A Rapid Colorimetric Procedure for Estimating the Amylose Content of Starches and Flours[1]

P. C. WILLIAMS, F. D. KUZINA, and I. HLYNKA, Board of Grain Commissioners, Grain Research Laboratory, Winnipeg 2, Manitoba

ABSTRACT

A rapid colorimetric test is described for estimating the amylose content of starches and flours. The principle of the test lies in the blue color developed by the addition of an iodine reagent to a solution containing the amylose under standardized conditions. The precision, rapidity, and simplicity of the procedure are considered to be superior to those of existing methods. Results are computed by means of a regression equation. An amperometric procedure was used as a standard method for amylose determination. The test has been applied to a series of starches varying very widely in amylose content and also to several straight-grade and patent flours. Possible applications of the procedure to some of the problems concerned with the baking and cooking quality of flour and semolina products are discussed.

In view of reports of a relation between the cooking quality of rice and amylose content (1,2), the possibility occurred to us of a similar varietal relation between the amylose content of wheat starches and such factors as cooking quality of spaghetti and the staling properties of breads. This communication describes a simple colorimetric test which can be used to screen large numbers of samples rapidly and accurately for amylose content. The test is based on the starch-iodine blue test which appears to have been originally applied to the routine testing of cereals by Roberts and co-workers (3). A modification of this test has since then been described by Halick and Keneaster (4).

The determination of amylose in starch and other products involves a gelatinization step as a preliminary to solubilization of the material. Because of certain difficulties associated with the gelatinization procedure, described by Halick and Keneaster (4), chemical gelatinization was investigated. A somewhat lengthy process for chemical gelatinization was outlined by Williams and co-workers (2). After some preliminary studies involving combinations of dimethyl sulfoxide with sodium and potassium hydroxide, it was found that a small sample of starch, flour, or even finely ground whole grain would disperse satisfactorily in 0.5N KOH in a few minutes, and the amylose was immediately measurable by means of an iodine reagent. For the purpose of comparison an amperometric test was perfected, based on the principles of the method of Bates et al. (5). Pure potato amylose was used throughout as the standard for both tests. While the amperometric method gave an absolute value for amylose, the rapid colorimetric test gave results in absorbance figures which could be translated into amylose content by means of a regression equation.

MATERIALS

Starches and Their Preparation

All starches were prepared in the laboratory, with the exception of the sorghum

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and maize starches, which were the kind gifts of T. J. Schoch of Corn Products Co., Chicago, Illinois, and C. H. Hullinger of American Maize-Products, Roby, Indiana, respectively. Whole grains were ground in a Wiley mill to pass through a 1-mm. sieve. Tapioca and sago were treated similarly; the roots and tubers were dried and wet-milled to a slurry in a Waring Blendor. Starch was washed out of "dough-balls" made with the meal, or from the slurries by enclosing the dough-ball or slurry in a bag made of 13xx flour silk. The washing-out process was carried out with tap water and was continued until no further starch could be washed out even after vigorous manipulation. The starch suspensions were centrifuged at 1,300 X g to remove fibrous debris and some soluble material. After removal of the surface layer of debris, the starches were resuspended in distilled demineralized water and recentrifuged at gradually increasing speed up to 5,000 X g. It was found that this method of centrifugation gave better separation of damaged granules and other cellular material. This process was repeated three times and was followed by two further centrifugations from 50% ethanol and absolute ethanol. The ethanol centrifugations effectively removed most of the water and prevented caking of the starches. Finally, the starches were filtered by suction and washed once with absolute ethanol and twice with anhydrous ether. It was found that high-vacuum drying of starches (without freezing, but in a freeze-dryer) was also quite satisfactory for drying starches without caking, but the alcohol-ether washing process resulted in a purer preparation.

REFERENCE AMPEROMETRIC METHOD

Amperometric titration apparatus was assembled after Kolthoff and Harris (6) with two modifications. The mercury-mercuric oxide-barium hydroxide half-cell of Samuelson and Brown (7) was used, together with a hook-type rotating platinum electrode (E. H. Sargent & Co.). The rotating platinum electrode was fitted with a suitable geared motor and the circuit was laid out as in Fig. 1. A Sefram

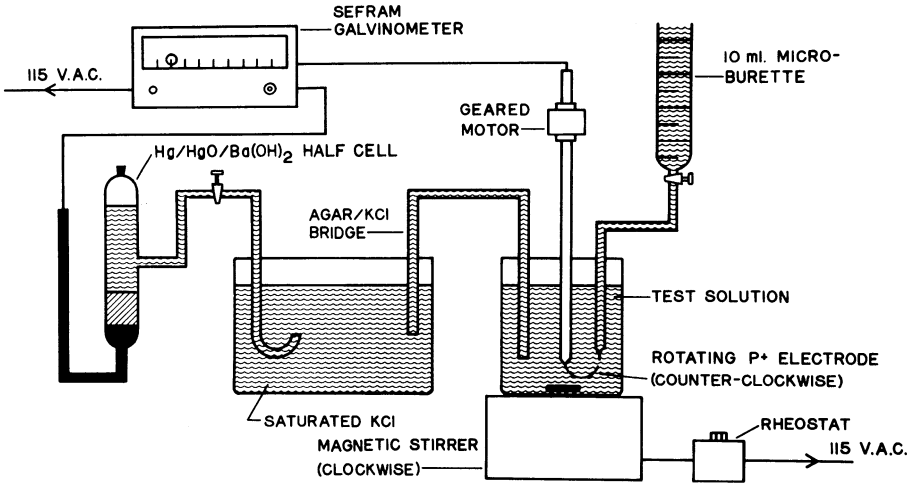

Fig. 1. Schematic diagram of circuit used for amperometric determination of amylose.

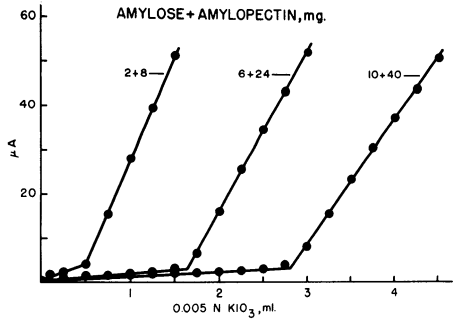

Fig. 2. Titration curves obtained on titration of increasing amounts of amylose at constant amylose-amylopectin ratio.

galvanometer was used for this work, but any sensitive galvanometer having a maximum deflection of about 100 microamp. would be suitable.

Procedure

The galvanometer was zeroed, with the use of water, a reagent blank, or an amylopectin blank. For each analysis, suitable samples of amylose, amylopectin, or starch were dispersed in 10 ml. of 0.5N KOH solution for 5 min. with a magnetic stirrer. Distilled water (75 ml.) was added, followed by 10 ml. of 1.0N HCl and 5 ml. of 0.4N KI solution, making the total volume up to 100 ml. The solution was titrated against 0.005N KIO_3 solution by means of the circuit illustrated in Fig. 1, and was stirred continuously with a magnetic stirrer working in the opposite direction to the rotating platinum electrode. This assisted in maintaining the sample in complete dispersion, and also stabilized reaction time. Potassium iodate solution was added in 0.25-ml. increments. Galvanometer readings were taken at zero time and 1.5 min. after the start of each addition of KIO_3. The rotating electrode was driven at 600 r.p.m. by a suitable motor. After each analysis, the bridge, electrode, and buret tip were rinsed with demineralized distilled water and immersed in a vial of distilled water.

Typical titration curves are illustrated in Fig. 2. The end point was determined by extrapolation. A perpendicular was dropped from the point of intersection of the two arms of the curve to determine the precise location of the end point. To check precision of the test a sample of 6 mg. pure amylose was analyzed each day during the course of the experiments. The amount of KIO_3 solution required ranged from 1.70 to 1.72 ml., which represented a variation from 19.06 to 19.28% iodine absorbed by the amylose with a mean value of 19.24% (N = 8, coeff. of variability = 1.2). The literature value for the iodine absorption of potato amylose is 19.5 (8), which gives the amylose used in the work a purity of 98.7%.

Calculation of Results

The amount of iodine absorbed by a given weight of amylose can be calculated as follows: $(63.46/W) \times T$ = % iodine absorbed, where W is the sample weight in mg. and T is the titration value in ml. 0.005N KIO_3 obtained for the amylose sample. The amount of amylose present in a starch sample is then calculated from the

TABLE I. CALIBRATION OF AMPEROMETRIC AND COLORIMETRIC METHODS FOR THE MEASUREMENT OF AMYLOSE IN STARCHES

Weight of Amylose mg.	Weight of Amylopectin mg.	Starch Equivalent[a] mg.	Iodine Affinity ml. KIO_3	Absorbance, 625 mμ	Color Developed
2	8	10	0.57	0.112	Light gray-blue
4	16	20	1.14	0.250	Medium blue
6	24	30	1.70	0.414	Medium dark blue
8	32	40	2.29	0.536	Dark blue
10	40	50	2.90	0.670	Very dark blue
12	48	60	3.46	0.792	Very dark blue

[a] "Starch equivalent" refers to the amount of starch at constant amylose:amylopectin ratio represented by the weights of amylose and amylopectin taken.

formula $(63.46 \times T^1)/X = 19.24$, where T^1 = titration value in ml. 0.005N KIO_3 obtained for the starch sample, 19.24 is average value obtained experimentally for the percentage of iodine absorbed by the standard amylose, and X is the weight of amylose present in the sample. From this formula, we get $X = (63.46 \times T^1)/19.24$, which means that X, the weight of amylose present = $3.298 \times T^1$. The percentage of amylose in a starch sample is given by $(X/W) \times 100$, where W is the weight of starch sample on moisture-free basis.

For the standardization of the test, pure amylose was analyzed in 2-mg. increments up to 10 mg. Amylopectin was added to simulate the conditions encountered in wheat starch, which contains about 20% amylose. Results of the preparation of the standard graph for amylose are included in Table I.

For analysis of the starch samples quoted below, a preliminary analysis was carried out to establish the approximate level of amylose present in the starch. Then a second analysis was carried out with the appropriate amount of starch necessary to give 6 mg. of amylose, the amount used as a standard throughout the work. Amylopectin blanks were found significant and in each case an amylopectin "blank" was analyzed, with the appropriate amount of amylopectin based on the amylose present. A commercial sample of amylopectin was used for this purpose.

RAPID COLORIMETRIC METHOD

In a later section, reference will be made to two colorimetric procedures. The first of these involved the determination of the amylose blue color in a series of starch samples in which the amount of starch was varied so as to give approximately 6 mg. of amylose in each determination. This involved an extra analysis to establish the approximate level of amylose present as a preliminary to the calculation of sample weight, and was carried out for the purpose of comparison with the amperometric test. The second represents the rapid procedure, which is based on analysis of a 20-mg. sample of weight regardless of the amount of amylose present. Experimental details are given only for the rapid method, which forms the subject of the paper. The only difference in procedure is the extra analysis to establish the approximate level of amylose present, and the subsequent weighing of a sample weight to give about 6 mg. of amylose.

Reagents

A. Stock iodine solution: potassium iodide (20 g.) was weighed into a 100-ml. beaker together with 2.0 g. resublimed iodine. The reagents were dissolved in the minimum of water and carefully diluted to 100 ml. in a volumetric flask.

B. Iodine reagent: 10 ml. of stock solution A was pipetted into a volumetric flask and diluted to 100 ml. with distilled water.

Procedure

Starch samples (20 mg.) were weighed into a 50- or 100-ml. beaker. Exactly 10 ml. 0.5N KOH solution (28.055 g. per liter) was added, and the starch was dispersed with a stirring rod or magnetic stirring bar for 5 min. or until fully dispersed. (Although most starch and flour samples disperse readily, a "difficult" sample may take as much as 20 to 30 min. to disperse.) The dispersed samples were transferred to 100-ml. volumetric flasks and diluted to the mark with distilled water, with careful rinsing of the beaker. An aliquot of the test starch solution (10 ml.) was pipetted into a 50-ml. volumetric flask, and 5 ml. 0.1N HCl (8.17 ml. AR conc. HCl per liter) was added, followed by 0.5 ml. of iodine reagent B. The volume was diluted to 50 ml. and the absorbance of the blue color was measured at 625 mμ after 5 min. The color was found to be stable for several hr. Several of the starch dispersions, together with the amylose control, were analyzed at intervals up to 30 days after dispersion. In all cases the stability of the dispersions was verified, indicating that the dispersions can be stored for several days if necessary, before analysis.

Calibration of the Colorimetric Test

Increments of amylose from 2 to 12 mg. were analyzed by the colorimetric method, but with appropriate amounts of amylopectin added to maintain the amylose content of the mixture at the 20% level in a manner similar to the practice adopted in the amperometric method. Results of the calibration are given in Table I and summarized in Fig. 3, in which amylose content is plotted against both absorbance and ml. iodate used up by the amylose. It is clear that the two

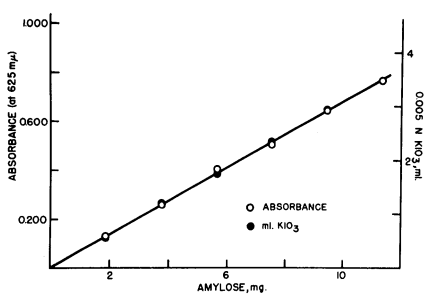

Fig. 3 (left). Calibration curves of amylose content as determined by amperometric and colorimetric procedures.

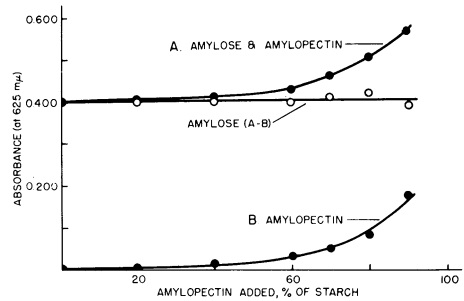

Fig. 4 (right). Effect of amylopectin on colorimetric procedure for the determination of amylose.

TABLE II. EFFECT OF AMYLOPECTIN ON ABSORBANCE OF A CONSTANT AMOUNT OF AMYLOSE

Weight of Amylose mg.	Weight of Amylopectin mg.	Ratio Amylose: Amylopectin	Starch Equivalent[a]	Absorbance, Amylose and Amylopectin	Absorbance, Amylopectin Alone	Net Absorbance, Amylose[b]
6	0	100:0	6	0.401	...	0.401
6	1.5	80:20	7.5	0.406	0.005	0.401
6	4	60:40	10	0.410	0.014	0.396
6	9	40:60	15	0.425	0.032	0.393
6	14	30:70	20	0.457	0.052	0.405
6	24	20:80	30	0.498	0.083	0.415
6	54	10:90	60	0.560	0.175	0.385

[a] "Starch equivalent" refers to the amount of starch at different amylose:amylopectin ratios represented by the weights of amylose and amylopectin taken.

[b] "Net absorbance amylose" is the absorbance of the amylose-amylopectin mixture less the absorbance contributed by the amylopectin. The absorbance of the amylopectin was determined separately.

procedures gave identical calibration slopes when plotted in terms of the units actually used for calibration.

Effect of Amylopectin Concentration on the Colorimetric Procedure

It was considered likely that where high ratios of amylopectin to amylose prevailed, deviations could occur from the calibration graph, which was prepared with a constant ratio of amylose:amylopectin. A series of samples of 6 mg. of amylose were carefully weighed, and amylopectin was added so as to vary the ratio of amylose to amylopectin from 100:0 to 10:90. Analyses were also carried out with the amounts of amylopectin alone that were used in this experiment. The results are given in Table II and Fig. 4. The absorbance of the 7 X 6-mg. samples of amylose studied when the value of the amylopectin solutions is subtracted from the total (amylose + amylopectin) values was remarkably constant and the absorbance of 6 mg. amylose had a mean over-all value of 0.400 with a standard error of 0.00254 absorbance units (coeff. of variability = 0.64).

Amylose Content of Various Starches

In view of the effect of amylopectin on the absorbance value of amylose, it was decided to carry out three series of determinations of the amylose content of selected starches. The first series (method 1) was analyzed by means of the amperometric procedure with correction for amylopectin. The amount of starch used was adjusted to contain approximately 6 mg. amylose after a preliminary analysis. This series was to be taken as the standard procedure. The second series (method 2) was analyzed by the colorimetric method, but once again the amount of starch was varied so that about 6 mg. amylose was present. Corrections for the amylopectin were applied to each separate starch sample by a separate analysis. The object of the study was to realize a rapid test for the estimation of amylose, and the third series (method 3) was analyzed with 20 mg. of starch throughout, with no correction for amylopectin, the results being computed by means of a regression

TABLE III. AMYLOSE CONTENT OF VARIOUS STARCHES AS DETERMINED BY THREE METHODS OF ANALYSIS

Starch	Weight of Starch Taken (Methods 1 and 2) mg. "as is"	Amylose Present in Sample Analyzed mg., d.b.	Amylose Content Method 1 (Amperometric) %, d.b.	Amylose Content[a] Method 2 (Colorimetric) %, d.b.	Amylose Content[a] Method 3 (Colorimetric) %, d.b.
Wrinkled pea	10	5.73	63.3	62.7	62.5
Smooth (field) pea	18	5.89	36.0	35.6	34.0
Bean	18	5.92	35.9	33.7	33.3
Potato	30	6.06	21.8	23.6	21.8
Sweet potato	30	5.60	20.5	22.4	19.8
Oat	30	5.02	18.5	21.8	18.6
Barley	30	5.95	21.9	24.8	21.9
Rice	35	5.18	16.9	20.2	18.2
Wild rice	40	5.61	16.3	15.9	16.8
Foxtail millet	30	5.66	20.7	23.6	20.7
Poso millet	30	5.86	19.2	20.4	20.0
Tapioca	37	6.05	18.0	20.7	15.9
Sago	37	6.02	17.9	20.8	17.3
Arrowroot	30	5.82	22.1	26.6	24.3
Parsnip	65	6.02	10.2	14.1	9.7
Buckwheat	27	6.08	25.2	25.6	23.0
Sorghum	37	5.95	18.3	22.2	19.8
Waxy sorghum	500	4.79	0.7	1.0	0.8
Maize	33	5.60	19.8	23.7	21.0
Amylomaize V	17	6.57	45.7	46.2	46.2
Amylomaize VII	12	5.50	53.9	56.2	55.4
Waxy maize Amioca	1000	3.95	0.2	0.1	0.5
HRS wheat	30	5.92	22.7	26.5	23.4
Durum wheat	30	6.02	22.8	26.3	23.2
SWW wheat	30	5.99	22.8	25.8	23.2
HRS wheat, wet-milled	30	6.02	22.5	26.6	22.4
HRS wheat, total starch[b]	35	6.15	19.6	23.0	20.1
Durum wheat, total starch	33	5.79	19.6	24.4	21.4
SWW, total starch	30	5.50	20.5	23.5	20.2
Rye	30	6.15	21.8	23.2	22.4
Triticale	30	5.68	20.3	22.0	20.8

[a]Method 2: amylose determined colorimetrically on a sample weighed on a basis of 6 mg. amylose, and corrected for amylopectin content (see text). Method 3: amylose determined colorimetrically on a 20-mg. sample of starch, uncorrected for amylopectin.

[b]"Total starch" refers to the whole of the material recovered from gluten-washing, after removal of crude dark fibrous material. It contains a certain amount of insoluble pentosan and represents starch of a lower degree of purity.

TABLE IV. REGRESSION EQUATIONS, STANDARD ERRORS OF ESTIMATE, AND COEFFICIENTS OF CORRELATION BETWEEN AMPEROMETRIC AND COLORIMETRIC METHODS FOR DETERMINATION OF AMYLOSE

	Amperometric Method 1	Colorimetric Method 2	Colorimetric Method 3
Method 1	...	Y = 79.03x − 5.07 ±1.48	Y = 85.24x − 13.19 ±1.37
Method 2	0.993	...	...
Method 3	0.994	...	...

equation rather than a standard curve. Full results, including statistical details of the relation of the results obtained by the amperometric method to those obtained by the colorimetric method, with or without correction for amylopectin, are contained in Tables III and IV. Figure 5 illustrates the relation between absorbance readings and the absolute amylose content of the starches as determined by the amperometric method with correction for amylopectin.

Application of the Test to Some Practical Aspects of Cereal Quality

Experiment 1. Three experiments were carried out in this series. The object of the first was to study the possibility of detecting major differences in the amylose content of whole corn grains, for the possible benefit of corn breeders. Five single grains of three types of corn—normal, amylose-free, and high-amylose—were ground singly to pass through a 0.5-mm. screen. The weight and moisture content of each kernel were determined. Moisture content was determined on 100-mg. samples of the finely ground kernel by drying for 1 hr. at 130°C. in an air oven. The average weight of a single kernel was about 210 mg. Samples of 20 mg. of finely ground meal were weighed into 50-ml. beakers and analyzed as described under "Procedure." The results are included in Table V. These preliminary results suggest that the test may provide a rapid, easily automated method for the screening of corn samples differing in amylose content.

Experiment 2. It is fairly well established that retrogradation of starch is in some

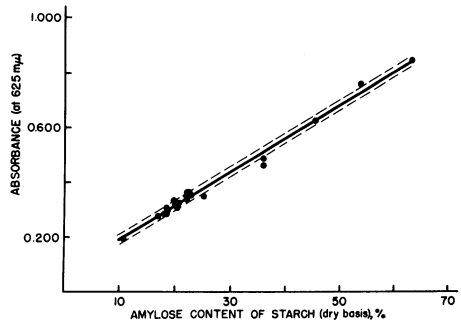

Fig. 5. Relation between colorimetric absorbance values and absolute amylose content of a series of starches as determined by the amperometric procedure with correction for amylopectin.

TABLE V. APPLICATION OF THE RAPID AMYLOSE TEST TO THREE DIFFERENT SERIES OF MATERIALS: ABSORBANCE VALUES[a]

Material	Absorbance	Material	Absorbance
Ground whole corn kernels		Fresh bread crumb A	0.222
A) Normal	0.253 (blue)	2 days old	0.209
	0.247	30 days old	0.168
	0.251		
	0.261	Fresh bread crumb B	0.220
	0.239	2 days old	0.214
Mean	0.250	30 days old	0.168
B) Waxy	0.082 (red-brown)	Fresh bread crumb C	0.228
	0.051	2 days old	0.223
	0.086	30 days old	0.168
	0.069		
	0.064		
Mean	0.070		
C) High-amylose	0.410 (blue)	Semolina A (Pelissier)	0.270
	0.486	Spaghetti A	0.265
	0.446	Cooked spaghetti A	0.253
	0.452	Semolina B (Ramsey)	0.257
	0.437	Spaghetti B	0.273
Mean	0.456	Cooked spaghetti B	0.265

[a] Amylose content may be computed by means of the formula $Y = 85.24x - 13.19$, where Y = amylose content and x = absorbance value.

way concerned with the staling of bread (9,10). Retrogradation of starch is associated with loss of the capacity of the starch to form colored complexes with iodine (11). The familiar deep blue complex formed with iodine is due largely to the properties of the amylose component (12). Consequently, it should be possible to monitor progressive retrogradation by measuring the progressive decrease in the capacity to form the iodine complex. The second experiment in this series involved a pilot-scale study of the staling of bread crumb. Three breads were baked by the conventional straight-dough procedure with 60-min. fermentation, 30-min. interproof, and 40-min. final proof. Fresh crumb was taken and immediately freeze-dried. Further samples of crumb were stored in plastic containers in a refrigerator. Samples were withdrawn at 4-day intervals, freeze-dried, and ground to a fine powder on a Moulinex mill. Samples of 20 mg. were analyzed for amylose by the procedure described above. Results, included in Table V, show that a gradual decrease in iodine complexing capacity did occur, although the rate of staling as judged by crumb firmness appeared to progress at a much faster rate.

Experiment 3. The final experiment in this short series was an attempt to establish a relation between cooking quality of spaghetti (firmness after about 15 min. of boiling) and amylose content. Spaghetti, semolina, and grain samples of four durum wheats, two graded blends, and two pure varieties were used in the experiment. "Cooked spaghetti" was boiled until the central white core of the spaghetti had disappeared; the time ranged between 13 and 15 min. The cooked material was immediately freeze-dried. All samples were reduced to pass through a 0.5-mm. screen, and 20 mg. was used for analysis. The results (Table V), however,

did not give promise of any conclusive relation between total amylose content and cooking quality of the spaghettis.

Conclusions. The three small-scale studies carried out with the rapid colorimetric test described for starch amylose content suggested that a useful potential field for practical application of the test to whole grain or flour, in addition to pure starches, appears to lie in the screening of high- and low-amylose samples of corn and other grains. It was, however, possible to detect differences in the amylose contents of such materials as flour, bread crumb, semolina, spaghetti and cooked spaghetti, and ground whole grains, as well as in pure starches, and the test would therefore appear to be of value wherever the rapid determination of amylose in a large number of samples is necessary. The amperometric procedure described, while being capable of a high degree of accuracy and precision, is considerably more time-consuming and requires rather specialized apparatus. As a standard procedure with which to compare the rapid colorimetric test described in this communication, however, the amperometric procedure was of immense value.

Acknowledgment

The pure potato amylose used in the standardization of both amperometric and colorimetric procedures was provided by A. W. MacGregor.

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