AUTOMATED METHOD FOR MEASURING ADDED SUCROSE IN SWEETENED CEREAL PRODUCTS WITH IMMOBILIZED INVERTASE[1]

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ABSTRACT

The colorimetric method is based on aqueous extraction of sucrose from the sweetened product. The filtered extract is split, one aliquot passed over a column containing a phenol-formaldehyde resin to which invertase has been attached, and a second aliquot over a column of resin to which no invertase has been attached (blank). Reducing sugars are then determined by a dinitrosalicylic acid colorimetric method. All steps after initial extraction are performed with a Technicon AutoAnalyzer. Sucrose contents in variously sweetened wheat-soy blends (WSB) and corn-soy-milks (CSM) were determined.

The U.S. Food for Peace program provides corn-soy-milk (CSM), wheat-soy blend (WSB), and sweetened (15%-sucrose) versions of these products (1,2) among others for world-feeding activities. Regulatory and quality assurance programs require a method for sucrose determination. Finley and Fellers (3) have reported both an automated and a manual method for sucrose determination in WSB and CSM based on the colorimetric reaction of sucrose with anthrone in 70% sulfuric acid. The concentrated sulfuric acid has drawbacks in safety and life-expectancy of the pumping tubes in the AutoAnalyzer.

This report presents a sucrose method that brings together basic work on insolubilized invertase (4) and an automated colorimetric procedure for determining reducing sugars based on their reaction with dinitrosalicylic acid (DNSA) (5).

MATERIALS AND METHODS

Duolite S-30 was obtained from Resinous Products Division of Diamond Shamrock Chemical Company and 25% aqueous glutaraldehyde from Union Carbide. The purified invertase was obtained as a liquid product, Sucrovert

[1]Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

(extracted from yeast), from Nulomoline Division of SuCrest Corporation. Dinitrosalicylic acid (DNSA) was obtained from Eastman Kodak Company.

DNSA reagent was prepared according to McCready *et al.* (5), by dissolving 10 g of 3,5-dinitrosalicylic acid, 0.5 g sodium bisulfite, and 2 g of phenol in 1% sodium hydroxide, and diluting to 1 l. with the same solvent. Dilution buffer to control pH on the columns was 0.1 *M* acetate buffer at pH 4.4.

The Duolite S-30 was washed with distilled water, soaked overnight in 0.1 *N* sodium chloride, rewashed with water to remove the salt and sized. The resin which passed 30-mesh was used for both the blank column and invertase column.

Immobilized invertase was prepared by adding 25 ml Sucrovert to 20 g of the washed, drained, and sized Duolite S-30 at 25°C. After 16 hr at 25°C, 2.0 ml of 25% glutaraldehyde was added and the mixture was held at 25°C for 3 hr, and at 5°C for an additional 16 hr.

Invertase activity was determined by incubating 1.00 g of wet, drained, resin-containing immobilized invertase in a 250-ml Erlenmeyer flask with 50 ml of substrate at 45°C with rapid, reciprocal shaking. The substrate was 0.40 *M* in sucrose and 0.05 *M* in sodium acetate, pH 4.5. Sequential aliquots (resin allowed to settle before sampling) were taken and analyzed for glucose by the glucose-oxidase chromagen procedure supplied by Worthington under the trade name Glucostat.

Columns for use in the AutoAnalyzer systems were prepared by packing wet, drained, resin-containing immobilized invertase (1.55 g) and an equal amount of plain resin in separate Pasteur pipets (5.5 mm × 9.5 cm) with glass wool plugs at both ends.

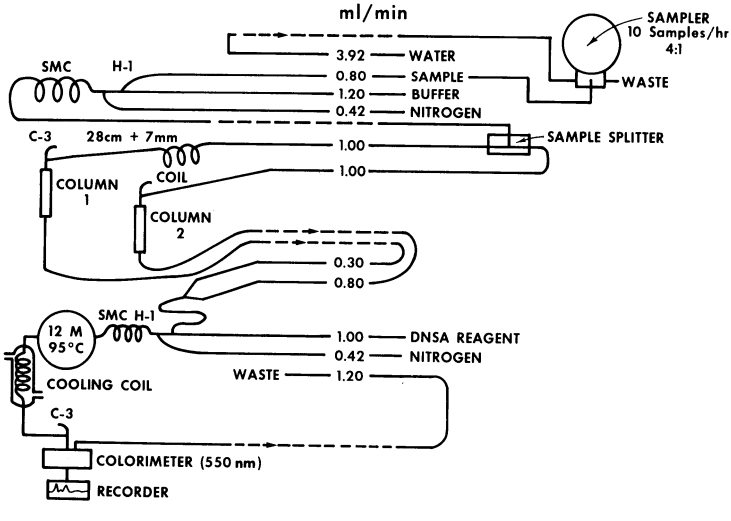

Fig. 1. AutoAnalyzer manifold for sequentially reading sample blank and invertase treated sample (sequential absorbance method). Letter-number combinations refer to Technicon symbols for parts. The Y tube and the 28 cm × 7 delay coil were fabricated in the laboratory. SMC is a small mixing coil. Column 1 contains immobilized invertase; column 2, resin only.

The Technicon AutoAnalyzer was set up in one of two ways. In the 'sequential absorbance method' (Fig. 1), a time-delay coil is incorporated in the enzyme-treated extract line which allows sequential reading of the blank- and enzyme-treated samples on a single spectrophotometer-recorder apparatus. The blank absorbance is subtracted from the absorbance of the enzyme-treated sample to yield the absorbance difference which is directly proportional to the reducing sugars produced by the invertase. These values are referred to as apparent sucrose.

In the second system called the 'ratio recording method' (Fig. 2), matched-flow cells are used in a ratio recording spectrophotometer allowing direct readout of the absorbance difference. The fact that the blank- and invertase-treated samples are read at the same time increases the capacity of the equipment and reduces calculations for determining per cent sucrose.

The spectrophotometer was a Beckman DK-2A equipped with a flow cell (two flow cells for the ratio recording method, Fig. 2) and connected to a Varian Model 14 strip-chart recorder. Absorbance was read at 550 nm. The AutoAnalyzer sampler was set to sample 10 samples per hr in the sequential-absorbance method and 30 samples in the ratio-recording method. A 1:4 sample to rinse ratio was used.

WSB and CSM were commercial samples prepared for export obtained from U.S. Dept. of Agriculture Consumer and Marketing Service.

To extract WSB and CSM, 10 g of sample and 75 ml of room-temperature

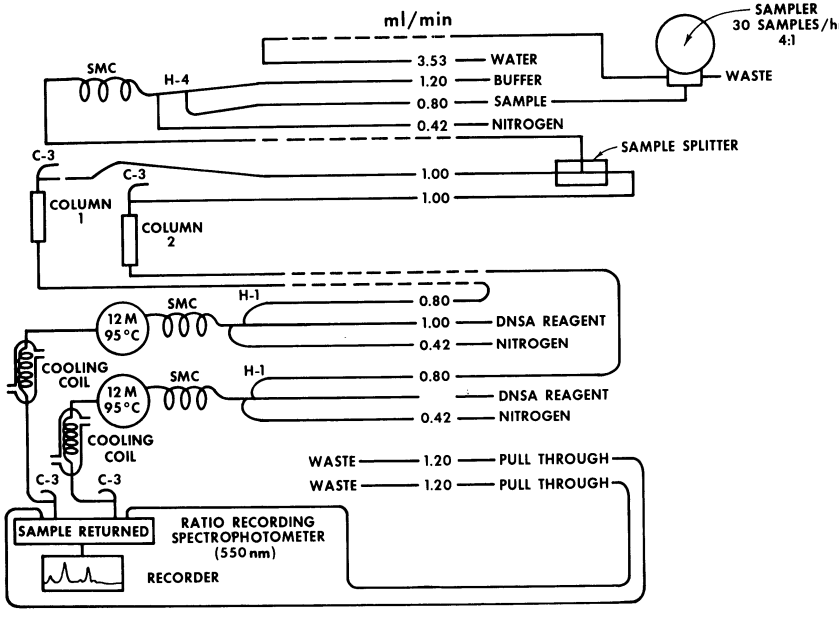

Fig. 2. AutoAnalyzer manifold for direct readout of sucrose (ratio-recording spectrophotometer method). Letter-number combinations refer to Technicon symbols for parts. SMC is a small mixing coil. Column 1 contains immobilized invertase; column 2, resin only.

distilled water were added to a 100-ml volumetric flask, shaken for 30 min on a wrist action shaker, and then diluted to 100 ml with water. After mixing, the extract was centrifuged at 2000 rpm for 10 min in a clinical centrifuge and a sample of supernatant placed in the AutoAnalyzer sampler for automated analysis.

TABLE I
Comparison of Sucrose Values for WSB and CSM by the Immobilized Invertase Sequential Absorbance Method and the Anthrone Method (3)

	% Sucrose[a]			
	Sequential Absorbance Method			Anthrone Method
Sample	Blank	Invertase Treated	Apparent Native Sucrose	Native Sucrose
WSB-1	2.4	6.3	3.9	3.9
WSB-2	2.5	6.4	3.9	3.9
WSB-3	2.2	6.0	3.9	3.8
CSM-1	4.7	7.5	2.8	2.8
CSM-2	5.0	7.9	2.9	2.8
CSM-3	4.7	7.5	2.8	2.8

[a]As-is basis.

TABLE II
Sucrose Determinations on Sweetened WSB and CSM Samples

	% Added Sucrose[a] Determined by	
Sample	Sequential Absorbance Method	Ratio Recording Method
WSB + 13% Sucrose	13.0	13.1
WSB + 14% Sucrose	14.0	14.0
WSB + 15% Sucrose	15.0	15.0
WSB + 15.5% Sucrose	15.6	15.5
Commercial S-WSB-1[b]	15.2	15.2
Commercial S-WSB-2[b]	15.1	15.0
CSM + 13% Sucrose	13.0	12.9
CSM + 14% Sucrose	14.0	14.0
CSM + 15% Sucrose	15.0	15.1
CSM + 15.5% Sucrose	15.4	15.5
Commercial S-CSM-1[b]	15.1	15.2
Commercial S-CSM-2[b]	15.1	15.1

[a]As-is basis.
[b]Added sucrose is 15% of the formulation.
Added sucrose is calculated using the following formulas:

$$Sa = \frac{(St - Snt)\ 100}{100 + Snt}$$

where

Sa = Added Sucrose
St = Total Sucrose
Snt = Native Sucrose (average values for unsweetened product: 3.9 for WSB and 2.8 for CSM)

RESULTS AND DISCUSSION

The enzyme activity of the immobilized invertase was 218 mg glucose produced per min per g of drained, immobilized enzyme, or 1.21 mmol sucrose hydrolyzed per min per gram of immobilized enzyme. This activity corresponds to 17% of that originally applied to the resin as Sucrovert. The amount of enzyme applied was in excess of the maximum that could be attached to ensure the highest invertase activity possible on the resin.

In column operation, the immobilized invertase hydrolyzed 74% of the applied sucrose in the concentration range of 1–3% under the flow, pH, and temperature conditions used in the analysis.

Columns were used daily during the experiments and stored overnight at 4°C. Under these conditions, there was no detectable loss in invertase activity over a 2-week period. Columns were susceptible to bacterial growth if they were not completely rinsed with buffer at the end of the day. Activity loss in 30 days was about 50% when the columns were left at room temperature. However, since it was routine practice to start a series of analyses by running a set of knowns and constructing a new standard curve, reproducible results on samples were possible even at 50% loss in column activity.

Standard curves for glucose, fructose, and sucrose were all linear through the range from 0.50 to 3.5% sugar in the standard solution.

As noted, the invertase treatment of standards and samples did not hydrolyze 100% of the sucrose. Linearity of the standard curve, however, shows that the extent of hydrolysis was constant under the conditions and concentrations studied. The calculated per cent hydrolysis for sucrose standards was initially 74%. As columns aged, the degree of hydrolysis dropped slowly. This was corrected by running standards daily.

Sucrose values for unsweetened CSM and WSB were determined by the sequential absorbance method. The absorbances were translated to per cent sucrose using the sucrose standard curve. The difference between the blank- and invertase-treated samples represents the reducing sugars generated by invertase action and are referred to as apparent native sucrose. The results for the various WSB and CSM are shown in Table I and are compared to the native sucrose content as determined by the anthrone method (3). The agreement is good.

Table II shows sucrose results obtained by both the sequential-absorbance method (Fig. 1), and the ratio-recording method (Fig. 2), on laboratory-sweetened WSB and CSM samples and commercially sweetened WSB and CSM. Both methods gave close estimations of the amount of sucrose added. In order to calculate added sucrose, a correction must be made for native sucrose. In the case of laboratory-sweetened products, the original WSB and CSM before sweetening were analyzed and found to contain 3.9% and 2.8% native sucrose, respectively. For the commercially sweetened samples, the native sucrose contents of the unsweetened WSB and CSM were used. The standard deviation on the commercial samples, determined in triplicate, was $\sigma = 0.09\%$ for both methods.

In conclusion, technology on immobilized enzymes (invertase) has been successfully combined with an established automated method for determination of reducing sugars resulting in an accurate, automated method for measuring added sucrose. When a ratio recording spectrophotometer is available, it offers

advantages of increased sampling rate and reduced calculations. Other methods for determining glucose or reducing sugars in an automated system should also be capable of combination with the immobilized invertase and work equally well.

Acknowledgment

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