An Evaluation of the Rapid Amylograph Method[1]

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ABSTRACT

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The operating procedure for the rapid amylograph method was studied. The optimum slurry composition was determined to be 26 g of wheat flour in 100 ml of distilled water. The rapid amylograph was less discriminatory than the standard amylograph test for flours with low to intermediate α-amylase activity but was a better differentiator for flours with high α-amylase activity. Comparison of the falling number and rapid amylograph methods indicated that the rapid method had about twice the range and was a better differentiator. An overall evaluation of the rapid amylograph method showed that it could be used in a wheat breeding program as an alternative to the falling number method but not to the standard amylograph method.

It is well known that wheat α-amylase can have a significant effect on the bread-making quality of flour. For this reason, an evaluation of α-amylase activity in flour is of concern to millers, bakers, and cereal chemists. In recent years, although several methods have been developed for the determination of α-amylase in flour (Barnes and Blakeney 1974, Marchylo and Kruger 1978, Paton and Voisey 1977), the viscometric methods of Brabender (1937) and Hagberg-Perten (1960, 1961, 1964) remain two of the most widely used α-amylase assays. The former method utilizes the amylograph to measure the effect of α-amylase on the peak viscosity of a cooked flour slurry. It requires a sample size of 65–100 g of flour and each test has a running time of 45 min. The latter procedure, known as the falling number method, measures the time in seconds required to stir and allow a viscometer-stirrer to fall a fixed distance through a hot flour slurry. In this case, a sample of 5–7 g of flour or wheat grist is employed and the running time can vary from 60–700 sec.

In some wheat breeding programs, up to 6,000 lines are evaluated in one crop year. To test these lines for α-amylase activity, the method of analysis must be rapid, require a small sample size, and be capable of differentiating samples in the intermediate to low activity range. The amylograph method does not meet the first two requirements and is thus of limited value to the plant breeder. In contrast, the falling number method meets the first two requirements and has been widely used in wheat breeding, but does not meet the third requirement.

Recently, Brabender Laboratories developed a rapid amylograph test that may fulfill wheat breeders' requirements. This procedure

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purportedly produces a starch curve in a fraction of the time (3–10 min) necessary for the standard amylograph, while using a relatively small flour sample of 21–26 g (Brabender Inc. Bull. CS 751, 1978; Seitz 1978). The purpose of this study was to investigate the operating procedure for the rapid amylograph and to determine if this method could replace the standard amylograph or falling number methods as the α-amylase test in a wheat breeding program.

MATERIALS AND METHODS

Flour Samples

Straight-grade flour samples were prepared in a Buhler laboratory mill from hard red spring wheat selections grown in western Canada during 1977. The wheat was conditioned to 16.5% moisture prior to milling.

Standard Amylograph Peak Viscosity Determination

The standard amylograph peak viscosity (AV) was determined on a Visco/Amylo/Graph (C. W. Brabender Instruments, Inc., South Hackensack, NJ) by the modified standard AACC amylograph method (1960). In this study, 65 g of flour and 450 ml of distilled water were used to obtain peak viscosities below 1,000 BU.

Rapid Amylograph Peak Viscosity Determination

The rapid amylograph peak viscosity (RAV) was measured by the standard amylograph equipped with a small sample bowl and a special sensor/stirrer (Brabender Inc. Bull. CS 751). In addition, instead of the direct radiation of heat, water in the annulus around the bowl was maintained at a constant temperature of 85°C and served as the heat transfer medium.

Falling Number Determination

This was performed on a 7-g flour sample by the standard ICC method.

α-Amylase Activity Determination

α-Amylase activity was assayed using the automated fluorometric procedure of Marchylo and Kruger (1978).

TABLE I
Repeatability of Rapid Amylograph Peak Viscosities for Three Flours of Different α-Amylase Activities

Enzyme Activity	Sample No.	Peak Viscosity (BU)	Analysis Time (Time to Reach Peak Viscosity) (min)
High	1	200	
	2	210	
	3	250	3.5
	4	190	
	5	220	
	Mean	222	
	SD[a]	26	
Medium	1	870	
	2	830	
	3	790	6
	4	820	
	5	840	
	Mean	830	
	SD	29	
Low	1	1,370	
	2	1,310	>15
	3	1,350	
	4	1,350	
	5	1,340	
	Mean	1,344	
	SD	22	
	Overall SD	23	

[a]Standard deviation.

Operating Procedure

The initial step in the rapid amylograph test was preparation of a flour-water suspension. Seitz (1978) suggested that this could be performed in an Erlenmeyer piston. This apparatus was not available so an alternative method was chosen. In this study, the flour-water suspension was prepared by slowly adding flour to the distilled water with continuous magnetic stirring. The resultant suspension or slurry was then stirred for a further 5–10 min to remove any lumps. This procedure minimized foaming, which was desirable since foaming leads to erratic results.

Some confusion exists in the literature concerning the optimum slurry composition required for this test (Brabender Inc. Bull. CS 751, 1978; Seitz 1978). Therefore, to ascertain the optimum slurry composition, five flours of varying AVs (140–1,000 BU) were analyzed with the rapid amylograph. Three separate slurry compositons of 26, 24, and 21 g of flour mixed with 100, 100, and 90 ml of distilled water, respectively, were tested for each of the five flours. In addition, a slurry composition (14.4 g flour + 100 ml water) equivalent to that used in the standard amylograph method was tested.

RESULTS AND DISCUSSION

The slurries prepared with 14.4 g of flour in 100 ml of water exhibited very low RAVs and little difference in RAV between the high and low amylograph viscosity flours. This precluded the use of

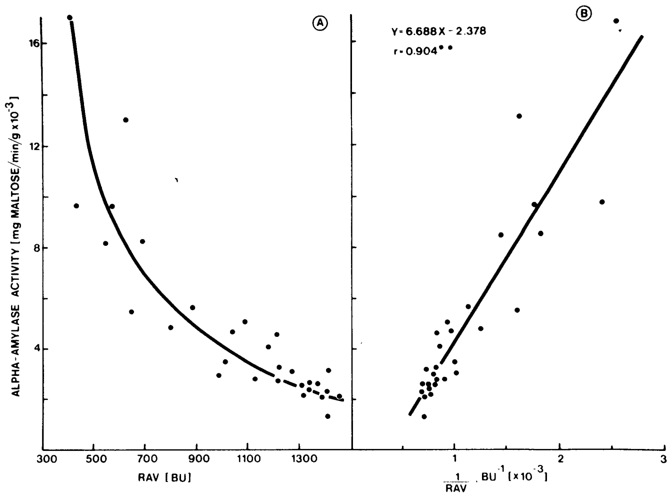

Fig. 1. Wheat α-amylase activity determined by the automated fluorometric method of Marchylo and Kruger (1978) vs A) rapid amylograph peak viscosity (RAV) and B) the reciprocal of RAV for 29 flours. **Significant at 1%.

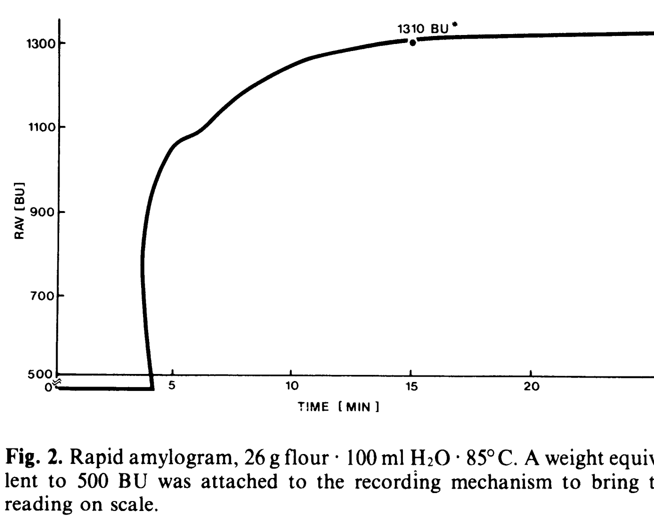

Fig. 2. Rapid amylogram, 26 g flour · 100 ml H_2O · 85°C. A weight equivalent to 500 BU was attached to the recording mechanism to bring the reading on scale.

this slurry composition. Of the remaining slurries, those prepared with 26 g of flour in 100 ml of water displayed the largest range in RAVs; on this basis, 26 g of flour in 100 ml of water was chosen as the optimum slurry composition. This agreed with the results of Seitz (1978).

The initial step in the evaluation of the rapid amylograph method was the determination of its repeatability. As indicated in Table I, the peak viscosities were found repeatable within relatively narrow limits and over a wide range in α-amylase activity. Thus, the rapid amylograph method has a reasonable degree of precision.

In the second step of the evaluation, 29 flour samples were analyzed for α-amylase activity using an automated fluorometric α-amylase assay (Marchylo and Kruger 1978) and the rapid amylograph method. As illustrated in Fig. 1A, a curvilinear plot was obtained when α-amylase activity was plotted against RAV. Curvilinear plots similar to this have been found when α-amylase activity has been plotted against AV (Barnes and Blakeney 1974, Marchylo and Kruger 1978, Tipples 1969) and falling number (Marchylo and Kruger 1978). Subsequently, the α-amylase activity was plotted against the reciprocal of the RAV, as suggested by Hlynka (1968), to obtain a straight line relation between α-amylase activity and RAV (Fig. 1B). A significant correlation between α-amylase activity and the reciprocal of the RAV was obtained indicating that the rapid method does measure α-amylase activity.

The rapid and standard amylograph methods were then compared. As was indicated by the manufacturer (Brabender Inc. Bull. CS 751), the rapid method was performed more quickly than the standard method. However, it was found that analysis time increased with decreasing α-amylase activity for the rapid method (Table 1). In addition, with flours of very low α-amylase activity, an actual peak viscosity was not reached. Instead, the rapid amylogram (Fig. 2) showed a gradual increase in RAV after the initial rapid increase in viscosity. As a consequence, the peak viscosity was recorded after an arbitrary length of time (15 min). Thus, the peak viscosity could be determined approximately 3–15 times more quickly with the rapid amylograph than the standard amylograph.

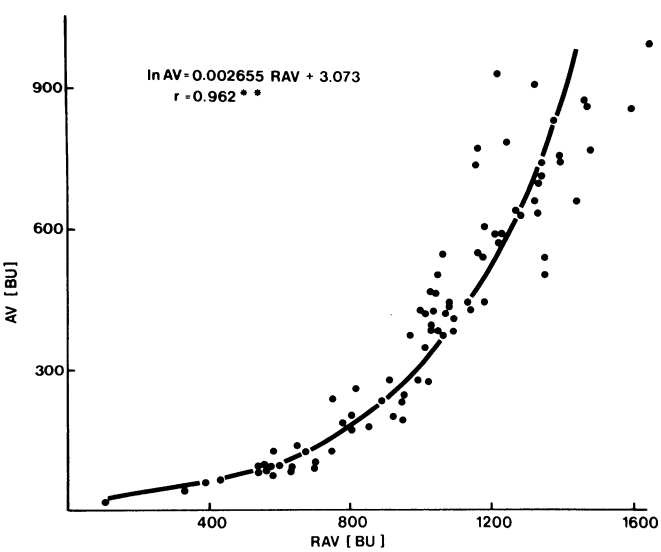

Fig. 3. Standard amylograph peak viscosity (AV) vs rapid amylograph peak viscosity (RAV) for 85 flours. **Significant at 1%.

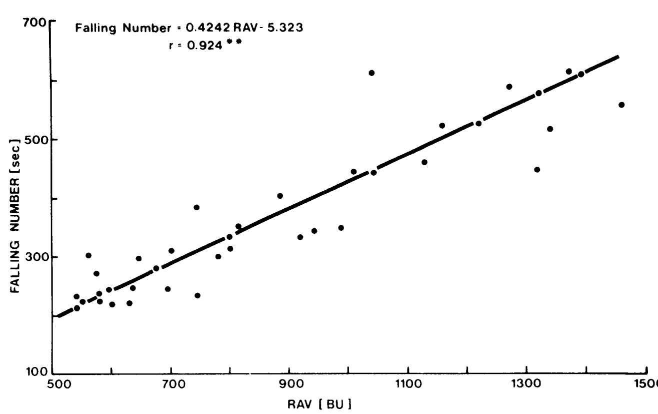

Fig. 4. Relation between the falling number reading and rapid amylograph peak viscosity (RAV) obtained for 41 flours. **Significant at 1%.

A total of 85 flour samples were then analyzed by the rapid and standard amylograph procedures. The AV increased exponentially when plotted against increasing RAV and a high degree of correlation was observed between the two methods (Fig. 3). The scatter in these data, which progressively increased with increasing AV and RAV, may be attributable to a number of variables. More specifically, it has been shown that factors such as pH, starch, and protein content, inherent differences in starch characteristics, and the extent of starch damage can have a significant effect on the peak viscosity of a standard amylograph (Anker and Geddes 1944, Meredith 1970). These variables could in turn also affect the RAV, which would contribute further to the observed scattering of data. The plot of AV against RAV (Fig. 3) also revealed that the rapid amylograph was more able to differentiate flour samples of high α-amylase activity, whereas the standard amylograph was a better differentiator for flours of intermediate to low activity.

Finally, the rapid amylograph method was compared with the falling number method. A total of 41 flour samples were analyzed by each technique. The analysis times for each test were comparable but the falling number procedure required less sample. A straight line relation, with a high degree of correlation, was obtained when falling number readings were plotted against RAV (Fig. 4). The range of the rapid amylograph method was about twice that of the falling number test as indicated by the slope of the regression line (Fig. 4). Taking into account the overall standard deviation of the rapid amylograph (23 BU) and that of the falling number (15 sec), it was also evident that the former method was a better differentiator over the entire activity range studied.

CONCLUSIONS

The rapid amylograph method provides a number of advantages over the standard amylograph procedure. It requires a smaller sample size (26 g) and also a faster running time (3–15 min). Unfortunately, it has the disadvantage of being less discriminatory at intermediate to low α-amylase activity levels (Fig. 3). This would be a drawback to the plant breeder, who must be able to differentiate between flours in this range of activity. For this reason, the rapid amylograph method would not serve as a suitable replacement for the standard amylograph procedure. On the other hand, the rapid method was a better differentiator than the standard method at high levels of α-amylase (Fig. 3). Thus, the rapid amylograph method would be particularly useful in these situations. The rapid amylograph method appears to be closely related to the falling number method. In particular, both techniques subject the flour slurry to similar heat abuse and although the falling number method does require a smaller sample, both tests have similar running times. Also, the readings from these two techniques are highly correlated but, as may be seen in Fig. 4, the range of the rapid method is approximately twice that of the falling number procedure. As a result, the rapid test is a better differentiator over the entire activity range studied. Thus, the rapid amylograph method could serve the plant breeder as a replacement for the falling number method.

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