Note on a Modified Method for the Quantitative Determination of Starch[1]

S. E. FLEMING[2] and R. D. REICHERT, Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan S7N 0W9

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The starch content of biological materials has usually been measured by quantitating the glucose after starch hydrolysis. An initial extraction of the material with aqueous methanol or ethanol may be required to remove low molecular weight carbohydrates containing glucose (Banks et al 1970). The starch in the residue must then be gelatinized to permit rapid and complete enzymatic hydrolysis. This gelatinization process may be effected by autoclaving (Shetty et al 1974, Thivend et al 1965) or by chemical treatments using dimethyl sulfoxide (Libby 1970, McGuire and Erlander 1966) or concentrated calcium chloride (Banks et al 1970, Earle and Milner 1944). A combination of α-amylase and glucoamylase enzymes has been used for digesting the starch to yield glucose (Libby 1970, Vose 1977). Glucostat reagent (Worthington Biochemical Corp., Freehold, NJ) has commonly been used in quantitating glucose to measure starch (Shetty et al 1974). Recently, however, this reagent has become unavailable and the reagent Statzyme has been offered as a substitute.

Our laboratory has found that calcium chloride dispersion provides higher yields of starch and more reproducible analytical results than gelatinization by autoclaving does. However, when Statzyme reagent was used to quantitate glucose in the presence of the reagents used in starch dispersion and hydrolysis, results were poorly reproducible and inaccurate. This note presents a modification that has overcome those problems.

MATERIALS AND METHODS

Samples for analysis included wheat starch (Wet-Star No. 4, Charles Tennant, Co., Winnipeg, Manitoba), potato starch (British Drug House, Canada Ltd., Toronto), a flour prepared by fine grinding dehulled field peas (Century variety), and dried and ground feces recovered after feeding laboratory rats a diet containing raw potato starch (Fleming and Vose 1979).

The dried samples were weighed to supply 10–20 mg of starch and were placed in glass centrifuge tubes (20 ml). The sides of the tubes were washed down with 80% aqueous methanol and the samples were wetted. This wetted residue was then extracted three times with 5-ml volumes of hot (60° C) aqueous methanol. Soluble carbohydrates were removed with the supernatants following centrifugation (2,200 × *g*, 15 min).

Calcium chloride solution (1 ml of 50% $CaCl_2$, pH 2) was added to the residue. The mixture was stirred and heated (15 min, 125° C) by immersion in a wax bath. During this time, material was scraped from the sides of the tubes with a glass rod and returned to the solution. Periodically, dropwise addition of water was required to maintain the original volume.

After cooling, potassium hydroxide (2 ml, 0.05*M*) and acetate buffer (4 ml, 0.1*N*, pH 4.8) were added to adjust the pH to 4.8. A mixture of α-amylase and glucoamylase (Tenase and Diazyme L-100, respectively, Miles Laboratories, Inc., Marschall Division, Elkhart, IN) was then added. The tubes were immersed in a water bath (48° C, 3 hr) and were occasionally stirred. The contents were made up to a predetermined volume with water and analyzed for glucose.

[1]NRCC No. 18037.
[2]Present address: Department of Nutritional Science, University of California, Berkeley, CA 94709.

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When glucose was quantitated using the Glucostat reagent, the samples were made up to 250-ml volumes. Two milliliters of this dilution were combined with 2 ml of Glucostat reagent and allowed to react for 10 min before 1 drop of 4*N* HCl was added to stop the reaction. The absorbance of the chromogenic complex was measured at 420 nm. Quantitation included a calibration factor for known quantities of glucose, which was incorporated into an equation similar to that reported by Banks et al (1970).

Quantitation of glucose using the Statzyme reagent (Worthington Biochemicals Corp., Freehold, NJ) required that the samples be made to a volume of 25 ml. The reagent was reconstituted in distilled water as recommended by the manufacturer, and this solution was preheated to 37° C. Sample aliquots of 30 μl were added to 3 ml of reagent solution and incubated 10 min at 37° C. The absorbances were measured at 500 nm.

The disodium salt of ethylenediamine tetraacetic acid (EDTA, Sigma Chemical Co., St. Louis, MO) was made to concentrations of 0.05, 0.1, and 0.2*M* with distilled water. When included in the analyses, volumes of 5 or 10 ml were added to the amylase-hydrolyzed samples and made to 25 ml with acetate buffer (0.1*N*, pH 4.8) before glucose analysis with Statzyme reagent.

RESULTS AND DISCUSSION

Purified wheat starch was found to yield 156.4–162.7% starch when the glucose content of the hydrolysate was determined by the Statzyme method (Table I). Adding the reagents used in the starch assay to the standards used to prepare the calibration curve (Fig. 1) lowered the absorption values, but the results were inconsistent and therefore unreliable. The addition of EDTA to the samples before glucose analysis improved both the accuracy and reproducibility of the analyses (Table I). At least 10 ml of 0.1*M* EDTA was required to provide accurate results. The calibration curves of absorption vs glucose concentration were the same for glucose solutions in water and for glucose solutions containing the starch dispersion reagent plus EDTA (Fig. 1). The effectiveness of EDTA in this analysis indicates that minerals, such as the calcium used for starch dispersion or other minerals naturally present in plant and animal products, are responsible for the excessively high absorption values that were observed. The EDTA presumably acts as a chelating agent to render the dissolved ions unavailable for complexation with Statzyme reagents.

Duplicate analyses were conducted on four starches, using both the Glucostat and Statzyme reagents for glucose analysis (Table II). Both wheat starch and potato starch were shown to contain in excess of 100% starch when the Statzyme method was used.

TABLE I
Influence of Ethylenediamine Tetraacetic Acid (EDTA) on Starch Determination Using the Statzyme Reagent for Glucose Analysis

EDTA		Starch Concentration[a] (%) in Replicate	
Volume (ml)	Molarity	No. 1	No. 2
0	0	162.7	156.4
5	0.05	159.7	164.3
10	0.05	111.6	102.6
10	0.10	103.5	101.7
10	0.20	100.5	102.1

[a]Calibration curve d in Fig. 1 was used in calculations.

TABLE II
Comparison of the Glucostat and Statzyme Methods of Measuring Glucose in the Determination of Starch Content

	Starch (%), as Determined[a] with Reagent			
		Statzyme, with Glucose in		
	Glucostat[b]	Water	Starch Dispersion Reagents	Starch Dispersion Reagents Plus EDTA[c]
Wheat starch	99.6	164.7	122.0	101.0
	101.4	172.5	120.2	98.6
Potato starch	102.9	163.8	96.9	98.0
	99.9	174.9	100.2	97.0
Field peas	53.1	···	···	56.3
	51.9	···	···	55.3[d]
Feces of rat	64.8	···	···	66.1
	65.1	···	···	66.6

[a]Using calibration curves shown in Fig. 1.
[b]With glucose in water.
[c]10 ml of $2M$ ethylenediamine tetraacetic acid.
[d]Standard deviation of six assays was 0.58. Mean and standard deviation of six assays on wheat flour by the Statzyme - EDTA method was 73.24 ± 0.426.

However, the addition of EDTA gave values in good agreement with those reported by the Glucostat procedure, and the results showed good reproducibility. Similarly, the addition of EDTA before Statzyme analysis for glucose gave values for field pea flour and rat feces that were in good agreement with those measured by the Glucostat method.

CONCLUSIONS

The newly available Statzyme reagent for glucose analysis can be used to quantitate starch content following dispersion with calcium chloride. To obtain accurate and reproducible results, however, EDTA must first be added to complex with the calcium.

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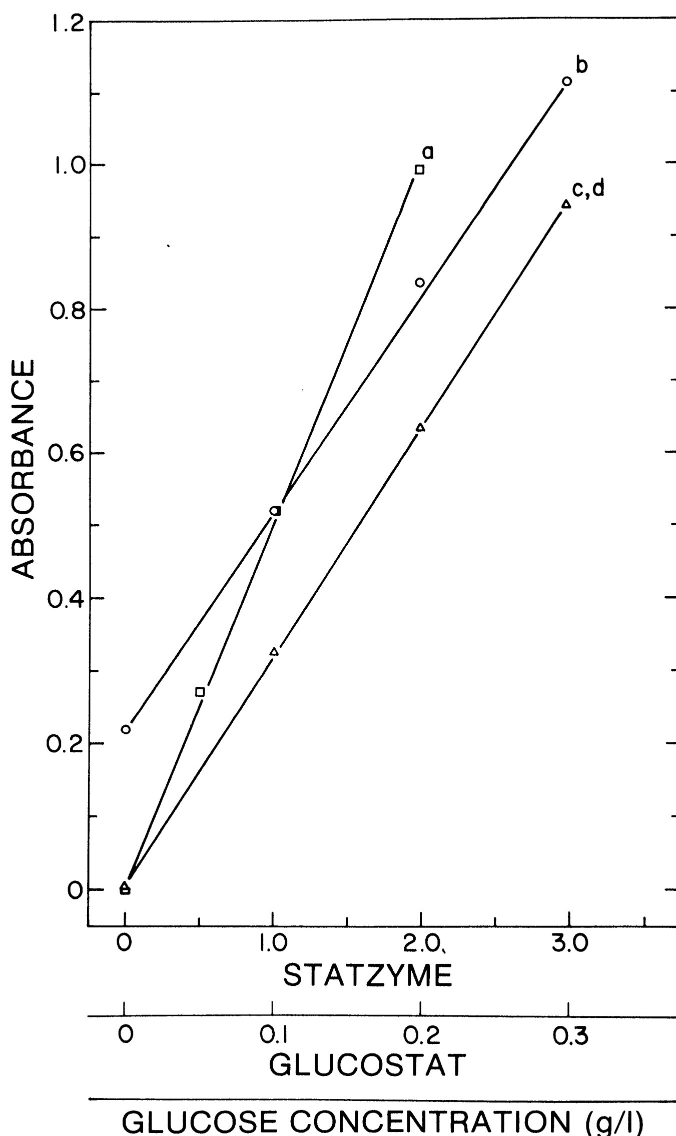

Fig. 1. Absorbance vs glucose concentration. **a,** Glucostat reagent, glucose in water; **b,** Statzyme reagent, glucose in reagents used in starch dispersion; **c,** Statzyme reagent, glucose in reagents used in starch dispersion plus ethylenediamine tetraacetic acid; **d,** Statzyme reagent, glucose in water.

LITERATURE CITED

BANKS, W., GREENWOOD, C. T., and MUIR, D. D. 1970. The characterization of starch and its components. II. The semi-micro estimation of the starch content of cereal grains and related materials. Staerke 22:105.

EARLE, F. R., and MILNER, R. T. 1944. Improvements in the determination of starch in corn and wheat. Cereal Chem. 21:567.

FLEMING, S. E., and VOSE, J. R. 1979. Digestibility of raw and cooked starches from legume seeds using the laboratory rat. J. Nutr. 109:2067.

LIBBY, R. A. 1970. Direct starch analysis using DMSO solubilization and glucoamylase. Cereal Chem. 47:273.

McGUIRE, J. P., and ERLANDER, S. R. 1966. Quantitive isolation and dispersion of starch from corn kernels without degradation. Staerke 18:342.

SHETTY, R. M., LINEBACK, D. R., and SEIB, P. A. 1974. Determining the degree of starch gelatinization. Cereal Chem. 51:364.

THIVEND, V. P., MERCIER, C., and GUILBOT, A. 1965. Application of glucoamylase to starch determination. Staerke 17:278.

VOSE, J. R. 1977. Functional characteristics of an intermediate amylose starch from smooth-seeded field peas compared with corn and wheat starches. Cereal Chem. 54:1141.

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