Note on the Determination of α-Amylase with the Perkin Elmer Model 191 Grain Amylase Analyzer

J. E. KRUGER,[1] P. M. RANUM,[2] and A. W. MacGREGOR[3]

Cereal Chem. 56(3):209-212

Methods for the determination of α-amylase in cereals include autolytic, starch-iodide (amyloclastic), dyed starch ("chromolytic"), saccharogenic-reducing methods, and coupled enzymatic assays and fluorometric methods. A very recently introduced turbidimetric method uses a prototype Perkin Elmer Model 191 Grain Amylase Analyzer and is based on the nephelometric procedure of Zinterhofer et al (1973), which is widely used in clinical medicine. We examined the practical applications of this method for analyzing α-amylase levels in cereals.

MATERIALS AND METHODS

Extraction of Wheat Flours

Flour (2 g) was extracted with 10 ml of 0.2M acetate buffer, pH 5.5, containing 10^{-3} M $CaCl_2$ as described by Marchylo and Kruger (1978). Enzyme supplemented flours were extracted with 0.5% sodium chloride and 0.02% $CaCl_2$.

Enzyme sources

The commercial enzymes used were: fungal α-amylase, 4,665 SKB units per gram (Calbiochem, Los Angeles, CA); and sweet potato β-amylase, type 1-B containing 1,140 Sigma units per milligram of protein (Sigma Chemical Co., St. Louis, MO). Wheat α-amylase was prepared by extracting 2 g of ground malted Manitou wheat with 10 ml of 0.2M acetate buffer, pH 5.5 containing 10^{-3} M $CaCl_2$ and heat-treatment at 70° C for 20 min to inactivate β-amylase, followed by centrifugation at 96,000 × g for 10 min.

α-Amylase Determination

Model 191 Grain Amylase Analyzer Method. The procedure for determining amylase with the Model 191 Grain Amylase Analyzer (Perkin-Elmer Corp., Coleman Instruments Div., Oak Brook, IL 60521) was as described by Lehane et al (1976). Amylopectin substrate was dissolved in 0.1M acetate buffer pH 5.5, containing 10^{-3} M $CaCl_2$, and the meter indicator was adjusted to 50 Nephelos units with the standard control.

Amylograph Viscosity Determination. This was performed on a Brabender Visco Amylograph using 65 g of flour and 450 ml of buffer (AACC 1976).

Falling Number Determination. The standard ICC Method (ICC, 1967) was followed using a 7 g sample of wheat.

Modified Amylograph Procedure. The procedure of Ranum et al (1978) was followed.

Saccharifying Activity. This was determined on a 2.5 g sample of barley and the results reported as degrees Lintner, dry basis (Meredith and Bendelow 1955).

Dextrin-Iodide Method. The procedure of Briggs (1961) as modified by MacGregor et al (1971) was followed.

Automated Fluorometric α-Amylase Determination. This was performed as described previously by Marchylo and Kruger (1978) with a fungal α-amylase source for standardization.

[1,3]Paper 411 of the Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main Street, Winnipeg, Manitoba, R3C 3G9.
[2]Pennwalt Corporation, Broadview, IL 60153.

0009-0352/79/000028$3.00/0
©1979 The American Association of Cereal Chemists

RESULTS AND DISCUSSION

The α-amylase levels in (i) wheat and wheat flours, in (ii) fungal and malted barley α-amylase supplemented flour, and (iii) in barley and barley malts were examined with the Model 191 Grain Amylase Analyzer (GAA) using amylopectin as substrate.

Wheats and Wheat Flour

Excess α-amylase in wheat flour can have a very deleterious effect on breadmaking quality. Levels of the enzyme are commonly measured using the falling number method on the whole wheat flour or amylograph on the milled flour. Any new methods should correlate at least roughly, therefore, with these two methods.

Table I illustrates the results obtained with the Model 191 Grain Amylase Analyzer for wheats with widely varying falling number and amylograph viscosity values. Results obtained with an automated fluorometric α-amylase method (Marchylo and Kruger 1978) are also shown.

Several salient points are quite obvious: (i) No correlation exists between results obtained with either the falling number method or the amylograph and the results with the Model 191 GAA. (ii) α-Amylase levels in flours should be roughly one-half of the levels in the whole wheat flours because substantial amounts of α-amylase in the bran layers are removed during the milling process. Examination of fluorometric α-amylase results indicates that this is the case. In contrast, results with the Model 191 GAA were very similar for both wheats and flours. (iii) Only the extremely sprouted wheat sample (L-9742) gave a noticeable increase in Model 191 GAA readings. Practically, this wheat as well as the two or three samples preceding it in Table I have excessively high α-amylase levels, indicating that discriminating between the low levels of α-amylase relevant to the grain trade would be difficult with this method.

The extremely high values obtained with the Model 191 GAA after the short reaction time of 1-min with samples varying widely in α-amylase suggested that interference from some other enzyme such as β-amylase affected the results, and experiments were done to establish if this was the case. The Model 191 GAA was connected to a recorder and the Nephelos mode was used after calibration with the 50 Nephelos standard. The results with extracts of sweet potato β-amylase and of fungal and malted wheat α-amylase are shown in Fig. 1A. β-Amylase activity had a very strong effect on the decrease in turbidity of amylopectin. The final turbidity of about 32 nephelos units was higher, however, than that of about 15 nephelos units resulting from fungal and wheat α-amylases attack. This indicates that the β-limit dextrin resulting from β-amylase degradation of amylopectin still has a definite turbidity. On the other hand, α-amylase hydrolyzes the substrate completely to simple sugars with no turbidity. Extracts of wheat (Table I) containing low, medium, and very high α-amylase activities were examined as described, using the Nephelos mode. As shown in Fig. 1B, all extracts produced rapid initial decrease in turbidity. This would explain the similarity of results of using the 1-min amylase mode of the Model 191 GAA. With increasing reaction time, the curve from the low α-amylase wheat L-9009 behaved very similarly to the sweet potato β-amylase curve (Fig. 1A) in arriving at a limiting nephelos of about 33. The high α-amylase wheat L-9742 proceeded to a final nephelos value of 15, as expected for α-amylase. Wheat L-9730 with intermediate α-amylase activity decreased in turbidity with time at a rate intermediate between the two other wheats. An extract of flour from the L-9742 wheat with high α-amylase activity

was evaluated and was very similar to the whole wheat extract. The results indicate that β-amylase must play an important role in affecting the results obtained with the Model 191 GAA for determining α-amylase. In wheats with low α-amylase, the resultant value is probably entirely due to β-amylase, and the apparatus may be potentially useful for determining β-amylase levels in different wheats that have no sprout damage. As shown in Fig. 2, a linear relationship exists between the increase in α-amylase and β-amylase concentrations and the rate of decrease in turbidity. It would be very difficult, however, to determine the amount of activity attributable to the individual enzymes and this could limit the usefulness of the method.

Further experiments were done to determine if α-amylase could be determined by selectively inhibiting the β-amylase with mercuric chloride and increasing the sensitivity of the Nephelos scale. Thus, addition of 5 μl of 1×10^{-2} $HgCl_2$ to the extracts depicted in Fig. 1A caused complete inhibition of the β-amylase reaction but had no effect on either the malted wheat or fungal α-amylase. Upon addition of $HgCl_2$ to the wheat extracts in Fig. 1B, only the L-9742 wheat showed any discernible decrease in nephelos with time. After increasing the sensitivity of the Nephelos Mode 10 times, however, addition of 5 μl of 1×10^{-2} $HgCl_2$ to extracts gave the results shown in Fig. 3.

Decreases in nephelos occurred with the wheat and flour extracts, as predicted by their falling numbers or amylograph viscosity. The curves were erratic, however, as might be expected with such small changes in nephelos. The required sensitivity was still not attainable because nephelos changes in wheat with a falling number of 265 or an amylograph viscosity of 190 could not be accurately measured. Consequently, α-amylase activity and Model 191 GAA values could not be correlated even when using the 2-min mode. The problem in adding $HgCl_2$ to extracts, of course, is that it does not always completely inhibit β-amylase or that it may partially inactivate α-amylase. Use of this chemical, therefore, does not seem a plausible solution to measuring α-amylase with the Model 191 GAA.

Preferential heat inactivation of the β-amylase is another possible way to determine α-amylase selectively. Heat inactivation curves of a sound wheat, followed by amylase determination on the Model 191 GAA, indicated 10% inactivation at 50° C, 50% at 57° C, and 90% at 64° C after 10 min. This provided corroborative evidence that β-amylase was the main enzyme measured with the Model 191 GAA, as wheat α-amylase is much more thermostable (Tkachuk and Kruger 1974). Some α-amylase could be expected to be inactivated at 64°C, however, and would lead to erroneous

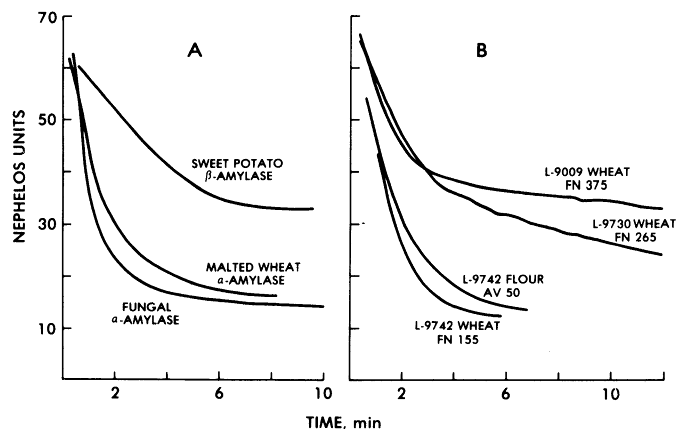

Fig. 1. A, Change in Nephelos with time caused by 5-μl additions of sweet potato β-amylase and 200 μl each of fungal and malted wheat α-amylases to 3 ml of amylopectin substrate. **B,** Change in Nephelos with time caused by 200 μl additions of extracts of L-9009, L-9730, and L-9742 wheats and L-9742 flour to 3 ml of amylopectin substrate.

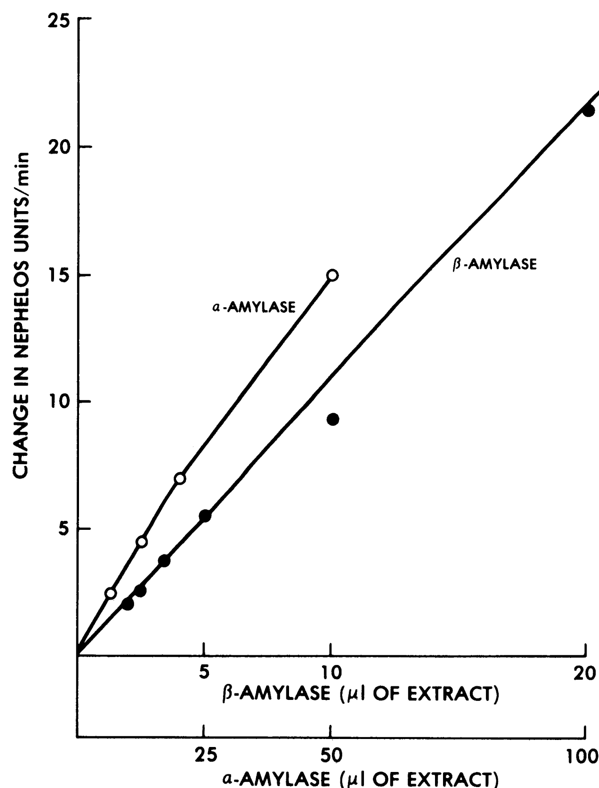

Fig. 2. Change in Nephelos with increasing concentrations of sweet potato β-amylase (•) and malted wheat α-amylase (o).

TABLE I
Comparison of α-Amylase Determinations by the Falling Number, Amylograph, Automated Fluorometric and Model 191 GAA Methods

	Wheats			Flours		
Sample	Falling No. (sec)	α-Amylase Fluorometric Method (mg of maltose/min/g $\times 10^{-3}$)	Model 191 Grain Amylase Analyzer (units/min)	Amylograph Viscosity (B.U.)	α-Amylase Fluorometric Method (mg of maltose/min/g $\times 10^{-3}$)	Model 191 Grain Amylase Analyzer (units/min)
L-9007	400	1.28	552	775	0.55	600
L-9009	375	1.02	498	630	0.62	602
L-9011	350	2.21	548	500	1.01	560
L-9680	345	1.90	570	445	0.98	567
L-9730	265	17.11	620	190	9.52	573
L-9731	225	23.30	660	185	16.08	660
L-9741	195	54.00	662	75	18.09	600
L-9742	155	167.1	880	50	73.04	650

results if heat-treatment of extracts was used before amylase analysis. Addition of excess β-amylase before addition of wheat or flour extracts also could be a possible solution to the use of this apparatus. Unfortunately, as shown in Fig. 1A, the nephelos of the resulting β-limit dextrin is quite low, and this would compress greatly the utilizable nephelos scale. Increased sensitivity of future models of this apparatus may overcome this problem, particularly if the stability can also be maintained.

Flour Supplementation

Flours low in α-amylase often require α-amylase supplementation to achieve optimum gas production and optimization of breadmaking quality.

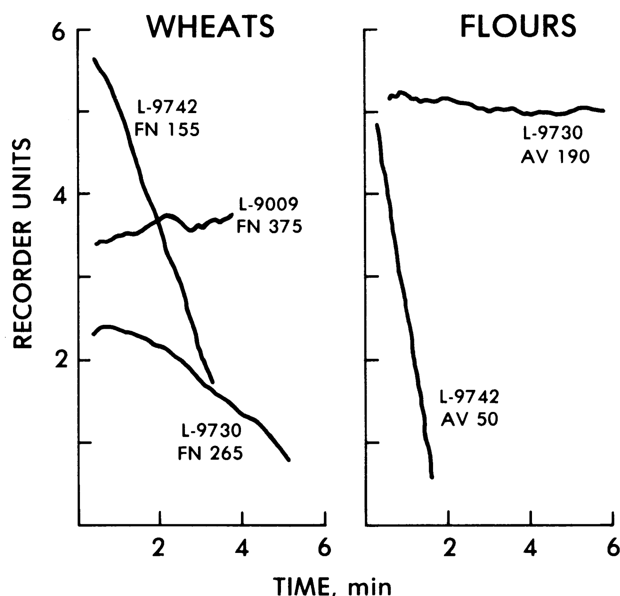

Fig. 3. Change in Nephelos with time caused by additions of 5 μl of $1 \times 10^{-2} M$ $HgCl_2$ and 200 μl of extracts of L-9009, L-9730, and L-9742 wheats and L-9730 and L-9742 flours to 3 ml of amylopectin. Nephelos scale expanded 10 times.

TABLE II
Model 191 GAA Values of Fungal, Malted Barley, and Malted Wheat α-Amylases Used in Flour Supplementation

Enzyme Source	α-Amylase Activity (SKB/g)	Enzyme Source in Solution (mg/100 ml)	GAA Reading (units/min)	Calculated[a] α-Amylase Activity (SKB/g)
Standard				
Fungal α-amylase				
(Calbiochem)	4,665	6	309	...
		8	422	...
		10	548	...
		12	642	...
		14	748	...
		16	883	...
Unknowns				
Fungal α-amylase				
(Miles Lab)	7,500	1	939	7,940–8,505
Fungal α-amylase				
(Novo Corp.)	5,800	1	722	6,100–6,540
Fungal α-amylase				
(Pennwalt)	5,400	10	620	6,240–5,616
Malted Barley	144	60	261	367–394
Malted Wheat	60	300	570	161–172

[a]Activity of sample = activity of "standard"/GAA reading of "standard" × concentration of "standard"/concentration of "unknown" × GAA reading of "unknown."

The Model 191 GAA was tested for its ability to measure such α-amylase supplements. Increasing concentrations of a standard fungal preparation resulted in a linear increase in Model 191 GAA values (Table II). These values were then used in calculating the α-amylase activities of a number of "unknown" supplements. Table II shows that fungal α-amylase supplements could be measured with a 5–15% error, but malted wheat and barley supplements could not be measured satisfactorily. In contrast to the fungal α-amylase supplements, the malted barley and wheat α-amylase supplements contained β-amylase, which undoubtedly affected the results. After addition of a supplement to a flour, the resulting α-amylase levels could not be measured. As shown in Table III, very small differences were found in Model 191 GAA values in a flour with varying supplementation levels of either fungal or barley malt α-amylase, again indicating the overriding influence of wheat flour β-amylase on the Model 191 GAA values.

TABLE III
Model 191 GAA Values Resulting from Supplementation of a Hard and Soft Wheat Flour with Fungal and Malted Barley α-Amylases

Flour with α-Amylase Added (SKB/100 g)	Source of α-Amylase	Model 191 Amylase Grain Analyzer (units/min)	Amylograph Regular	Amylograph Modified
Hard wheat; bakers flour				
0	Malted barley	447		1,100
16	Malted barley	512	575	
22	Malted barley	497	500	
29	Malted barley	509	435	
19	Fungal	498		260
29	Fungal	491		195
Soft wheat; cookie, cracker flour				
0	Malted barley	367		915
13	Malted barley	408	1,250	
19	Malted barley	399	1,020	
25	Malted barley	433	880	
32	Malted barley	399	780	
6	Fungal	397		650
12	Fungal	361		425
18	Fungal	354		300
24	Fungal	361		255
48	Fungal	408		120

TABLE IV
Comparison of Saccharifying Activity (Barleys), α-Amylase by the Dextrin-iodide Method (Barleys and Malt), and the Model 191 GAA α-Amylase Activity in the Presence and Absence of 5 μl of $10^{-2} M$ Mercuric Chloride

Sample	Barley Saccharifying Activity (°Linter/g)	α-Amylase Dextrin-Iodide Method Activity (IDC units/g × 10^{-3})	Nephelos Activity[a] Without Hg++	Nephelos Activity[a] With Hg++
Barley				
1	124	0.23	380	0
2	162	0.29	540	0
3	163	0.43	520	0
4	183	1.42	620	Trace
5	193	0.48	580	0
Malt				
1	...	63	78×10^2	76×10^2
2	...	121	114×10^2	100×10^2
3	...	121	104×10^2	104×10^2
4	...	123	102×10^2	100×10^2
5	...	158	140×10^2	142×10^2

[a]Activity is expressed as change in Nephelos/min/g.

Barley and Barley Malts

In the brewing and malting industry, barleys are examined before and after malting for their saccharifying activity. Mature barleys contain mainly β-amylase, whereas the malted barleys contain a large excess of α-amylase in relation to β-amylase. Because of this, it appeared that the Model 191 GAA might find applicability for more rapidly determining levels of the two enzymes if there was little contamination of one enzyme by the other. Barleys and malts were assayed for amylase by diastatic power (Meredith and Bendelow 1955) and/or the IDC Method (Briggs 1961) and with Model 191 GAA. The results are shown in Table IV. Mercuric chloride also was added in the Model 191 GAA method to inactivate the β-amylase preferentially. The barley α-amylase values were very low and the saccharifying values reflected the levels of β-amylase. The Model 191 GAA method, therefore, could measure such β-amylase levels. Addition of $HgCl_2$ eliminated any meter readings, confirming that β-amylase was being measured. Sample 4 had higher α-amylase than the other samples, and this barley also had a higher reading with the Model 191 GAA. Caution would be required, therefore, to ensure that barleys were low in α-amylase if the Model 191 GAA replaced saccharifying activity determinations. Malted barleys had very high α-amylases and the Model 191 GAA values were correspondingly large. Addition of $HgCl_2$ confirmed that such values were almost entirely due to α-amylase. Although the lowest and highest α-amylase malts ranked in the same order with the Model 191 GAA, a larger number of samples should be tested to establish the correlation between the two methods.

CONCLUSIONS

The Model 191 GAA has potential usefulness to the cereal industry for measurement of amylase. For example, it can satisfactorily measure the potency of fungal α-amylase supplements. With amylopectin as substrate, it can measure various concentrations of α- or β-amylase. The analyzer cannot, however, differentiate between the effects of these two enzymes, which are both normally present in cereals. This limits its present usefulness, particularly for measurement of α-amylase. Selective inactivation with $HgCl_2$ or heat-treatment cannot guarantee that β-amylase is completely destroyed or that α-amylase has not been affected. Manufacturer refinements to the prototype method and instrument such as developing a β-limit dextrin substrate and increasing the sensitivity of the analyzer may ultimately provide a simple, rapid assay of α-amylase.

ACKNOWLEDGMENTS

The authors wish to thank M. Retzik, Perkin Elmer Corporation, for loan of the Model 191 Grain Amylase Analyzer and substrate.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1976. Approved Methods of the AACC. Method 22-10. The Association: St. Paul, MN.

BRIGGS, D. E. 1961. A modification of the Sandstedt Kneen and Blish assay of α-amylase. J. Inst. Brew. 67:427.

INTERNATIONAL ASSOCIATION FOR CEREAL CHEMISTRY. 1967. Determination of "falling number" (according to Hagberg-Perten) as a measure of the degree of alpha-amylase activity in grain and flour. ICC standard No. 107.

LEHANE, D. P., WISSERT, P. M., LUM, G., and LEVY, A. L. 1976. Nephelometric measurement of alpha-amylase and lipase activity in biological fluids. In: Manual of Procedures for the Seminar on Clinical Enzymology. p. 104. Institute for Clin. Sci.: Philadelphia, PA.

MacGREGOR, A. W., LaBERGE, D. E., and MEREDITH, W. O. S. 1971. Changes in barley kernels during growth and maturation. Cereal Chem. 48:255.

MARCHYLO, B., and KRUGER, J. E. 1978. A sensitive automated method for the determination of α-amylase in wheat flour. Cereal Chem. 55:188.

MEREDITH, W. O. S., and BENDELOW, V. 1955. Reliability of prediction tests for malting quality of barley. Can. J. Agric. Sci. 35:252.

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

TKACHUK, R., and KRUGER, J. E. 1974. Wheat α-amylases. II. Physical Characterization. Cereal Chem. 51:508.

ZINTERHOFER, L., WARDLAW, S., JATLOW, D., and SELIGSON, D. 1973. Nephelometric determination of pancreatic enzymes. I. Amylase. Clinica Acta 43:5.

[Received December 4, 1978. Accepted February 6, 1979]