Enzymatic Procedure for Determination of Starch in Cereal Products[1]

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ABSTRACT

An enzymatic procedure was developed for measuring the starch content of cereal products. The procedure requires about 4 hr to perform and employs standard laboratory equipment. It incorporates a short gelatinization step at 100°C, high temperature (85°C) α-amylase hydrolysis, and conversion of starch to glucose at 60°C with glucoamylase. Readily available commercial enzymes were employed. The new procedure provides accurate starch values as shown by comparison with standard procedures. Precision of the test is approximately ±1.5% starch. Results indicate its applicability to a wide range of cereal products.

In 1970, the AACC Starch and Pentosans Committee began an investigation into new and improved methods for determining starch in cereal products. After surveying the literature, it was decided to examine a procedure that incorporated the use of enzymes specific in their catalytic action on starch and glucose. It was believed that relatively pure enzymes were available that would eliminate problems inherent in starch methodology existing before 1965.

For instance, the use of glucoamylase in hydrolyzing starch to glucose could eliminate the problem of inversion caused by acid hydrolysis (AACC Method 76-10). Also the use of glucose oxidase would reduce the uncertainties associated with empirical methods of measuring glucose, such as reducing sugars (AACC Methods 76-30A, 80-60, 80-68; Underkofler et al 1943) and certain colorimetric techniques (Dubowski 1962, Saunders et al 1970). The use of enzymes could eliminate the problem of materials other than starch contributing to the angle of rotation as measured by polarimetric procedures (AACC Method 76-20).

Of the methods using glucoamylase and glucose oxidase (Libbey 1970, Rutthoff et al 1966, Thivend et al 1965), the method according to Thivend et al (1965) was selected for examination because of its acceptance in Europe as a standard starch procedure and for its recognized utility in this country (Thivend et al 1972).

The Thivend method employs the use of an autoclave to gelantinize starch. Due to limited availability of autoclaves to most members of the Starch and Pentosans Committee, the gelatinization procedure was modified. After three years of collaborative studies, the Committee decided that the modified method was not acceptable. The original procedure was then examined by those members who had autoclaves. Results led to recommendation of the Thivend procedure for First Approval for the AACC Approved Methods.

However, the usefulness and acceptability of the Thivend procedure do not help laboratories that do not have autoclaves. Consequently, we decided to report on an enzymatic starch method that we developed and have used successfully for several years. This procedure uses an ordinary water bath to gelatinize starch followed by hydrolysis with both α-amylase and glucoamylase.

Preliminary results of this procedure were discussed at the 61st Annual Meeting (Baur and Alexander 1976). An extensive study of the rapid version of the procedure was conducted during the past year, and these results are being reported.

MATERIALS AND METHODS

Cereal Products

Corn flour, corn germ, sorghum grits, corn-soy-milk (CSM), soy-fortified sorghum grits (SFSG), and hominy feed (Tables I and III) were obtained from Krause Milling Co.; wheat clears (Tables I and III) from the Pillsbury Co.; and corn starch (Tables I and III) from Grain Processing Corp. Corn starch, waxy corn starch, sorghum feed, and wheat clears (Table II) were obtained from B. D'Appolonia, North Dakota State University.

The starches (Table IV) were obtained from the following: Waxy sorghum starch and 20F and 80F acid modified starches from Corn Products Co., wheat starch from General Mills, waxy corn starch I from American-Maize Products, waxy corn starch II from National Starch and Chemical Corp., and potato starch from Penick and Ford.

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Enzymes

The enzymes normally used in the procedure are HT-1000 and Diazyme L-100, both obtained from Miles Laboratories, Inc. HT-1000 is an α-amylase (EC 3.2.1.1 α-1,4-glucan glucanohydrolase) of bacterial origin; Diazyme L-100 is a glucoamylase (EC 3.2.1.3 α-1,4-glucan glucanohydrolase) of *Aspergillus niger*. The hydrolysis temperatures employed were indicated as optimum by the supplier. The other enzymes shown in Table V were obtained as follows: α-amylases: Thermamyl 60L from Novo Enzyme Corp., H-39 from Rohm and Haas, and WC-8 from Wallerstein Co.; glucoamylases: AMG 150 from Novo Enzyme Corp.

Starch Procedure

Starch-containing material (1.000 g or less) was ground to pass a 60-mesh screen. The sample was washed into a 250-ml volumetric flask with 200 ml of distilled water.[2]

The flask, fitted with a lead ring, was placed into a boiling water bath (12 × 15-in. bath from Precision Scientific or equivalent) and held for 30 min from the time the flask reached 62° C.[3] The flask was shaken for 10 sec every 2 min for the first 10 min; thereafter, every 5 min.

The flask was cooled to 85° C, 5.0 ml of 0.10% α-amylase (HT-1000) solution was added, and the mixture was hydrolyzed for 15 min in a water bath at 85° C.

The flask was cooled to 60° C, 1.0 ml of glucoamylase (Diazyme L-100) was added, and the mixture was hydrolyzed for 30 min in a water bath at 60° C, then cooled to room temperature and neutralized to phenolphthalein end point with 10% NaOH, and finally diluted to mark with distilled water and filtered.

An aliquot containing between 1 and 9 mg of glucose was removed and percentage reducing sugars was determined according to Underkofler et al (1943).[4]

Percentage starch was calculated according to the following equation:

$$\% \text{ Reducing sugars (as-is)} = \frac{A \times 250 \times 100}{B \times C}$$

where A = weight of reducing sugars in aliquot (mg),
B = volume of aliquot (ml),
C = sample weight (mg).

Reference Starch Procedures

By-difference starch values were obtained by determining values for percent H_2O, protein, fat, crude fiber, ash, and pentosans, calculating the sum of these values, and subtracting the sum from 100%. Standard AACC methods were used to obtain constituent analyses. Polarimetric starch values were obtained by standard AACC Method 76-20.

[2] With pregelatinized products, the sample may be treated with 5 ml of methanol or ethanol prior to the addition of water to eliminate dispersibility problems.
[3] Use a blank flask containing water and a thermometer throughout the procedure.
[4] Other procedures for determining percentage glucose could be employed. Values for an enzyme blank should be subtracted in calculating percentage reducing sugars.

TABLE I.
Comparison of Starch Values for Eight Cereal Products: New Procedure, By-Difference, and Polarimetric Methods

Product	% Starch (Dry Basis)		
	New Procedure[a]	By-Difference[b]	Polarimeter[c]
Corn flour	86.8	86.8	86.4
Corn germ	31.1	50.2	32.3
Wheat clears	74.5	73.7	73.7
Corn-soy-milk	48.9	60.2	
Soy-fortified sorghum grits	70.2	76.6	71.8
Sorghum grits	84.4	86.3	85.9
Hominy feed	49.7	60.6	49.9
Corn starch	99.8	98.3	

[a] Average of six replicates for all products except corn starch where average of 11 replicates.
[b] Sum of H_2O, protein, fat, crude fiber, ash, and pentosans subtracted from 100.
[c] Average of two analyses made by two different laboratories.

RESULTS AND DISCUSSION

The new starch procedure required somewhat less than 4 hr to analyze a series of 5–6 ground samples. More samples could be tested with little additional time required if more or larger water

TABLE II.
Comparison of Starch Values for Four Cereal Products. New Procedure vs. Starch and Pentosan Method[a]

Product	% Starch (Dry Basis)	
	New Procedure[b]	Starch and Pentosan Method[c]
Corn starch	100.0	98.2
Sorghum feed	44.0	44.6
Wheat clears	76.7	75.1
Waxy corn starch	100.0	98.2

[a] Thivend (1965) procedure modified by the Starch and Pentosan Committee to use a pressure cooker for 4 hr at 121° C for gelatinization.
[b] Average of five replicates except for waxy corn starch where average of three replicates.
[c] Average of five replicates.

TABLE III.
Precision of New Starch Procedure for Eight Cereal Products[a]

Product	% Starch (Dry Basis)	Standard Deviation (%)	Range (%)
Corn flour	86.8	0.89	2.4
Corn germ	31.1	0.85	2.2
Wheat clears	74.5	0.93	2.3
Corn-soy-milk	48.9	0.89	2.2
Soy-fortified sorghum grits	70.2	0.77	2.1
Sorghum grits	84.4	0.48	1.2
Hominy feed	49.7	0.85	2.4
Corn starch	99.8	1.30	3.2

[a] Values calculated from the average of six replicates for each product except corn starch where 11 replicates were obtained.

TABLE IV.
Starch Content of Seven Industrial Starches[a]

Product	% Starch (Dry Basis)	Standard Deviation (%)	Range (%)
Wheat starch	99.0	0.89	2.1
Waxy corn starch, I	99.0	1.09	2.4
Waxy corn starch, II	99.5	0.88	1.9
Waxy sorghum starch	100.8	0.94	2.0
Potato starch	99.0	1.02	2.3
20F Acid-modified corn starch	98.9	1.28	3.5
80F Acid-modified corn starch	98.8	0.97	2.2

[a] Values calculated from the average of four replicates except for waxy sorghum starch and 80F corn starch where five replicates were obtained.

TABLE V.
Comparison of Several Different Enzymes in the New Starch Procedure[a,b]

α-Amylase	Glucoamylase	% Starch (Dry Basis)[c]
Thermamyl 60L	Diazyme L-100	87.6
H-39	Diazyme L-100	88.1
WC-8	Diazyme L-100	86.9
HT-1000	Diazyme L-100	87.5
HT-1000	AMG-150	88.7

[a] Enzyme concentrations were identical except with Thermamyl 60L where 0.1 ml per 100 ml was used. Conversion temperatures were identical except with H-39 where 70° C (recommended by supplier) was used.
[b] Starch content was determined on a sample of corn flour.
[c] Average of two replicates.

baths were employed. This time period is comparable to other published starch methods.

The accuracy of the new procedure was comparable to other published methods. A comparison of values obtained by the new procedure with by-difference and polarimetric methods is shown in Table I. Data for the high starch-containing products (corn flour, wheat clears, sorghum grits, and corn starch) were comparable with by-difference values. However, larger differences existed for samples with low starch content (corn germ and hominy feed) and the soy-containing products (CSM and SFSG). These differences can be explained by the fact that the by-difference method of calculating starch does not account for nonreducing sugars present in corn germ, hominy feed, and the soy-containing products. Also, high by-difference values are expected for high fiber containing products, particularly corn germ and hominy feed, because of low fiber values due to the standard test for crude fiber.

Starch values by the new method were comparable to the polarimetric method, but it should be pointed out that the average polarimetric values shown in Table I do not really reflect the variation obtained between the two laboratories. Values for individual products differed by as much as 5%, apparently the result of difficulties in performing the test. Modifications (centrifugation and filtration) of the standard polarimetric procedure were necessary because of cloudy $CaCl_2$ suspensions, particularly with the less refined flours. Use of the new method clearly avoids these problems.

Values observed by the new procedure also compared well with our results obtained with the Thivend method (Thivend et al 1965) as modified by the Starch and Pentosans Committee. Results with two commercial starches and two less refined cereal products are shown in Table II.

The precision or reproducibility of the new procedure is based primarily on the precision of the method for detecting glucose (in our case reducing sugars) (Underkofler et al 1943). As indicated earlier (Baur and Alexander 1976), this was determined to be a range of 2.5% starch. Values obtained in our current study (Tables III and IV) indicated an average range of 2.3%, a maximum range of 3.5%, and a maximum standard deviation of 1.3%. Results with a series of seven industrial starches (Table IV) were similar to those obtained with the original eight products examined (Table III).

A small group of different α-amylases and glucoamylases was examined to determine the use of various commercial enzymes in the new procedure. Values obtained were compared to the standard enzymes described in the Starch Procedure under Materials and Methods. Results shown in Table V indicate that starch values for all enzymes examined were comparable.

Although reducing sugars were to detect glucose in our work, most common methods for measuring glucose should also be applicable. Our experience with commercial glucose oxidase preparations was not very encouraging (Baur and Alexander 1976).

Because of the nature of the cereal products of interest to us, no provision was made in the procedure for a sample blank. With products containing measurable amounts of reducing sugars, a sample blank (sample taken through the procedure but without enzyme hydrolysis) should be included, along with the enzyme blank, to ensure meaningful starch values.

No pH adjustments were made with the starch dispersions prior to enzyme hydrolysis. Our experience with the products of this investigation indicated that the natural pH ranges of 4.5 to 6.5 did not adversely affect the reactions. pH can have a significant effect on enzyme activity, however, and it may need to be adjusted for optimum results with a particular cereal product or enzyme preparation.

Although the use of an autoclave may be the preferred method of dispersing starch prior to enzyme hydrolysis, our new method appears to be a satisfactory substitute, employing only common laboratory equipment. It is postulated that the combination of gelatinization in a water bath at 100° C plus α-amylase treatment at 85° C takes the place of the autoclave procedure used in other methods (Rutthoff et al 1966, Thivend et al 1965, 1972).

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