Enzymatic Determination of Starch in Wheat Fractions[1]

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ABSTRACT

The Donelson-Yamazaki enzymatic procedure for estimation of damaged starch in soft wheat flour has been modified for use in the determination of starch in wheat fractions by a preliminary heat-gelatinization of the starch in the sample. The procedure is applicable to small quantities of sample. Starch determinations were made on wheat meals, flours, starches, and brans, and the data were compared with starch values obtained by the polarimetric procedure. The data were very highly correlated, and both methods performed comparably with respect to precision.

Several methods have been described for starch determinations in wheat and flour. The Association of Official Agricultural Chemists has approved only the polarimetric method for wheat flour (1, p. 165); however, a direct acid hydrolysis method and a combined diastase-acid hydrolysis method have been described for grain and stock feeds (1, p. 289). Recommended sample sizes for these procedures range from 2 to 5 g.

The introduction of micro milling techniques has necessitated the development of micro testing procedures which are applicable to small quantities of wheat and wheat flour. For this purpose, a rapid, enzymatic procedure for starch determinations has been devised for use with 0.10- to 0.20-g. samples. The method is a modification of a recently published method (2) for determining damaged starch in soft wheat flour. The procedure is outlined in this paper, and data are presented to illustrate the close association between these starch determinations and those from the official polarimetric procedure.

MATERIALS AND METHODS

Basically, the procedure relies on heat-gelatinization of dilute starch suspensions and subsequent determination of the gelatinized starch as damaged starch. The important modifications of the damaged starch test include: 1) reduction in sample size from 1.0 to 0.10 or 0.20 g.; 2) heat-treatment to gelatinize the starch; 3) reduction in enzymatic digestion time from 15 to 5 min.; and 4) a specific starch conversion factor of 1.58.

Sample Size

Maximum sample weight for starch, flour, and meal is 0.10 g. Bran samples may be 0.20 g. because of their inherently low starch content.

Enzyme

The enzyme employed is Rhozyme 33, Rohm and Haas Co., which is a fungal (*Aspergillus oryzae*) diastatic enzyme preparation standardized by the manufacturer at 5,000 SKB units/g. Studies with several samples of the

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Mention of specific equipment or products does not constitute endorsement by the U. S. Government over products or companies not named.

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enzyme obtained over a 2-year period have shown that it performs in this procedure without significant variation from batch to batch.

Rhozyme 33 was the only enzyme used in this work, because it was our aim to keep the reagent requirements identical with those for the damaged-starch procedure. Substitution of other enzyme systems may possibly be made after their hydrolytic characteristics with gelatinized starch suspensions have been investigated.

Gelatinization Time

The gelatinization of starch in dilute concentrations is very rapid, and under the conditions of the test requires only 2 min. in a boiling-water bath, as illustrated in Table I. About 97% of the total gelatinization, measured as "reducing sugars" resulting from enzymatic hydrolysis, occurs within 1 min. and remains uniform after 2 min. Thus, the 2-min. boiling-gelatinization period was adopted.

TABLE I

EFFECT OF BOILING TIME ON GELATINIZATION OF VARIOUS WHEATEN MATERIALS
(Dry-weight basis)

BOILING TIME	REDUCING SUGAR			
	Starch	Flour	Meal	Bran
min.	*mg./g.*	*mg./g.*	*mg./g.*	*mg./g.*
0.5	411	347	207	155
1	594	479	380	198
2	613	486	401	203
5	620	490	397	.....
10	617	486	402	199
15	615	483	397	.....

Digestion Time

The rapid hydrolysis of dilute, gelatinized starch suspensions by the enzyme system employed is well illustrated by the digestion curves in Fig. 1.

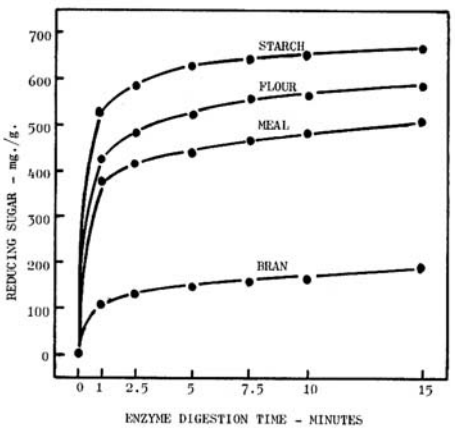

Fig. 1. Curves showing relation between amylase-substrate reaction time and reducing sugar produced for wheat starch, flour, meal, and bran.

The hydrolysis proceeds rapidly, initially, but approaches a steady rate after 5 min. In most cases a 2.5-min. digestion would suffice for these starch determinations. However, the 5-min. digestion was adopted because it is less critical and permits digestion of several samples at one time. (Although the curves in Fig. 1 seem to suggest that a longer digestion period is more practical, our studies have shown that the apparent starch content tends to increase if the digestion time is lengthened substantially beyond 5 min.)

Conversion Factor

In order to express results in terms of starch content, a conversion factor of 1.58 is applied to the reducing-sugar values. This factor represents the reciprocal of the mean fraction reducing-sugar yield from a group of 30 wheat starches which were gelatinized and hydrolyzed in the manner described above. An average hydrolysis of 63.4% (± 0.36% reducing sugars) was obtained on a pure, dry starch basis.

Sample Granulation

Granulation is a factor to be considered with enzymatic starch determinations where wheat meals are being analyzed. Data illustrating the effect of granulation on the apparent starch content of meals from various wheat classes is given in Table II. The data show that granulation is a critical

TABLE II
EFFECT OF GRANULATION OF MEALS FROM VARIOUS WHEAT CLASSES ON APPARENT STARCH CONTENT
(Starch contents on dry-weight basis)

Wheat Class	Granulation		Starch Content	
	Screen Opening	Meshes	Enzymatic	Polarimetric
	in.	*no./in.*	%	%
Durum	0.024	32	56.1	64.1
	.012	62	60.0	64.2
	.008	88	64.1	63.2
Hard	.024	32	63.8	65.3
	.012	62	63.8	65.0
	.008	88	65.1	64.7
Soft	.024	32	65.1	65.3
	.012	62	64.8	65.3
	0.008	88	65.2	64.8

factor with durum samples and is important to a lesser extent in the hard wheats. For both classes, meals should pass through an 88-mesh sieve. Soft wheats do not present serious grinding difficulties, and a 32-mesh grind appears to be sufficient for them.

Starch and flour samples are of suitable granulation and do not need further treatments. Bran samples should be ground finely enough to ensure representative sampling.

Correction for Blanks

Naturally occurring reducing components which are present in wheat fractions will tend to give slightly elevated starch values if corrections are not made for them. The average reducing values for the samples in-

cluded in this work are (mg. reducing sugars/g.): starch, 0.0; flour, 2.0; meal, 3.0; bran, 6.0. The final reducing sugar value should be corrected for the appropriate blank before the starch conversion factor is applied.

SPECIFICATIONS FOR THE PROPOSED METHOD

Reagents

Prepare reagents as described for the diastatic activity of flour (3).

Gelatinization

Suspend appropriate weight of sample in 20 ml. water in 25 × 200-mm. culture tube. Cap lightly with foil and immerse in vigorously boiling water bath for 2 min. Cool rapidly to 30°C. It is necessary to complete the determinations with minimum delay from this point.

Starch Determination

Add 25 ml. buffer and 0.10 g. Rhozyme 33 enzyme preparation (both at 30°C.) to gelatinized suspension and mix uniformly by inverting several times. Incubate at 30°C., for 5 min. At the end of this period add 3.0 ml. 1:9 sulfuric acid and 2.0 ml. 12% sodium tungstate solutions. Mix by inversion, allow to stand for 2 min., and then filter through Whatman No. 4 filter paper, discarding the first 8–10 drops. Pipet 5.0 ml. of the filtrate into a 100-ml. centrifuge tube containing exactly 10.0 ml. of 0.05*N* potassium ferricyanide solution. Immerse the centrifuge tube in a vigorously boiling water bath for exactly 20 min., keeping the liquid level in the tube submerged in the boiling water throughout. Cool the tube and contents under running water and transfer the solution to a 125-ml. Erlenmeyer flask. Rinse the tube with 25 ml. acetic acid reagent and add this wash to the contents in the Erlenmeyer flask with thorough mixing. Add 1.0 ml. potassium iodide solution, followed by 2.0 ml. starch indicator solution, and mix thoroughly. Titrate with 0.05*N* sodium thiosulfate to complete disappearance of the blue color. Subtract the volume of sodium thiosulfate used from that of the reagent blank—previously determined by running the procedure as outlined but omitting the sample—to obtain the volume of potassium ferricyanide reduced to potassium ferrocyanide by the reducing sugar in the filtrate. The net value corresponds to a quantity of reducing sugar which may be determined from the AOAC maltose conversion table (3). Determine reducing sugar in sample, correct for reducing sugar blank, and multiply result by 1.58 to obtain starch content of the sample.

RESULTS AND DISCUSSION

For comparative purposes, the AOAC polarimetric procedure for starch (1, p. 165) was chosen as the standard of performance. The two methods were compared for a series of 30 starches, 40 flours, 18 brans, and 39 meals. All determinations were made in duplicate.

Representative comparative starch data for wheat starches, flours, and meals are presented in Table III. The starch samples had been isolated enzymatically from wheat kernels and are lower in starch content than prime starches obtained from flours by wet-fractionation. The wide range of starch values for the meal samples is due to the fact that the wheats varied widely in protein content. Invariably, the starch contents were inversely related to protein levels.

TABLE III

COMPARISON OF POLARIMETRIC AND ENZYMATIC STARCH DETERMINATIONS FOR WHEAT STARCHES, FLOURS, AND MEALS
(Dry-weight basis)

SAMPLE	POLARIMETRIC STARCH CONTENT	ENZYMATIC STARCH CONTENT	SAMPLE	POLARIMETRIC STARCH CONTENT	ENZYMATIC STARCH CONTENT	SAMPLE	POLARIMETRIC STARCH CONTENT	ENZYMATIC STARCH CONTENT
	%	%		%	%		%	%
Starch No.			Flour No.			Meal No.		
1	93.8	94.8	1	77.0	76.3	1	54.5	54.5
2	95.3	96.1	2	77.0	77.9	2	61.2	60.4
3	95.1	96.2	3	78.6	80.1	3	63.5	64.1
4	94.3	94.0	4	79.6	81.2	4	55.8	56.1
5	94.3	94.0	5	77.1	77.4	5	58.9	57.2
6	94.9	95.3	6	75.6	76.9	6	62.2	61.1
7	94.1	93.9	7	77.3	77.9	7	56.5	55.1
8	94.4	94.8	8	78.1	78.2	8	58.7	58.5
9	93.7	94.2	9	79.7	79.5	9	63.2	62.7
10	95.5	95.3	10	80.2	79.8	10	62.9	63.7

TABLE IV

COMPARATIVE RESULTS FOR POLARIMETRIC AND ENZYMATIC STARCH DETERMINATIONS ON BRANS
(Dry-weight basis)

BRAN No.	STARCH CONTENT COMMERCIAL SAMPLE		BRAN No.	STARCH CONTENT LABORATORY SAMPLE	
	Polarimetric	Enzymatic		Polarimetric	Enzymatic
	%	%		%	%
1	18.4	18.8	1	14.3	15.1
2	23.7	23.9	2	14.3	14.6
3	23.6	24.5	3	14.8	15.2
4	26.6	26.8	4	9.3	9.7
5	25.5	25.4	5	14.3	14.6
6	30.2	30.2	6	17.0	17.6
7	26.8	26.7	7	23.8	23.5
8	26.8	26.5			
9	22.2	22.4			
10	26.4	26.7			

Starch values for bran samples are given in Table IV. The "laboratory" samples are from millings made on an Allis-Chalmers experimental mill. The commercial samples were obtained from commercial sources. Over-all, experimentally milled brans averaged 25.1% starch compared with 15.6% starch for commercial millings.

Both methods performed satisfactorily with respect to precision. The standard deviation for the polarimetric method was $s = 0.49\%$ for pooled samples, compared to 0.58% for the enzymatic procedure. Comparable precision data for sample groups are presented in the table below.

A very highly significant correlation coefficient of $+0.99$ ($n = 127$) was obtained between the two methods when all the samples were pooled. This practice, however, tends to produce an abnormal distribution of the data favoring a stronger correlation. A better, more indicative practice is to

Sample	Standard Deviation	
	Polarimetric	*Enzymatic*
	%	%
Starch	0.92	1.13
Flour	0.74	0.66
Meal	0.39	0.79
Bran	0.63	0.43

treat each group of samples independently and obtain correlations between the methods within each group. These correlations are given in the table below.

Sample	*n*	*r*
Starch	30	0.90**
Flour	40	0.93**
Meal	39	0.97**
Bran	18	0.99**

SUMMARY

An enzymatic method for the determination of starch in wheat fractions has been described. Although this method is empirical and dependent upon strict adherence to treatment steps and to the use of a unique conversion factor, it nevertheless offers a rapid, convenient method for starch which agrees well with an officially approved procedure. No specialized equipment is needed and the materials and techniques are common to most cereal laboratories. An operator should be able to complete 35 to 50 determinations per day.

Acknowledgment

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