RAPID METHOD FOR THE DETERMINATION OF DIASTATIC ACTIVITY OF CEREAL FLOURS USING THE OTTAWA STARCH VISCOMETER[1]

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Cereal Chem. 54(5) 1007-1017

ABSTRACT

The operational conditions of the Ottawa Starch Viscometer were modified so that the instrument may be used as as a rapid means of determining the diastatic activity of wheat flours with a 70 g slurry sample. Approximately 35 samples per normal day may be handled using this accelerated procedure. The instrument is accurate and has the sensitivity to discern differences between those wheats of intermediate malt index, an advantage for cereal breeding activities.

Several AACC methods are approved for the determination of diastatic activity in cereal flours. Methods 22-15 and 22-16 incorporate either chemical or enzymatic hydrolysis and estimation of the reducing sugars produced. They have the advantage of a small sample size (5 to 25 g of flour) but are time-consuming (1.5 to 4 hr). Method 22-11 uses only 10 g of flour and is based upon the production of gas by fermentation but takes 5 hr to complete. A widely accepted method is 22-10, which employs the Amylograph to measure the effect of starch-liquefying enzymes on the peak viscosity of a cooked flour slurry. It requires 65 to 100 g of flour and each test takes about 45 min.

In cereal breeding programs, it is not uncommon to make selections from some 4000 to 6000 lines. However, due to the time required, only a small proportion (<10%) is analyzed for diastatic activity by the Amylograph method. The majority are assessed by the falling number method, which is rather inaccurate and can only discriminate between high and low activity with confidence. Breeders need to rate those samples that fall in the intermediate activity range since these may possess qualities which could be crossed with other genetic lines to produce varieties with a spectrum of desirable characteristics.

The cereal breeder needs a method for diastatic activity assessment that is rapid (<15 min) and consumes small amounts of flour (<10 g). The Amylograph (C. W. Brabender Inc., South Hackensack, N.J.) is available with an optional attachment (an additional bowl and paddle) which must be inserted into the existing instrument, and can produce "rapid amylograms" in 10 min using a 110 g slurry sample. This has been applied to the measurement of diastatic activity of various flours (1). While the added cost for these accessories may be justified for existing Amylographs, it may be prohibitive to purchase the entire assembly specifically for the "Rapid Amylograph" technique.

The Ottawa Starch Viscometer (OSV) was previously described (2) and was designed to eliminate some of the difficulties experienced with the Amylograph, particularly in the rate of heat transfer to the slurry, the amount of sample required, and the configuration of the bowl and paddle. The OSV, in its normal operational mode, produces rapid cooking curves for starch-based products

[1]Contribution No. 296 from Food Research Institute and No. 638 from Engineering Research Service.
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using a 70 g slurry sample. The intent here was to learn whether or not the OSV could be used to determine the diastatic activity of wheat flours and to examine the correlation of its results with variants of the standard Amylograph procedure.

MATERIALS AND METHODS

Twenty samples of different hard red spring (HRS) wheats, with a known wide range of diastatic activity, were obtained from the Grain Research Laboratory, Winnipeg, and milled on a micro-flour mill, as previously described (3). Each clean flour was immediately sealed in vials and stored at −20° C until required. Moisture was determined by the vacuum oven method (4). All flour-water concentrations were calculated on a moisture-free basis and were equivalent to that used in the AACC Method 22-10 for diastatic activity of HRS wheats (4).

The OSV was operated at 200 rpm with a paddle giving a 1-mm shearing gap

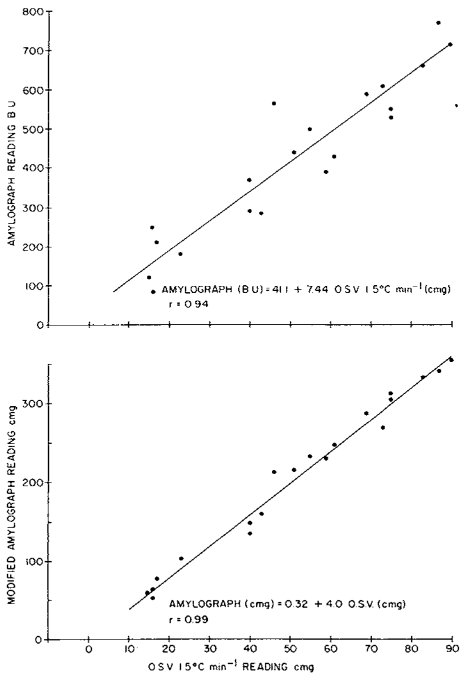

Fig. 1. Scatter diagrams of the peak viscosity readings obtained in the OSV against those from the Amylograph (top) and modified Amylograph (bottom) when the three instruments increased the temperature at 1.5° C/min for 20 flours.

and a water bath temperature in the range 25° to 97°C. The instrument was calibrated to give a full-scale deflection at the recorder when 166 cm-g was applied to the electronic torque detector. A modified Amylograph with electronic torque (viscosity) recording was used as a standard for comparison as its readings were known to be accurate and repeatable (5). The slurry temperature was monitored by thermocouples connected to an automatic data logger (Model 800, Kaye Instruments Inc., Cambridge, Mass.) that recorded at 10-sec intervals.

To accelerate the test, the OSV water bath can be programmed to increase in temperature at any desired rate or, more simply, the test can be started at a preselected elevated temperature and the bath then allowed to heat up at a rate determined by the heater size. This was demonstrated by recording the slurry temperature at the edge of the mixing paddle during wheat flour tests, where the initial water bath temperature was 50°, 55°, and 60°C. For the purposes of the following tests the OSV accelerated rate was 3.8°–3.9°C/min (hereafter designated as 4°C/min), but it was recognized that other rates could be achieved by increasing or decreasing the heater size. The heating rates were not optimal, but were selected arbitrarily so that the total time would not exceed 10 min.

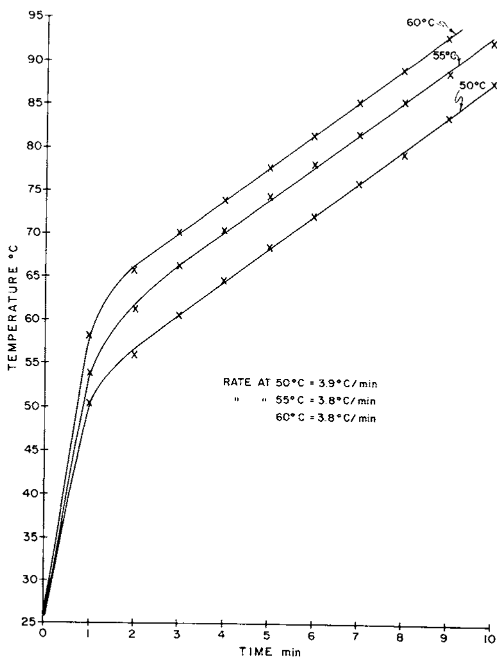

Fig. 2. Increase of flour slurry temperature with time at the edge of the mixing paddle in the OSV for three initial water bath temperatures used to accelerate the test for one flour.

RESULTS AND DISCUSSION

Since the usual method of performing Amylograph tests employs a controlled heating rate of 1.5° C/min, a temperature programmer was attached to the heater of the OSV water bath and adjusted to give the same rate. The 20 wheat flours tested in the OSV and the modified Amylograph gave the results shown in Fig. 1 (lower). Linear regression analysis showed a high correlation between the two instruments ($r = 0.99$; $P > 0.01$). For comparison, the samples were also tested in a standard Amylograph and these results were highly correlated with the OSV readings (Fig. 1, upper) but at a lower level ($r = 0.94$; $P > 0.01$). There was greater scatter in the relation between the OSV and Amylograph (Fig. 1, upper) compared to the OSV and modified Amylograph (Fig. 1, lower), which reflected the improved accuracy of the modified Amylograph.

The time taken for the tests at 1.5° C/min was approximately 45 min. It was hypothesized that this time could be reduced by increasing the heating rate. In the normal OSV test procedure (2), the water bath is maintained at 97° C to minimize the time to obtain starch pasting curves. Under these conditions, using wheat flours, there is insufficient time for the enzyme to break down the starch. The

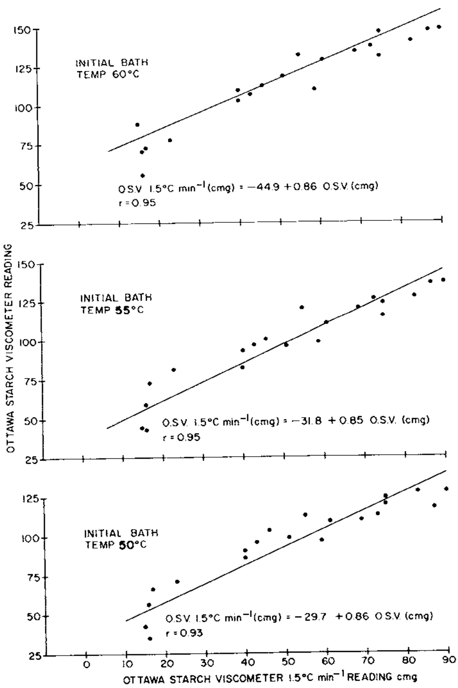

Fig. 3. Relations between the OSV peak viscosity readings obtained at a heating rate of 1.5° C/min and the readings from the accelerated test procedure at three initial water bath temperatures for 20 flours.

results (Fig. 2) show that the flour slurry was heated rapidly to the initial selected bath temperature and then increased linearly with time at 3.8° to 3.9° C/min. In comparison, the Amylograph heated the slurry linearly with time at 1.5° C/min, starting at the initial slurry temperature. The fact that the Amylograph heater, its shroud, and the machine were cold before the first test sample or hot from previously tested samples did not change these conditions by more than 2° C. However, at the end of the 45-min test, the gradient of temperature within the slurry across the bowl radius was up to 12° C, reflecting the poor heat transfer characteristics of the radiant electric sample heating system. By contrast, the entire sample rapidly reached and remained at a uniform temperature in the OSV bowl (2).

The 20 flours were tested in the OSV under the accelerated conditions. These results were highly correlated with the OSV (Fig. 3), modified Amylograph (Fig. 4) and standard Amylograph (Fig. 5) readings obtained at 1.5° C/min. A summary of the data (Table I) shows that all the readings were highly correlated with each other and the regression lines between the 1.5° C/min readings, and those obtained at the three starting temperatures were parallel. This was anticipated in view of the parallel heating curves obtained (Fig. 2). The starting

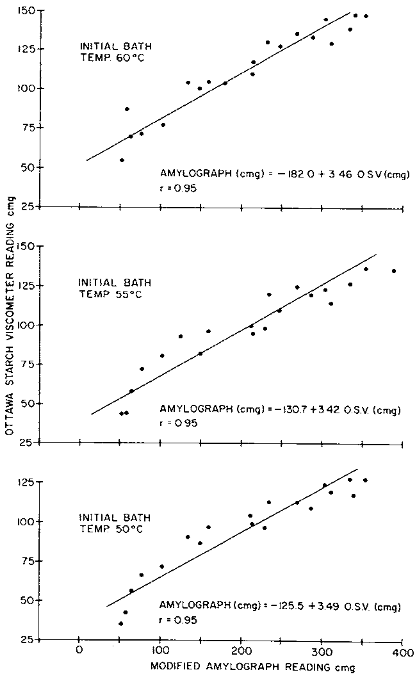

Fig. 4. Relations between the modified Amylograph peak viscosity readings and the readings from the accelerated test procedure at three initial water bath temperatures for 20 flours.

temperature did not affect the slope of the relationships, but had a marked effect on the intercept (Table I). The starting temperature selected appeared to have little effect on the level of correlation.

A high degree of correlation was also observed between the readings from the modified and standard Amylographs (Fig. 6). The scatter in these data was

TABLE I
Regression Equations of the Form Y = a + bx Relating the Readings Obtained by the Different Methods Based on Readings from 20 Different HRS Flours

Y	X	a	b	r
Modified Amylograph 1.5°C/min^{-1}	OSV—50°C	−125.5	3.49	0.95
	OSV—55°C	−130.7	3.42	0.95
	OSV—60°C	−182.0	3.46	0.95
	OSV—1.5°C/min^{-1}	0.32	4.00	0.99
	Amylograph	2.00	0.69	0.95
OSV, 1.5°C/min^{-1}	OSV—50°C	− 29.7	0.86	0.93
	OSV—55°C	− 31.8	0.85	0.95
	OSV—60°C	− 44.9	0.86	0.95
	Modified Amylograph 1.5°C/min^{-1}	0.80	0.25	0.99
	Amylograph	1.30	0.17	0.94
Amylograph	OSV—50°C	−142.0	4.63	0.91
	OSV—55°C	−160.3	4.65	0.94
	OSV—60°C	−221.9	4.63	0.92
	Modified Amylograph 1.5°C/min^{-1}	28.3	1.31	0.95
	OSV—1.5°C/min^{-1}	28.8	5.22	0.94

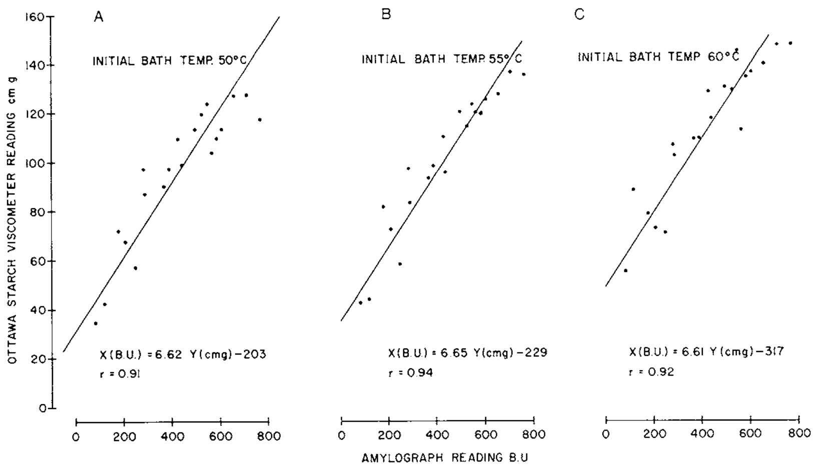

Fig. 5. Relations between the Amylograph peak viscosity readings and the readings from the accelerated test procedure at three initial water bath temperatures for 20 flours.

attributed to the inaccuracies in the standard Amylograph recording apparatus compared to the modified instrument.

The readings of peak viscosity (torque) and slurry temperature at this point

TABLE II
Repeatability of OSV Readings Using an Initial Temperature of 60°C and 4°C min^{-1} Rate of Temperature Increase for Three Flours of Different Diastatic Activity

Enzyme Activity	Sample No.	Peak Viscosity cm-g	Temperature at Peak °C
High	1	51.77	75.6
	2	47.59	75.6
	3	49.93	75.4
	4	54.77	76.5
	Mean	51.015	75.775
	SD	3.0319	0.4924
Medium	1	123.41	89.9
	2	123.58	89.8
	3	120.07	89.5
	4	121.91	89.9
	Mean	122.2425	89.775
	SD	1.6311	0.1892
Low	1	159.99	92.3
	2	160.15	92.4
	3	160.32	93.0
	4	154.47	92.4
	Mean	158.7325	92.525
	SD	2.8448	0.3201

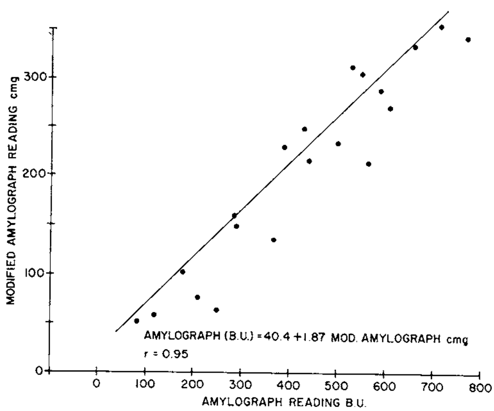

Fig. 6. Relation between peak viscosity in the Amylograph and modified Amylograph both programmed at 1.5°C/min for 20 flours.

were found repeatable within narrow limits for the OSV procedure starting at elevated temperatures (Table II). This reflects the precision with which the OSV operates, using a 70 g slurry sample (2).

Typical viscosity-time curves obtained from the OSV, modified Amylograph, and Amylograph using a 1.5°C/min heating rate and the accelerated test procedure in the OSV for high- (Fig. 7) and low- (Fig. 8) diastatic activity flours demonstrate the advantages of the OSV accelerated test. The curves for the three methods performed at the 1.5°C/min heating rate were quite similar in shape, and the temperature at the peak viscosity was the same within close limits (±1.4%) for each flour. The testing time and time to reach the peak viscosity were also comparable, but reflected the differences between the two initial slurry temperatures used in the three instruments (23.5° and 30°C). The temperature at the peak viscosity was slightly altered by the accelerated OSV procedure. The most active flours showed peak viscosity temperatures up to 3°C above those found for the same samples at lower heating rate. However, it was noted that, as enzyme activity decreased, the peak temperature appeared to pass through a slight maximum, the least active flours showing values up to 3°C below those run at 1.5°C/min. However, neither the increased heating rate nor the higher initial bath temperatures had much effect on the peak viscosity correlations between instruments and methods. The reasons for these temperature anomalies are not presently clear; results do show (Fig. 2) that the heating rate becomes constant only after the first 2 min. There is, therefore, the possibility that the variation in heat transfer rate within the first 2 min affects the final peak temperature, but only a more refined study using programmed heating rates would serve to resolve this question.

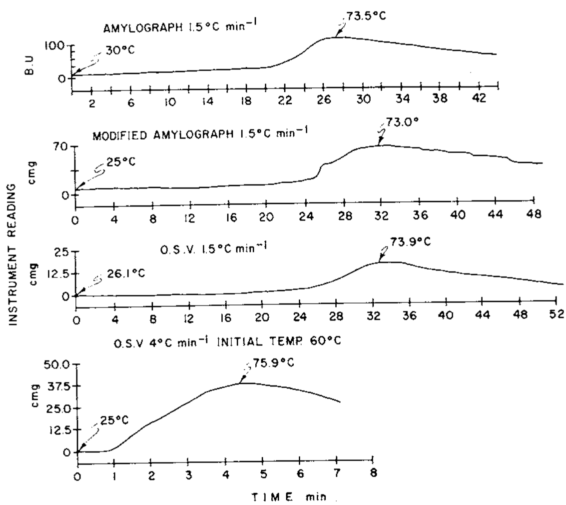

Fig. 7. Typical curves obtained for a high activity flour using the various instruments and techniques.

When the OSV was started at an elevated temperature and a 4° C/min heating rate applied, the process was speeded up, reducing the time to reach the peak viscosity to 4.5 min for the highest and 8.5 min for the lowest activity flours. This allowed the testing of 35 samples per 7.5-hr working day compared to the 8 tested at 1.5° C/min. A point of interest was that the curve peaks for all the flours were clearly discernable in the accelerated procedure for both low- and, more importantly, high activity flours (Fig. 7, 8). The apparent peak viscosity was

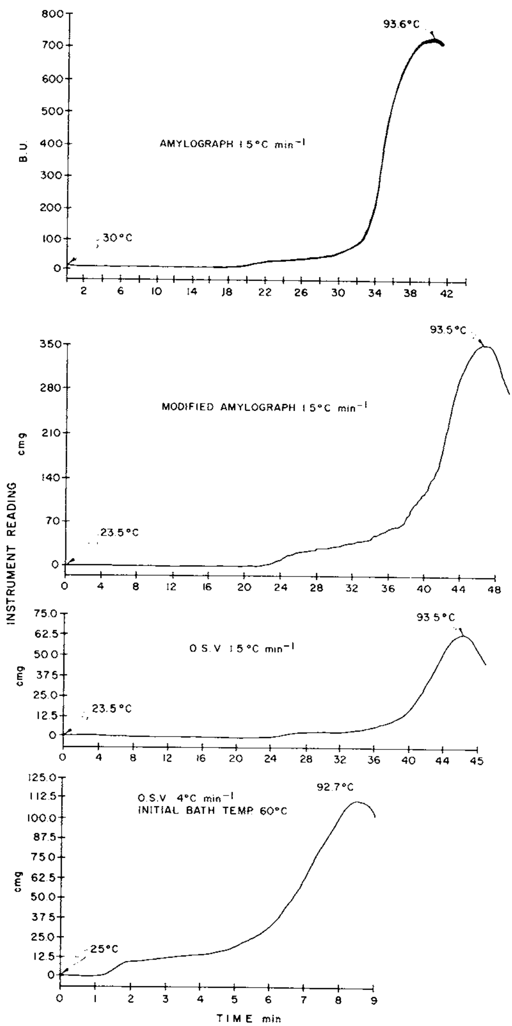

Fig. 8. Typical curves obtained for a low activity flour using the various instruments and techniques.

greater using the OSV accelerated procedure compared to the OSV 1.5° C/min rate. These increased readings were repeatable within narrow limits (Table II), and increased with increasing initial temperature (Table III).

A summary of the results (Table III) shows that the variation and range of peak viscosity readings within the group of 20 flours were reduced when the accelerated procedure was applied. This was attributed to the different temperature-time integral the samples experienced in the shorter test. Enzyme activity is time- and temperature-dependent. The slurry must be heated above the temperature necessary to activate the process, but remain below the inactivation temperature, to allow sufficient starch liquefication in order to discriminate between levels of activity. In the accelerated procedure, the combinations of conditions (initial temperature and heating rate) are infinite and the conditions selected (50°, 55°, 60°, and 4° C/min) appeared to be satisfactory. The range of readings within the 20 flours was 82 to 97%, compared to 145 to 160% in the 1.5° C/min procedures (Table III). The precision and resolution possible with the OSV apparatus are such that, theoretically, the reduced range of readings should not reduce the discrimination between samples. This was supported by the high degree of correlation between the results from the accelerated procedure and each of the 1.5° C/min methods (Table I).

CONCLUSION

An additional application of the Ottawa Starch Viscometer has been demonstrated, namely that of determining the diastatic activity of wheat flours. By modifying the operational conditions to accelerate the test, it was possible to complete evaluation of each flour in a time not exceeding 8.5 min for the least active samples. Less than 12 g of flour in a total slurry weight of 70 g is required, and this can theoretically be reduced to 3 g of flour in 17.5 g of slurry by utilizing a

TABLE III
Summary of Results Comparing Results from Six Methods for 20 Flours

Instrument[a]	OSV	OSV	OSV	OSV	Modified Amylograph	Amylograph[e]
Temperature at start of test, °C	50	55	60	25	25	30
Heating rate[b], °C/min^{-1}	4	4	4	1.5	1.5	1.5
Units of measurement	cm-g	cm-g	cm-g	cm-g	cm-g	cm-g
Mean peak viscosity[c]	95.2	98.7	112.6	51.7	207.2	298.9
SD	27.4	28.1	27.9	25.1	101.4	139.8
CV, %	28.8	28.5	24.8	48.6	48.9	46.8
Range, %[d]	96.5	93.9	81.6	145.1	146.3	160.3

[a]OSV = Ottawa Starch Viscometer.
[b]See text.
[c]Mean of 20 different wheat samples.
[d]$\frac{\text{Maximum-Minimum}}{\text{Mean}} \times 100\%$.
[e]Converted from BU.

25-g bowl (2). Using four identical measuring bowls (2) allows approximately 35 analyses per average working day and represents substantial time saved over the Amylograph Method. The method has the sensitivity to discern differences in wheats of moderate diastatic activity and may allow the breeder a wider choice by using certain crosses which may have been previously rejected on the basis of their intermediate malt indices (by falling number). The results suggest that the accelerated OSV procedure may be useful for other research activities and quality control applications.

Acknowledgments

The authors wish to acknowledge the assistance of H. Hamilton of the Food Research Institute who performed the tests, and R. Tkachuk of the Grain Research Laboratory, Winnipeg, Manitoba, who provided the test wheats.

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[Received December 10, 1976. Accepted March 15, 1977]