The level of phytate phosphorus in the mill fractions from the HMP 1700 bulk are given in Table VII. The level in the germ was quite high and the level in the endosperm was low as might be expected.

Registry No. Riboflavin, 83-88-5; thiamin, 59-43-8; niacin, 59-67-6; phytic acid, 83-86-3.

LITERATURE CITED

Abdelrahman, A.; Hoseney, R. C.; Varriano-Marston, E. *Cereal Chem.* **1983**, *60*, 189.
Adrian, J.; Sayerse, C. *Br. J. Nutr.* **1957**, *11*, 99.
American Association of Cerial Chemists "Approved Methods of the AACC"; AACC: St. Paul, MN, 1969; Methods 86-50 Niacin, approved April 1966, 86-70 Riboflavin, approved April 1968, and 86-80 Thiamin, approved May 1960.
Chavan, V. M. *Indian Counc. Agric. Res. Farm Bull.* **1958**, 48.
Chitre, R. G.; Desai, D. B.; Raut, V. S. *Indian J. Med. Res.* **1955**, *43*, 575.
Hinton, J. J. C.; Peers, F. G.; Shaw, B. *Nature (London)* **1953**, *172*, 993.
Hulse, J. H.; Laing, E. M.; Pearson, D. E. "Sorghum and the millets"; Academic Press: New York, 1980.
James, E. M.; Norris, F. M.; Wokes, F. *Analyst (London)* **1947**, *72*, 327.
Pai, M. L.; Mehta, B. V.; Shah, H. C. *J. Maharaja Sayajirao Univ. Baroda* **1958**, *7*, 41-48; *Chem. Abstr.* **1959**, *53*, 22657*i*.
Rachie, K. O.; Majmudar, J. V. "Pearl Millet"; The Pennsylvania State University Press: University Park, PA, and London, 1980.
Rama Rao, G.; Swaminathan, M. *Bull. Cent. Food Technol. Res. Inst., Mysore* **1953**, *3*, 68.
Samson, M. F.; Adrian, J. *Agron. Trop. (Paris)* **1971**, *26*, 1090.
Tangkongchitr, U.; Seib, P. A.; Hoseney, R. C. *Cereal Chem.* **1981**, *58*, 226.
Vogel, S.; Graham, M. "Sorghum and Millet: Food Production and Use"; International Development Research Center, Ottawa, Canada, 1979.

Received for review May 16, 1983. Revised manuscript received September 19, 1983. Accepted October 16, 1983. This work was supported by Grant AID/DSAN/XII/G-1049 from the Agency for Internatonal Development, Washington, DC. Contribution No. 83-13J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan, KS 66506

Determination of Total Available Glucose in Corn Base Materials

Clifford C. Budke

A method is described for the determination of total available glucose in corn base materials. The procedure involves gelatinization with water at 122 °C, conversion of starch and glucose polymers to free glucose with α-amylase and amyloglucosidase at 55 °C, and determination of the glucose content by using a Yellow Springs Instrument Co. Model 27 analyzer. This instrument uses an immobilized film of glucose oxidase to convert β-D-glucose to hydrogen peroxide, which is measured electrochemically to provide an absolute measurement of the glucose content. The method eliminates the inaccuracy and uncertainty associated with many of the chemical methods for determining glucose and is applicable to a wide variety of corn base materials. The procedure may be applicable to other agricultural products.

Interest in obtaining fuel and energy from nonpetroleum sources has increased in the past few years. Conversion of biomass materials to alternative fuels has received particular attention. It is estimated (*Chem. Week*, 1983) that consumption of corn for this purpose during the current year will increase about 3 times over the 1980 level. The use of corn base materials for the production of fermentation ethanol requires an accurate determination of total available glucose for process optimization and to monitor the quality of the feed materials.

Methods traditionally used for the determination of total available glucose or fermentable carbohydrates involve the hydrolysis of starch and lower α-D-glucose polymers with acid or amylolytic enzymes followed by the determination of glucose by chemical or enzymatic procedures. Of the chemical methods, modifications of those employing reduction by copper (Munson and Walker, 1906; Lane and Eynon, 1923; Zerban and Sattler, 1938) or alkaline ferricyanide (Friedemann et al., 1967) are more commonly used whereas glucose oxidase techniques are generally considered the most useful of the enzymatic methods. AOAC procedures have been published that use these methods for the determination of starch (AOAC, 1980, methods 7.808 and 14.075).

The copper reduction procedures for glucose determination are subject to interference from other reducing substances, require stringent analytical conditions, and are time consuming. On the other hand, enzymatic methods using glucose oxidase are relatively simple, are rapid, and have the distinct advantage of being specific for glucose. Numerous modifications of the glucose oxidase method have been reported including those using colorimetric (Banks and Greenwood, 1971; Norton and Smith, 1967) and electrometric measurement (Trop and Grossman, 1975; Enfors, 1981).

During the course of recent work, we used the Yellow Springs Instrument Co. (YSI) Model 27 analyzer for the routine determination of free glucose at various stages of the saccharification–fermentation process. This instrument (Taylor et al., 1977; Mason, 1983) uses a film of glucose oxidase immobilized on a membrane to convert β-D-glucose to hydrogen peroxide, which is measured electrochemically and related to the total glucose content. The instrument is calibrated with an equilibrated dextrose solution. Conversion of polymerized glucose to the free monomer results in an equilibrium mixture of the anomers that can then be directly analyzed. The method is simple, fast, and accurate and gives a digital readout of the glucose content of the sample solution.

U.S. Industrial Chemicals Co., Division of National Distillers and Chemical Corp., Research Department, Cincinnati, Ohio 45237.

0021-8561/84/1432-0034$01.50/0

Application of the YSI analyzer for the accurate determination of total available glucose in the corn base materials after conventional gelatinization and enzyme conversion of α-D-glucose polymers is reported in this paper. To achieve the required accuracy, the YSI calibration range was extended from 200 to 600 mg of glucose/100 mL and a rigorous standardization procedure was adopted. The method has been used for the routine assay of various corn base feedstocks such as whole corn, grits, meal, and starch. The values obtained from numerous samples have been consistent with the overall composition balance and expected yields and conversions. They were in reasonable agreement with those obtained from conventional procedures using copper reduction.

EXPERIMENTAL SECTION

Apparatus and Materials. A Yellow Springs Instrument Co. (YSI) Model 27 analyzer equipped with a glucose oxidase membrane (YSI No. 2365) and a modified pH 7.3 phosphate buffer (YSI No. 2357) was used. Standards and sample solutions were injected into the analyzer with a 25-μL syringe (YSI No. 2361). This instrument is available from the Yellow Springs Instrument Co., Yellow Springs, OH 45387.

The α-amylase enzyme Novo Termamyl 60L, activity = 60 kilo Novo units/g, was obtained from Novo Laboratories, Inc., Wilton, CT. Amyloglucosidase enzyme Diazyme L-150, activity = 150 Diazyme units/g, was obtained from Miles Laboratories, Inc., Elkhart, IN. Both enzymes were stored under refrigeration.

ACS reagent grade dextrose was dried under vacuum for 6 h at 70 °C and stored in a desiccator. This material was then used to prepare standard dextrose solutions containing 400, 500, and 600 mg of dextrose/100 mL. The standard solutions were allowed to equilibrate for 4–5 h after preparation and then stored under refrigeration. The standards were warmed to ambient temperature and equilibrated several hours before use.

Acetate buffer was prepared by dissolving 330 g of sodium acetate in approximately 1 L of water. A total of 240 mL (251 g) of acetic acid was then added, and after being cooled, the solution was diluted to 2 L with water. The pH of this solution, when diluted 1:9, was approximately 4.8.

Procedure. *Sample Preparation.* The moisture content of a composite sample of the corn, meal, grits, or starch was determined by vacuum drying an approximately 10-g sample for 16 h at 70 °C. Where required, a portion of the composite sample was reduced to −40 mesh in a Wiley mill and blended, and the moisture content was again determined as described.

Analysis. Samples (−40 mesh) containing 0.8–1.2 g of total available glucose were weighed and quantitatively transferred into 200-mL volumetric flasks. Approximately 100 mL of water was added to each and the flasks were vigorously swirled to wet the samples. The walls of the flasks were then washed down briefly with water to remove adhering particles, and the solutions were autoclaved for 2 h at 122 °C.

After the solutions were cooled to ambient temperature, 20 mL of acetate buffer and 0.2 mL of calcium chloride reagent (6.25 g of $CaCl_2$/100 mL) were added to each and the solutions mixed. One milliliter each of α-amylase and amyloglucosidase enzyme solutions was then added, and the solutions were mixed and hydrolyzed for 2 h in an oven at 55 °C.

The sample solutions were then cooled to ambient temperature, diluted to volume with water, and thoroughly mixed. A portion of each solution was filtered through a dry Whatman No. 42 filter to remove insoluble matter consisting mainly of protein, fat, and fiber, and 3–5 mL of the clear filtrates were collected in small vials that were capped and reserved for the glucose determination. The prepared solutions were allowed to equilibrate for approximately 2 h before analysis.

Before analysis, the YSI instrument was calibrated with a 500 mg/100 mL dextrose standard and the linearity checked by analyzing standards containing 400 and 600 mg of dextrose/100 mL. Values obtained were consistently within 1% of theory when the instrument was functioning properly. Each sample was then analyzed in duplicate for glucose content. As the YSI analyzer is calibrated to provide a direct digital readout of glucose content in mg/100 mL and the samples had been diluted to 200 mL, the sum of the two readings equaled the total glucose in the sample. Between samples, the analyzer was again calibrated with the 500 mg/100 mL dextrose standard. Before the calibration was set at 500 mg/100 mL, the YSI digital readout was observed. These values were consistently within 1% of theory.

A blank was determined by transferring the prescribed amounts of enzymes, calcium chloride, and acetate buffer into a 200-mL volumetric flask. Exactly 1.0000 g of dextrose was then added and the solution diluted to 100 mL with water. After being warmed for 2 h at 55 °C, the solution was cooled to ambient temperature, diluted to volume with water, and mixed. The solution was then analyzed in duplicate for glucose. The blank contribution, calculated from the increase in glucose content, was subtracted from the total glucose contents of the prepared sample solutions.

Calculation. The total available glucose content of the original sample, reported as percent starch, was calculated according to the equations

$$\%\ \text{starch, dry weight} = \frac{(R - B) \times 0.9 \times 100 \times 100}{1000 \times S \times [100 - M_a]}$$

$$\%\ \text{starch, as received} = \frac{\%\ \text{starch, dry weight,} \times [100 - M_b]}{100}$$

where R = total mg of glucose found in sample solution = sum of two YSI sample glucose determinations, B = mg of glucose found in reagent blank = sum of the two YSI blank glucose determinations minus 1000, S = weight of sample, g, M_a = moisture content of analytical sample, %, and M_b = moisture content of original sample, %. The theoretical factor for conversion of glucose to starch is 0.9.

RESULTS AND DISCUSSION

While YSI claims a linear response to 500 mg of glucose/100 mL and recommends calibration with a 200 mg/100 mL standard, our experience has consistently shown that the instrument responds linearly to 800 mg of glucose/100 mL. Accordingly, to increase the accuracy of the determination, the analyzer was routinely calibrated with a 500 mg/100 mL glucose standard and sample weights were adjusted to approximate this level when prepared for analysis. By checking observed values of 400 and 600 mg/100 mL glucose standards before sample analysis, we could monitor linearity within this working range.

Glucose exists as an equilibrium mixture containing mainly the α-D (37%) and β-D (63%) forms in aqueous solution. As glucose oxidase responds to β-D-glucose only, it is essential that the dextrose standards and sample solutions are equilibrated prior to analysis. To establish the

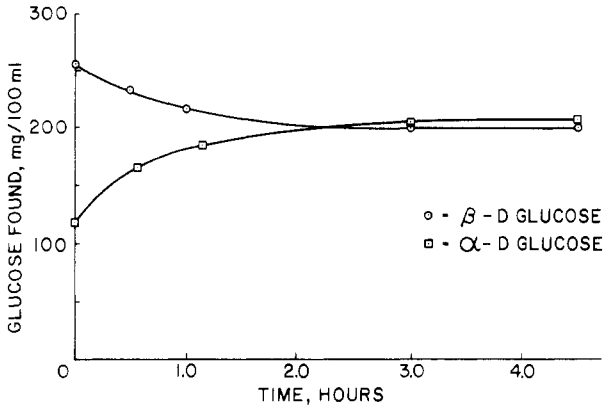

Figure 1. Equilibrium of α-D-glucose and β-D-glucose.

time required for the anomers to equilibrate, solutions containing approximately 200 mg/100 mL α-D glucose (Sigma Chemical Co., 95% α–5% β) and β-D-glucose (Sigma Chemical Co.) were prepared. The glucose contents were then determined over a period of several hours by using the YSI analyzer. As shown in Figure 1, no increase in glucose content was observed after 3 h. It should be noted that several minutes elapsed in preparation of the sample solutions and analysis before the initial (0 h) YSI readings were obtained. The rate of mutarotation would also be affected by the temperature (37 °C) of the YSI sample chamber and catalyzed by the phosphate buffer and the equilibrium rate would differ in pure aqueous solution.

After conversion of corn base samples for 2 h at 55 °C and subsequent dilution and filtration, the prepared sample solutions were routinely allowed to equilibrate for approximately 2 h before analysis. No increase in glucose content was observed after this time.

A mixture of α-amylase and amyloglucosidase was used for the conversion since the combination is reported to be more effective in completely cleaving the α-1,3, α-1,4, and α-1,6 linkages in glucose polymers (Blake, 1978). Commercial amyloglucosidase preparations are normally contaminated with small amounts of α-amylase and have been used alone in numerous procedures for the hydrolysis of starch. However, as the α-amylase levels vary widely (Marshall, 1975) and the rate of hydrolysis is greatly reduced at very low α-amylase levels (Blake, 1978), an additional amount of α-amylase was added to ensure complete hydrolysis.

Because the enzymes used for the starch conversion contained varying amounts of glucose or glucose polymers, a blank correction was required. To eliminate the somewhat erratic readings obtained at very low glucose levels, a known amount of dextrose was added to the blank before hydrolysis and the blank value determined from the increase in glucose content. The blank contribution normally ranged from 0 to 10 mg of glucose. Enzymes that yielded a blank contribution greater than 25 mg were not used.

No attempt was made to establish the minimum quantities of enzymes or the minimum time required for gelatinization and conversion in order to obtain complete hydrolysis. However, the reproducibility of the results with varying sample weights, the nearly theoretical values obtained for starch, and the fact that higher glucose values were not obtained when the amount of enzymes and conversion times were increased are sufficient evidence that hydrolysis was complete.

Satisfactory precision was obtained by the method as shown in Table I where typical samples of commercial corn starch and grits were analyzed in replicate. Satisfactory precision was also obtained on whole corn and corn meal samples.

Table I. Determination of Total Available Glucose in Starch and Grits Samples

sample wt, g	YSI glucose, mg/100 mL		recalibration[a]	% starch (dry wt basis)
Starch Sample 4850-25 (11.52% Water)				
0.9550	462	463		98.52
			499	
0.9564	467	466		99.23
			500	
1.0283	499	501		98.92
			499	
1.0048	490	491		99.31
			503	
1.0592	515	513		98.72
			498	
1.0709	521	521		98.97
			499	
1.1122	543	542		99.23
			502	
1.1035	532	534		98.26
			497	
1.1771	570	572		98.68
			500	
1.1565	560	562		98.68
			502	
1.0176	493	495		98.76
			499	
			av =	98.84
			σ =	0.33
95% confidence limits for av = 98.84 ± 0.22				
Grits Sample 2170-43 (11.96% Water)				
1.0759	455	456		86.56
			500	
1.0733	453	456		86.58
			502	
1.1234	474	475		86.36
			500	
1.1320	477	477		86.15
			497	
1.1897	505	506		86.87
			501	
1.1852	500	503		86.51
			501	
1.2578	532	532		86.48
			501	
1.2223	518	517		86.56
			500	
1.3103	553	552		86.21
			499	
1.3146	553	555		86.16
			499	
1.2511	531	532		86.86
			500	
			av =	86.48
			σ =	0.25
95% confidence limits for av = 86.48 ± 0.17				

[a] YSI analyzer recalibrated with 500 mg/100 mL glucose standard after duplicate sample readings. Values shown are YSI readings for standard just prior to resetting the calibration.

As shown in Table II, the starch assay was in close agreement with the overall composition balance of 100.1% obtained by summation of the starch and minor protein, oil, fiber, and ash constituents. The lower composition balance (95.9%) obtained for the grits sample can be attributed to the presence of other corn components such as phytic acid, sucrose, fructose, and hemicellulose. The level of these components is considerably higher in meal and whole corn.

Total available glucose values obtained from numerous samples of starch, grits, meal, and whole corn were con-

Table II. Composition Analysis of Grits and Starch Samples

component, %	sample: starch 4850-25	sample: grits 2170-43
moisture[a]	11.52	11.96
protein, dry wt[b]	0.56	7.98
oil, dry wt	0.53[c]	0.72[d]
fiber, dry wt[e]	0.09	0.38
ash, dry wt[f]	0.09	0.34
starch, dry wt[g]	98.84	86.48
total, dry wt	100.11	95.90

[a] Determined by vacuum drying an approximately 10-g sample for 16 h at 70 °C. [b] AOAC (1980), method 14.068. [c] Corn Refiners Association Method B-20. [d] AOAC (1980), method 7.056. [e] AOAC (1980), method 7.061. [f] AOAC (1980), method 14.006. [g] YSI method, total available glucose as starch.

sistent with expected glucose yields and conversions. They were also in reasonable agreement with fermentable carbohydrate values obtained by a copper reduction procedure commonly used in the distillery industry (NDPC Method for Fermentable Starch in Grain). Using this method, a sample is hydrolyzed by refluxing with dilute HCl. The solution is then cooled, neutralized with NaOH, diluted to volume, and filtered. Total carbohydrate sugar, as glucose, is determined titrimetrically on an aliquot of the filtrate by a modified Munson–Walker procedure. A portion of the hydryolysate is then fermented with yeast and filtered, and an aliquot of the filtrate is analyzed for nonfermentable carbohydrates in the same manner. Fermentable carbohydrates, as starch, are determined from the difference. By use of this method, duplicate starch values obtained on a dry weight basis for the samples in Table I were 85.4 and 84.7% for grits and 97.0 and 95.4% for starch.

As the rate of enzyme hydrolysis is dependent on the chain length of the glucose polymer with lower oligosaccharides being less readily hydrolyzed (Norman, 1979), a brief study was made to ascertain if maltose and other oligosaccharides are quantitatively converted to glucose under the analytical conditions. Samples of maltose and Staley Neto 7300 corn syrup were used. The latter is an acid/enzyme conversion syrup containing approximately 7% dextrose, 33% maltose, 24% maltotriose, and 36% higher saccharides on a dry weight basis. The solids content is approximately 80%.

When dissolved in water, converted, and analyzed for glucose by the proposed method, the maltose sample gave a nearly theoretical equivalent of 104.2% glucose while the Neto 7300 syrup showed a glucose content of 81.2%, in agreement with the approximate sample composition. No oligomers or higher saccharides were detected in the hydrolyzed samples by liquid chromatography, confirming complete conversion to glucose. The absence of maltose and isomaltose also indicates that the reverse reaction, the enzyme-catalyzed condensation of glucose molecules, is negligible under the conditions outlined.

Registry No. Glucose, 50-99-7; α-amylase, 9000-90-2; amyloglucosidase, 9032-08-0.

LITERATURE CITED

AOAC "Official Methods of Analysis", 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.
Banks, W.; Greenwood, C. T. *Staerke* **1971**, *23* (7), 222.
Blake, C. J. "The Detection and Determination of Starch in Foods—a Literature Review"; British Food Manufacturing Industries Association: Leatherhead, Surrey, England, 1978; Scientific and Technical Survey No. 109, p 14.
Chem. Week **1983**, *132* (3), 10.
Enfors, S. O. *Enzyme Microb. Technol.* **1981**, *3* (1), 29.
Friedemann, T. F.; Witt, N. F.; Neighbors, B. W. *J. Assoc. Off. Anal. Chem.* **1967**, *50* (4), 944.
Lane, J. H.; Eynon, L. *J. Soc. Chem. Ind., London* **1923**, *42*, 32-7T.
Marshall, J. J. *Staerke* **1975**, *27* (11), 377.
Mason, M. *J. Assoc. Off. Anal. Chem.* **1983**, *66* (4), 981.
Munson, L. S.; Walker, P. H. *J. Am. Chem. Soc.* **1906**, *28* (6), 663.
NDPC Method for Fermentable Starch in Grain "Manual of Official Laboratory Control Methods"; National Distillers Products Co.: New York, NY.
Norman, B. E. In "Microbial Polysaccharides and Polysaccharases"; Berkeley, R. C. W.; Gooday, G. W.; Ellwood, D. C., Eds.; Academic Press: New York, 1979; Chapter 15.
Norton, R. L.; Smith, P. R. "Technical Circular No. 334"; British Food Manufacturing Industries Research Association: Leatherhead, Surrey, England, 1967.
Taylor, P. J.; Kmetec, E.; Johnson, J. *Anal. Chem.* **1977**, *49*, 789.
Trop, M.; Grossman, S. *J. Assoc. Off. Anal. Chem.* **1975**, *55* (6), 1191.
Zerban, F. W.; Sattler, L. *Ind. Eng. Chem., Anal. Ed.* **1938**, *10*, 669.

Received for review April 1, 1983. Revised manuscript received September 9, 1983. Accepted October 26, 1983.