing effect of rising temperature. The amylograms of untreated flour (curve A) and those containing normal levels of fungal α-amylase (curve B) reach a minimum, called minimum viscosity (MV), and then start coming back. This increase in viscosity is due to the gelatinization of the starch component in the 30 g of tested flour. Amylograms with high levels of α-amylase (curve C) indicate an extensive reduction of paste viscosity, but the reversion of the curve subsequent to the MV point is not present, probably due to the low level of sensitivity of the instrument at this region. Instead, only a plateau is observed.

The MV for fungal α-amylase treated flours, which normally occurs after 22 to 26 min (58–64° C) was found to be a useful reference index for estimating α-amylase activity. This point is relatively easy to read; as shown in Table I, it is inversely correlated with the level of added α-amylase activity. Figure 2 demonstrates this relationship to be linear when the log of MV is plotted against

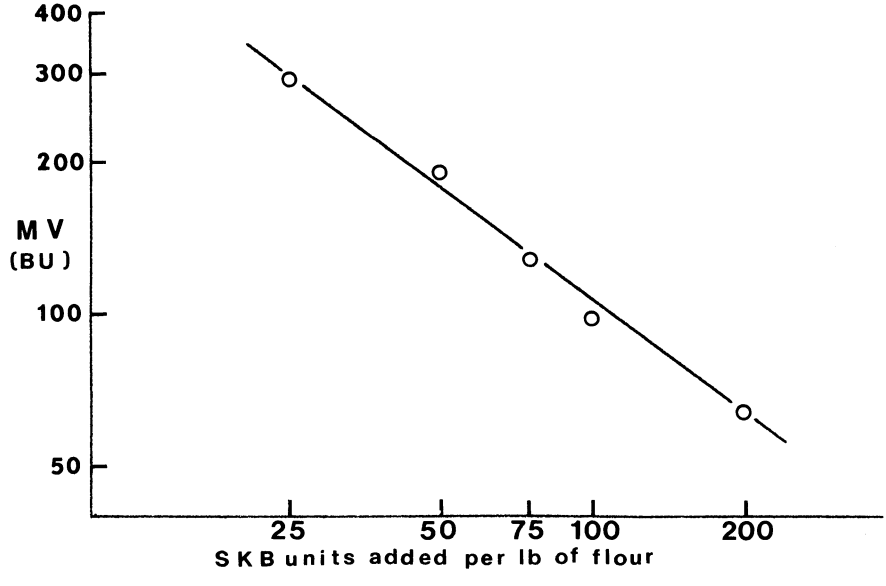

Fig. 2. Relationship between minimum viscosity (MV) of modified amylograph test and amount of added fungal α-amylase activity is linear when plotted on log-log scale.

the log of added α-amylase activity. The same correlation was reported (5) to exist between the peak viscosity in a conventional amylogram and α-amylase activity. This log-log relationship makes the MV, like peak viscosity, sensitive to small amounts of α-amylase. As the level of α-amylase increases, the sensitivity of the tests drops.

Cereal amylase. A minimum viscosity point does not generally occur with flours supplemented with barley malt (Fig. 3). Instead, a plateau (A) or a change in slope of the curve (B) are detected at approximately the same time as the MV point would be expected for fungal α-amylase–treated flours. The reason for this difference is the high residual activity of the cereal α-amylase at this temperature range, which is able to degrade the gelatinizing starch from the 30 g of flour while the fungal α-amylase is nearly inactive at this point.

The plateau viscosity can be considered an equivalent minimum viscosity (EMV), since its value is nearly identical to the MV for equal levels of added α-amylase activity as is evident from Table I. The EMV can be used as an index of α-amylase activity in the same manner as the MV for fungal α-amylase–treated flours.

A direct linear relationship exists between EMV and the peak viscosity (PV) of the conventional amylogram, permitting an interconversion of EMV and PV values. The approximate formula for this transformation is: $PV = 2MV + 100$.

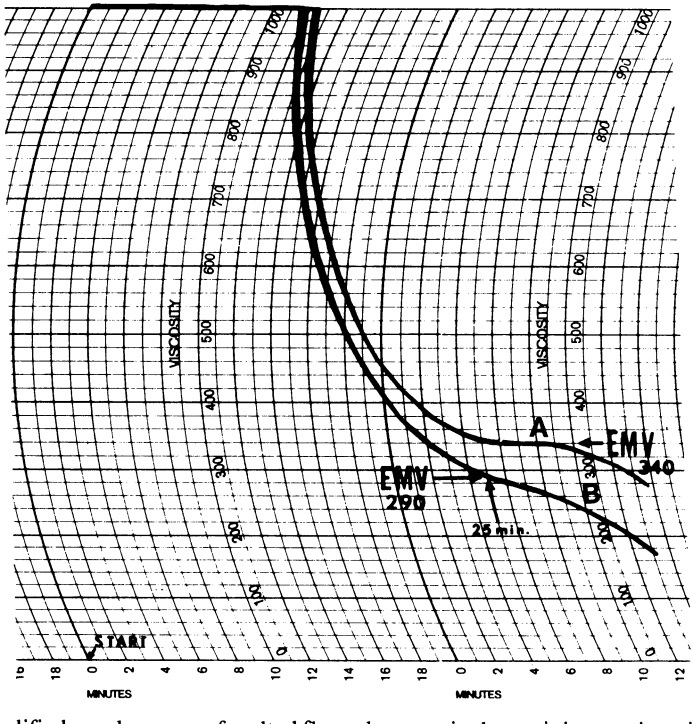

Fig. 3. Modified amylograms of malted flour show equivalent minimum viscosity (EMV), which may be in form of plateau (A) or inflection point (B).

Care should be taken in applying this formula to other flours, since it may vary with the flour. For the same flour, however, the MV (for fungal α-amylase) equals EMV (for cereal α-amylase) at the same activity level of added α-amylase, and both values can be converted to the PV, applying an appropriate conversion formula. For example, for the flour used in Table I, a fungal α-amylase treatment giving an MV of 200 BU is expected to correspond to a malted flour showing peak viscosity of 500 BU.

The fact that modified amylograms of fungal α-amylase–treated flour differ in appearance from those of malted flours can be used as a means of detecting the source of enzyme present. The enzyme type used can be verified further by running a conventional amylograph test with the flours. If it shows a high peak viscosity characteristic of untreated flour along with a low MV, one can conclude that it was treated with an enzyme of fungal source.

Modified Amylograms of Different Types of Flours

The modified amylograph test can be applied to determine the level of natural α-amylase activities in untreated flours. The MV range of untreated flours is from 500 to 1,500 BU. The test can also be used on ground wheat samples (Table II) to get an indication of the degree of sprout damage in the wheat.

The MV of a treated flour depends on both the natural and added levels of α-amylase activities of flours. Low natural activities in flours (indicated by high MV values) are expected to affect the MV values only slightly. Untreated flours showing MV values of 650, 710, 850, 965, and 1,100 BU required additions of 45, 64, 75, 75, and 75 SKB units of fungal α-amylase, respectively, to achieve an MV of 200 BU. These results suggest that an MV of 850 BU or more is needed to assume that the contribution of the natural activity of flour is negligible.

Modified amylograms of soft wheat flours are shaped differently from those of hard wheat flours (Fig. 4). At comparable levels of added α-amylase, the MV values of soft wheat flours are higher than are those of hard wheat flours and the viscosity buildup after the MV is more pronounced. Both differences can be attributed to variations in the degree of starch damage. α-Amylase is known not

TABLE II
α-Amylase Activity in Wheat[a]

Crop Year	Grade[c]	Falling Number (sec)	Modified Amylograph MV or EMV[b] at 25 min (BU)
1973	CW-1	479	985 (MV)
1973	CW-2	391	800 (MV)
1973	CW-3	234	200 (EMV)
1974	CW-1	379	710 (MV)
1974	CW-2	315	380 (EMV)
1974	CW-3	284	330 (EMV)
1974	CW-1	402	680 (MV)
1974	CW-2	293	300 (EMV)

[a]Wheat samples ground according to AACC falling number method (4).
[b]MV = minimum viscosity, EMV = equivalent minimum viscosity.
[c]CW = Canadian Wheat grading code.

to attack native starch granules readily, but it will degrade damaged starch (6). The higher the proportion of damaged starch in the flour, the more it is degraded during the amylograph test and the less of it can contribute to the viscosity buildup. Since hard wheat flours generally contain more damaged starch than do soft wheat flours, flours from hard wheat show less viscosity buildup. A fungal α-amylase–treated, double pin-milled hard wheat flour, which contains a high level of damaged starch, shows a modified amylogram pattern similar to that of malted hard wheat flour with no clearly defined MV (Fig. 4).

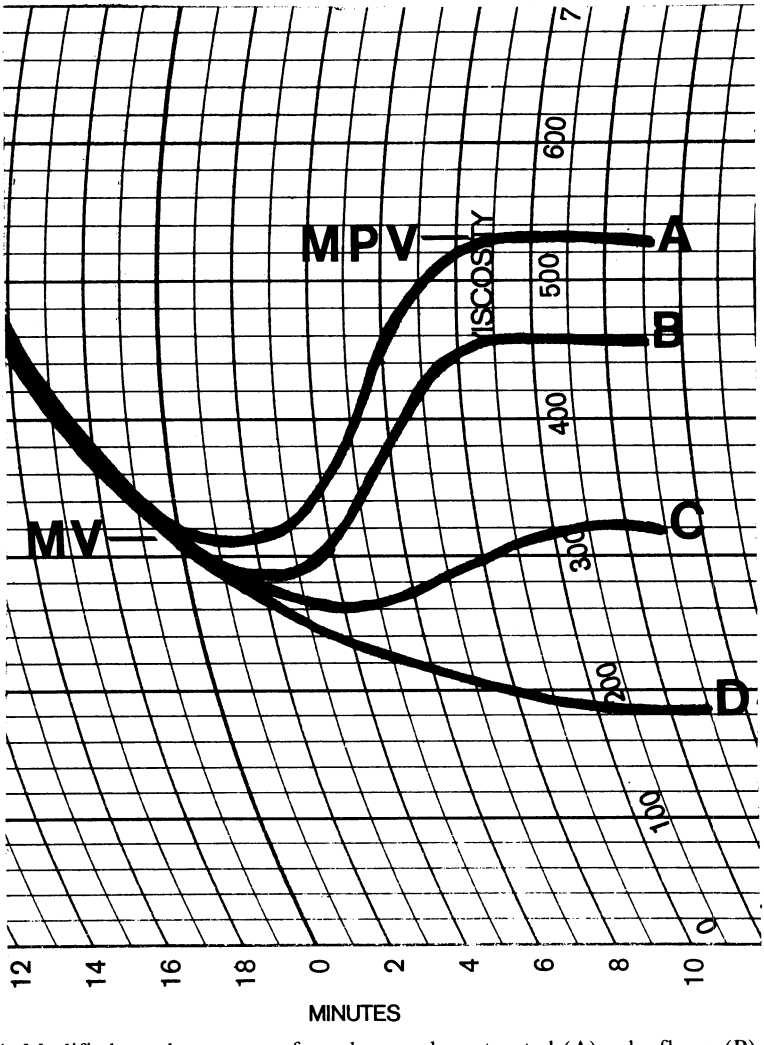

Fig. 4. Modified amylograms on fungal α-amylase–treated (A) cake flour, (B) cookie-cracker soft wheat flour, (C) bread flour, (D) double pin-milled hard wheat flour. MV = minimum viscosity, MPV = modified peak viscosity.

Modified Amylograph on Enzyme Extracts

The level of α-amylase activity in various enzyme preparations can be estimated by the modified amylograph procedure with use of a standard curve.

The enzyme is added as an extract, as described in the methods section, and the test is then run in the normal manner using an untreated flour of low diastatic activity (an MV over 850 BU). The standard curve is prepared by running various aliquots of a standard enzyme preparation containing a known amount of α-amylase activity. Examples of standard curves, plotted on a log-log scale, are shown in Fig. 5. The activity in an unknown enzyme source can then be determined from its MV value by comparison with the standard curve.

Factors Influencing Modified Amylograph Results

The results of a ruggedness test (7) run on the modified amylograph test are shown in Table III. Of the seven factors tested, weight of the starch, starting

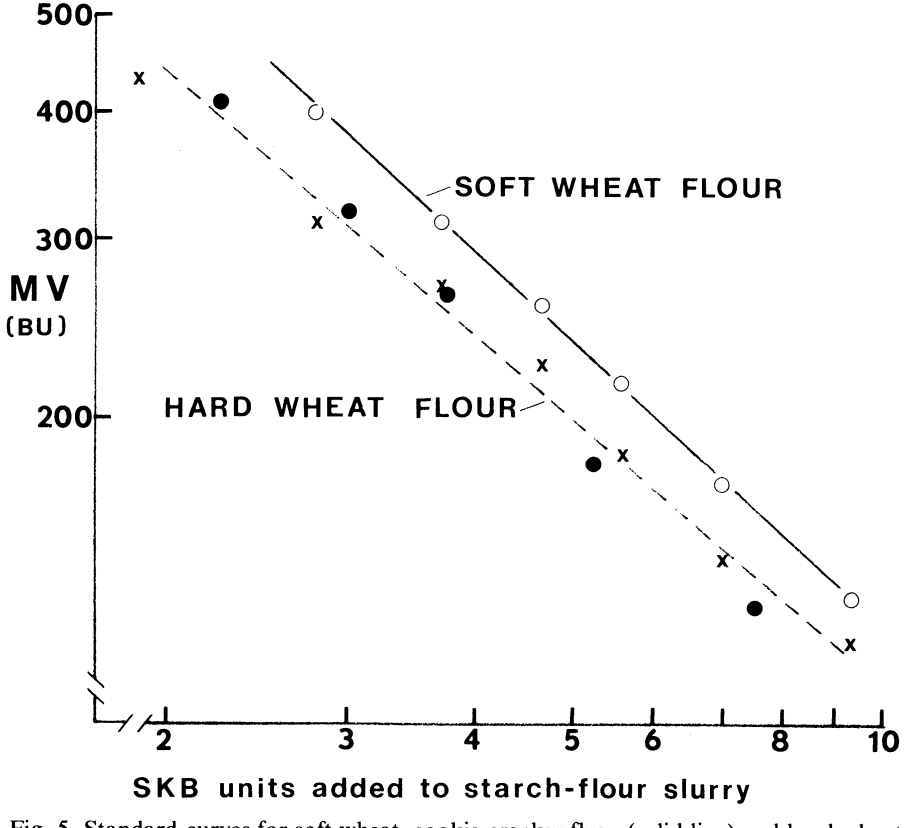

Fig. 5. Standard curves for soft wheat, cookie-cracker flour (solid line) and hard wheat bread flour (dashed line) made by adding extracts of fungal α-amylase preparation to amylograph buffer solution. Two fungal enzyme standards are shown, one containing 4,665 SKB units/g (X and O) and one containing 53,900 SKB units/g (•). MV = minimum viscosity.

temperature, and pH had significant effects.

Using the correct type and amount of pregelatinized wheat starch is important. Samples of pregelatinized wheat starch from four different manufacturers were run with the same flour treated with fungal α-amylase. One sample of pregelatinized wheat starch failed to show an MV; the other three gave typical curves but differed in the MV value obtained. The inherent paste consistency of the starch, which may account for these differences, can be controlled by an amylograph method which uses 40.0 g of starch in 460 ml of water. The amylograph is run with the temperature held at 25° C, and the viscosity read after 30 min. The viscosity of the pregelatinized wheat starch used in this study was 240 BU when tested by this procedure.

The modified amylograph procedure calls for a pregelatinized wheat starch containing 8% moisture. Deviations from this moisture level should be corrected, since as shown in Table III, a 0.5-g lowering in the amount of starch resulted in an increase of 32 BU in the MV obtained. (The corrected weight of the starch is 70 ×

TABLE III
Ruggedness Test on Modified Amylograph

Factor[a]	MV Change[b] (BU)
Weight of starch	31.9*
A. 70.0 g	
a. 69.5 g	
Mixing method	−9.4
B. As per specified procedure	
b. Starch dumped in all at once	
Mixing time	−13.1
C. 2-1/2 min	
c. 2 min	
Time to start amylograph	5.6
D. After 5 min	
d. After 8 min	
Amount of slurry put in bowl	6.9
E. Regular amount	
e. 1 tbs less	
Starting temperature	−24.4*
F. 25° C	
f. 30° C	
Buffer (pH)	−114.4*
G. Regular buffer used (4.3 pH)	
g. No buffer used	

[a]Capital letter, per regular procedure; lower case letter, change from regular procedure.
[b]A negative value indicates that the change from the first to the second condition gave a higher minimum viscosity (MV) value.
*Significant difference at 5% level.

92/[100 − percent of moisture in the starch]. The volume of dilute amylograph buffer = 460 − [corrected weight of the starch − 70].)

When distilled water was used instead of the buffer solution, the MV increased 114 BU. In the modified amylograph procedure, pH control appeared to be more critical than it did in the regular procedure since there is less buffering provided by the flour.

The changes made in the other four factors tested did not produce significant differences in the MV value. This would suggest that the specified mixing procedure is perhaps more stringent than it would need to be.

TABLE IV
Collaborative Study on the Modified Amylograph Test

Value	Number of Laboratories	Mean	Standard Deviation
MV[a] on fungal α-amylase treated			
All results	20	170	38
Outliners eliminated*	16	177	22.8
Intralaboratory			8.5
Interlabroatory			21.2
Zero line corrected	18	183	22.3
Intralaboratory			7.8
Interlaboratory			20.9
PV[b] malted flour (regular amylograph test)			
All results	20	559	67
Outliners eliminated*	17	555	48
EMV[c] on malted flour			
All results	19	190	40
Outliners eliminated*	17	199	30

[a]MV = minimum viscosity.
[b]PV = peak viscosity.
[c]EMV = equivalent minimum viscosity.
*At 80% confidence level.

TABLE V
Second Collaborative Study on the Modified Amylograph Test

	α-Amylase Activity Added (SKB/g)		
	41	68	164
Number of laboratories	11	11	12
Mean minimum viscosity	385	259	105
Standard deviation (SD)	49	32	22
Mean difference between replicates	24	15	6
Total intralaboratory SD (repeatability): 13.0			
Total interlaboratory SD (reproducibility): 36.0			

Collaborative Study

The results of AACC collaborative studies on the modified amylograph test are summarized in Tables IV and V. In the first study (Table IV), the collaborators were supplied with identical samples of pregelatinized wheat starch, a fungal α-amylase–treated flour, and a malted flour. The intralaboratory standard deviation was 8.5 BU; the interlaboratory standard deviation was 21.2 BU after eliminating outliners at an 80% confidence level (4 of 20 laboratories). Two of the four outliners would have been within the 80% confidence range were their zero viscosity properly adjusted.

In the second study (Table V), the collaborators ran three flour samples in duplicate, supplemented with fungal α-amylase at levels of 41, 68, and 164 SKB units/lb. The intralaboratory standard deviation was 13.0 BU; the interlaboratory standard deviation was 36.0 BU. The repeatability of the test is thus fairly good and the reproducibility is similar to the regular amylograph procedure and should be adequate for mill control purposes.

Acknowledgments

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