Analysis of Starch in Wheat Milling Fractions[1]

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ABSTRACT

Starch in wheat materials can be determined by chemical, polarimetric, or enzymatic methods. The present work compares the results obtained on a series of wheats and their milling fractions when different analytical methods were used. All methods agree fairly well on high-starch materials. However, with high-fiber fractions, polarimetry gives low values and mixed enzymes give high apparent starch values. Only enzyme methods specific for starch are recommended for analysis of high-fiber wheat products.

At the time we undertook some work on the metabolizable energy of wheat milling fractions, no simple enzymatic procedure for starch determination was available, although several have subsequently appeared (1,2). We devised our own analysis which involves gelatinization by autoclaving, digestion with alpha-amylase (crystalline from hog pancreas), colorimetric determination of reducing sugar, and calculation of starch content from a curve prepared from a wheat-starch standard.

When the method was used to analyze wheat samples whose starch content had recently been determined polarimetrically (3,4,5), the enzymatic results agreed as well as expected for whole wheat, flour, and red dog but were higher than the reported values for shorts, bran, and germ. Consequently, we analyzed another sample of whole wheat and its derived milling fractions by a variety of starch procedures including our own, to see what sort of agreement could be expected in starch analyses with wheat materials.

MATERIALS AND METHODS

The initial wheat and milling fractions examined were seven of the nine samples studied by Farrell et al. for the Millers' National Federation (4,5).

The study of comparative analytical methods was made on wheat materials supplied by International Milling from a special milling of a No. 2 grade HRS wheat from North Dakota (1967).

Starch used for a standard was prepared from a HRS wheat. The starch contained 11.3% moisture and 0.05% nitrogen.

A suspension of crystalline alpha-amylase from hog pancreas (alpha-amylase in half-saturated NaCl containing 0.003M $CaCl_2$ from Sigma Chemical Co., St. Louis, Mo.) had an activity of 940 units per mg. (one unit liberates 1 mg. of maltose from starch in 3 min. at pH 6.9 at 20°C.), and was supplied at a concentration of 42 mg./ml. Other enzymes used were from Rohm & Haas, Philadelphia, Pa.

All samples except the flours were ground in a Wiley mill through a 20-mesh screen. In some cases they were ball-milled, but we found this unnecessary for the degree of accuracy required. Samples (whole wheat, flour, and red dog, 50 mg. each; shorts, germ, and bran, 100 mg. each; starch standard, 30 mg.) in 5 ml. of water and 1 ml. of 0.15M sodium chloride solution were autoclaved for 20 min. at

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15 lb. p.s.i.g. The solutions were cooled and diluted with 4 ml. of pH 7 barbiturate buffer (0.02M sodium barbiturate made up in 0.15M sodium chloride) (6); then 4 μl. of the pig alpha-amylase preparation and 1 drop of toluene were added. Control samples without amylase were also prepared. The mixtures were shaken at 70°F. overnight. After centrifugation, 1 ml. of supernatant was removed and analyzed for reducing sugar by the colorimetric dinitrosalicylic acid (DNSA) procedure (7). Analysis of control samples gave an initial reducing value that was deducted from the alpha-amylase digestion values. Starch values were calculated from the corrected absorbances by using a standard curve constructed from enzymatic digests of pure wheat starch analyzed by the DNSA method. When eight identical samples of clear flour were analyzed by the described method, starch values ranged from 61.9 to 63.1%, with a mean of 62.4% and a standard deviation of 0.4%.

Other methods used to determine starch in the samples included the polarimetric procedure employed in the Millers' National Federation study (3,4,5), the iodine "blue value" method (8), the phenol-sulfuric acid method (9), and the anthrone method (10), and two recently developed enzymatic methods–Donelson and Yamazaki's procedure (2) using Rhozyme 33, a fast acting alpha-amylase, and the procedure of Friedemann et al. (1) using Rhozyme S, a powerful glucoamylase.

Pentosan was determined quantitatively in the calcium chloride polarimetric extracts by an orcinol procedure (11). Pentosan or hemicellulose was also isolated from the calcium chloride extract of bran by a dialysis, digestion procedure. After its specific rotation had been determined, it was hydrolyzed (1N H_2SO_4, 100°, 1 hr.) and paper-chromatographed in a n-butanol:pyridine:water system (6:4:3, by volume).

RESULTS AND DISCUSSION

The mean differences between our enzyme and the polarimetric method for the initial seven samples of whole wheats and their five milling fractions are shown in Table I. The differences are small and random for whole wheat, flour, and red dog, but are large and unidirectional for shorts, bran, and germ. The disagreement is greatest in the high-fiber fractions and least in the low-fiber fractions.

The starch contents of another single set of wheat milling fractions were then determined by several different chemical and enzymatic methods (Table II). For the most part, the results from the different methods again agreed better on the high-starch, low-fiber fractions than on the low-starch, high-fiber materials. The

TABLE I. DIFFERENCES FOUND IN STARCH ANALYSIS OF WHEAT MILLING FRACTIONS BY ENZYMATIC AND POLARIMETRIC METHODS

	Mean Difference between Methods[a] %[b]		Mean Difference between Methods %
Whole wheat	−4.7	Shorts	+20.8
Flour	+5.2	Bran	+57.7
Red dog	−0.4	Germ	+25.7

[a]Based on milling samples from seven separate wheats.

[b]Based on polarization value = 100%. +, Pig alpha-amylase value higher; −, Pig alpha-amylase value lower.

TABLE II. COMPARATIVE RESULTS OF STARCH CONTENT OF WHEAT MILLING FRACTIONS USING DIFFERENT ANALYTICAL METHODS

Sample	Polarization	Iodine Blue Value	Phenol-Sulfuric Acid[a]	Anthrone	Rhozyme S	Pig Alpha-Amylase	Rhozyme 33
Wheat starch[b]	88.3	88.7	...	88.7	...	88.7	...
Patent flour	67.3	72.0	72.4	71.5	68.7	67.9	68.6
Flour	60.0	63.4	64.3	67.5	61.4	62.5	61.4
Whole wheat	52.3	60.0	57.8	55.9	58.9	58.4	56.4
Red dog	36.0	45.2	44.3	44.0	47.4	42.2	44.2
Shorts	12.1	15.7	21.6	17.0	21.3	16.8	17.5
Germ	14.2	16.6	22.2	18.3	20.6	19.2	18.5
Bran	4.3	6.2	14.7	9.8	11.0	8.9	8.5
Bran[c]	3.1	6.8	17.3	...	...	...	...

[a]Calculated as glucose.

[b]11.3% Moisture.

[c]Extracted with four successive 15-ml. portions of $CaCl_2$ solutions.

most consistent agreement over-all was obtained with two of the alpha-amylase methods.

Generally the iodine "blue values" and the anthrone values were quite close to those of the two enzymes. Iodine is quite specific for amylose; and anthrone, although not specific for glucose, is not subject to much interference from pentoses. Polarization values were low on shorts, germ, and bran, but phenol-sulfuric acid and the glucoamylase Rhozyme S values tended to be high with this group.

It is clear that the observed discrepancies are all related to the nonstarch carbohydrate present in high-fiber products. This material can be solubilized along with the starch when calcium chloride solution or other extraction agents like $HClO_4$ is used as extractant. In 1948, Clendenning (12) pointed out that hemicelluloses in wheat endosperm and bran probably interfere in the polarimetric determination of starch in wheat products because they are soluble in calcium chloride. He suggested that the apparent starch content of wheat bran could be 1.5 to 2% low because of the negative rotation ($[\alpha] = -35°$) of the hemicellulose he isolated from wheat bran. Fraser and Holmes (13) re-emphasized this point in a paper 10 years later. We should like to point out that to interfere, a hemicellulose must not be hydrolyzed to a positively rotating sugar during the 30 min. of boiling in calcium chloride at pH 2. Otherwise, liberated xylose and arabinose, with their low positive rotations, would contribute to slightly high values of apparent starch. Even though sucrose is completely hydrolyzed and raffinose is partially hydrolyzed in boiling calcium chloride solution, the wheat hemicellulose is not entirely hydrolyzed, since residual material having a high negative rotation ($[\alpha]_D^{25} = -82°$, H_2O) was isolated from the extract after removal of calcium and starch. Literature values for the rotation of wheat hemicellulose range from -35° (12) to -100° to -110° (14). Hydrolysis and paper chromatography of this residual fraction showed it was mostly xylose with a small amount of arabinose. In Table III pentose data, run on the calcium chloride solutions used for the polarization measurements, confirm that hemicelluloses or pentosans are low in extracts from low-fiber fractions and increase significantly in high-fiber fractions.

We conclude that polarimetric starch analyses of the high-starch, low-fiber fractions agree with those obtained by other methods, but only because there is a very low level of interfering substances. For low-starch, high-fiber products, polarization values for starch are different from those obtained by other methods. According to Fraser and Holmes (13), these differences can be equalized if artificial values of $[\alpha]_D = +161$ and $[\alpha]_D = +213$ are used for starch in bran and germ respectively. These values are based in part on a relatively low rotation of $[\alpha]_D$ of

TABLE III. PENTOSE CONTENT OF WHEAT MILLING FRACTIONS IN $CaCl_2$ EXTRACTS

	Pentose[a] %		Pentose[a] %
Patent flour	0.5	Shorts	8.7
Clear flour	0.5	Germ	6.8
Whole wheat	2.0	Bran	8.7
Red dog	5.5		

[a]Calculated as arabinose.

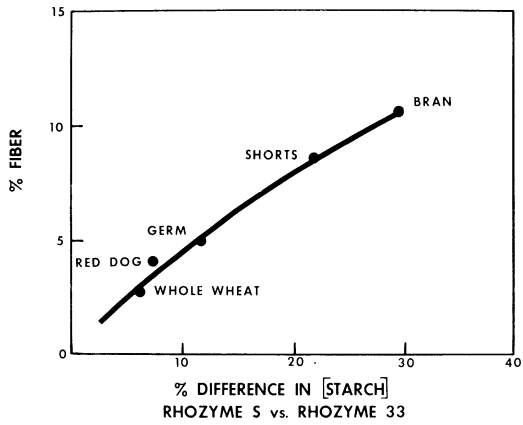

Fig. 1. Starch analysis in wheat mill fractions. Percent difference between starch values determined by Rhozyme S and Rhozyme 33 methods vs. fiber content.

$-25°$ to $-35°$ for wheat hemicellulose but are not sufficient to adjust the differences observed in the present set of samples. Using Fraser and Holmes values would only raise the polarimetric values of starch in bran by 26% instead of the needed 58% and would decrease starch in germ 5% instead of raising it the needed 26%. As the British authors point out, ". . .it must be borne in mind that the composition of commercial samples of bran and germ will vary, and so the specific rotation of the starch will have to be altered according to the different quantities of interfering matter."

The phenol-sulfuric acid results are high because the method measures all soluble carbohydrate, both starch and hemicellulose. Results with the Rhozyme S enzyme are also high because the glucoamylase preparation contains other enzymes including hemicellulase and cellulase (2) which give a measure of total carbohydrate instead of starch only. Paper chromatography shows xylose and arabinose spots along with glucose in bran hydrolysates prepared under the digestion conditions recommended for starch analysis by this procedure. Figure 1 shows that the percent difference between starch values determined by Rhozyme S and Rhozyme 33 is directly proportional to the fiber contents of the various fractions found by proximate analysis.

The results obtained with either pig alpha-amylase or Rhozyme 33 agree consistently. The pig alpha-amylase prepared from crystalline material is highly specific for starch. The Rhozyme 33 is not a pure enzyme, but when the prescribed hydrolysis conditions are used, not enough xylose or arabinose is generated to interfere with the analysis of bran starch. If longer hydrolysis times are used, paper chromatograms show that xylose and arabinose are liberated by weak hemicellulase action, and too high apparent starch figures are obtained.

The results of the present investigation conflict with those of Donelson and Yamazaki (2) about the agreement between Rhozyme 33 enzymatic and polarimetric methods of starch measurement in wheat bran. These investigators show that both methods give almost identical starch values ($r = 0.99^{**}$); the present study shows considerable difference. The explanation for this anomaly is not readily apparent.

We conclude that there are a variety of excellent methods for determining starch

content of low-fiber wheat milling fractions. For the high-fiber fractions, however, care must be exercised in selecting the method for starch determination, and no polarimetric method is recommended. Because of interference from nonstarch polymeric carbohydrate in these materials, we recommend a method using highly specific amylases which generate reducing sugars from starch only under appropriate experimental conditions.

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