Sucrose Determination by a Modified Anthrone Method. Application with Sweetened Wheat-Soy Blend and Corn-Soy-Milk[1]

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ABSTRACT

The colorimetric method developed is based on extraction of sugars with 80% ethanol, destruction of interfering reducing sugars with Fehling solution, and reaction of remaining sucrose with anthrone in sulfuric acid at 40°C. Absorbance of the sucrose-anthrone complex is read at 610 nm. and compared with the absorbance of a similarly treated standard sucrose solution. An automated method using a Technicon AutoAnalyzer is also described. Application of the method to determine added sucrose in sweetened wheat-soy blend and sweetened corn-soy-milk for quality assurance and regulatory purposes is described.

The U.S. Department of Agriculture recently released specifications for two new products for use in the Food for Peace Program (1,2). The products are sweetened wheat-soy blend (WSB) and sweetened corn-soy-milk (CSM). A simple sucrose method was needed for use with these high-protein, precooked, supplemental foods for quality assurance and regulatory purposes. Since added sucrose was the desired value, compensation for the native sucrose of the cereals and soy had to be taken into account.

The anthrone method for determining carbohydrates has been known for many years. Van Handel (3) reported direct micro-determination of sucrose in the presence of other carbohydrates. The procedure involved destruction of the reducing sugars with hot concentrated alkali followed by reaction with anthrone at low temperature. Jorgensen and Jorgensen (4) have used hypobromite to destroy reducing sugars followed by the anthrone reaction. Samotus and Kujawski (5) reported the use of both dinitrophenol and anthrone to quantitate mixtures of sugars in potato tubers. Pinnegar and Whitear (6) reported an automated anthrone procedure for determining total carbohydrate in worts and beers.

The present work describes a simplification of the anthrone method for determination of sucrose and the application of the method to determine added sucrose in sweetened WSB and sweetened CSM.

MATERIALS AND METHODS

The method is based on extraction of sugars with 80% ethanol followed by destruction of reducing sugars with Fehling solution. The use of Fehling solution eliminates the need to evaporate to dryness as reported in the procedure of Van Handel (3) where reducing sugars are destroyed by alkali. Upon destruction of reducing sugars, the remaining sucrose is reacted with anthrone in sulfuric acid and absorbance read at 610 nm.

[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.

Manual Method

Reagents: 1) 80% ethanol. 2) Fehling solution according to Shriner et al. (7). Solution A: 36.4 g. copper sulfate pentahydrate is dissolved and diluted with water to 500 ml. Solution B: 173 g. sodium acid tartrate and 70 g. of sodium hydroxide are dissolved and diluted with water to 500 ml. Equal parts of solutions A and B are mixed daily to meet daily needs. 3) Anthrone reagent according to Van Handel (3). Carefully add 760 ml. of sulfuric acid to 300 ml. of water; cool to room temperature and dissolve 1.5 g. anthrone in the diluted acid.

Method: 1) Weigh 1.0 g. of product into a 100-ml. volumetric flask. Add 50 ml. of 80% ethanol and gently boil for 15 min. Concurrently, run a reagent blank and a standard which is 1 ml. of a 15% w./v. sucrose solution (15 g. sucrose made up to 100 ml. with water). 2) Cool to 20°C. and dilute to 100 ml. with 80% ethanol. 3) Filter through S&S 576 filter paper or equivalent. 4) Add 1 ml. of filtrate to 9 ml. of Fehling solution in a test tube and heat in boiling water bath for 15 min. Cool to 20°C. 5) To 1 ml. of this solution in a clean test tube, rapidly add 10 ml. of anthrone reagent. Hold 30 min. at 40°C. 6) Read absorbance at 610 nm. against a reagent blank. 7) Calculate percent total apparent sucrose:

$$\text{Percent total apparent sucrose} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 15 \qquad (1)$$

Automated Method

Reagents: 1) 80% ethanol. 2) Fehling solution. Dilute Fehling solution for Manual Method with an equal volume of water. 3) Anthrone reagent. Carefully add 880 ml. of sulfuric acid to 190 ml. water and cool to room temperature. Dissolve 0.375 g. of anthrone in the diluted acid.

Method: Figure 1 is a schematic diagram of the Technicon AutoAnalyzer sampler, pump, and heating bath that is employed. The spectrophotometer was a Beckman DK-2A equipped with a flow cell. It was modified to accommodate a 2-mm. flow cell and was connected to a Varian Model-14 stripchart recorder. Samples were run at 20 per hr. with 1-min. sampling and 2-min. rinsing. Absorbance was recorded at 610 nm.

Determination of Added Sucrose in Sweetened WSB or Sweetened CSM

Method: 1) Determine percent total apparent sucrose in sweetened WSB (or sweetened CSM) as in either method above. 2) Determine percent total apparent sucrose in WSB (or CSM) or use the value of 3.78% for WSB (or 2.77% for CSM). This value represents the average obtained for several WSBs or (CSMs); see Table I. 3) The percent added sucrose was calculated using the following equation:

$$\%AS_{SP} = \%TS_{SP} - \frac{100 - \%TS_{SP} + \%TS_{UP}}{100} \%TS_{UP} \qquad (2)$$

where: $\%AS_{SP}$ = Percent added sucrose in sweetened product.

$\%TS_{SP}$ = Percent total apparent sucrose in sweetened product.

$\%TS_{UP}$ = Percent total apparent sucrose in unsweetened product.

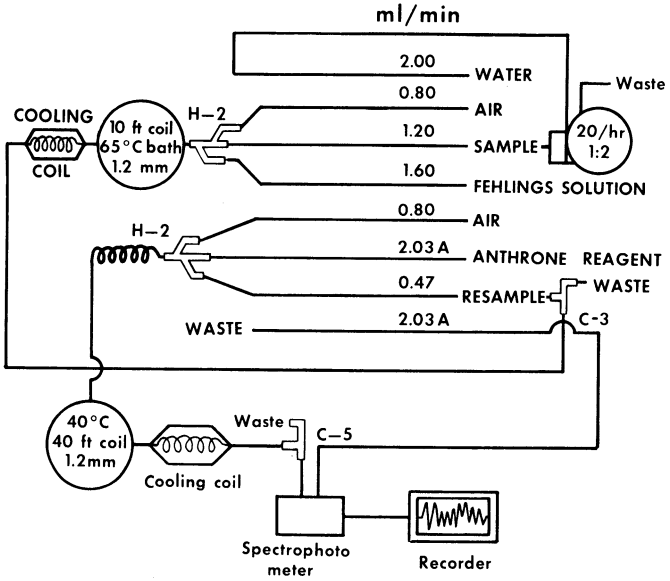

Fig. 1. Manifold for AutoAnalyzer for determination of sucrose by anthrone procedure. Connector numbers refer to Technicon numbers. A = acidflex tubing.

This formula accounts for the difference in blank due to the 15% of blank replaced by sucrose. Because of the large sucrose additions (15%), the correction becomes necessary.

RESULTS AND DISCUSSION

The standard curves in Fig. 2 show that the anthrone reaction with sucrose follows Beer's Law over the range of interest.

TABLE I. TOTAL APPARENT SUCROSE CONTENT OF WSBs AND CSMs BY THE MANUAL AND AUTOMATED ANTHRONE METHODS

	Percent Total Apparent Sucrose, as-is		
	Manual method		Automated method
WSB-1	3.74		3.91
WSB-2	3.60		3.88
WSB-3	3.60		3.82
WSB-4	3.71		3.98
WSB-Average	3.66	3.78[a]	3.90
CSM-1	2.72		2.81
CSM-2	2.80		2.82
CSM-3	2.72		2.77
CSM-4	2.58		2.75
CSM-5	2.68		2.70
CSM-6	2.78		3.00
CSM-7	2.75		3.00
CSM-Average	2.72	2.77[a]	2.83

[a] Average of Manual and Automated Methods.

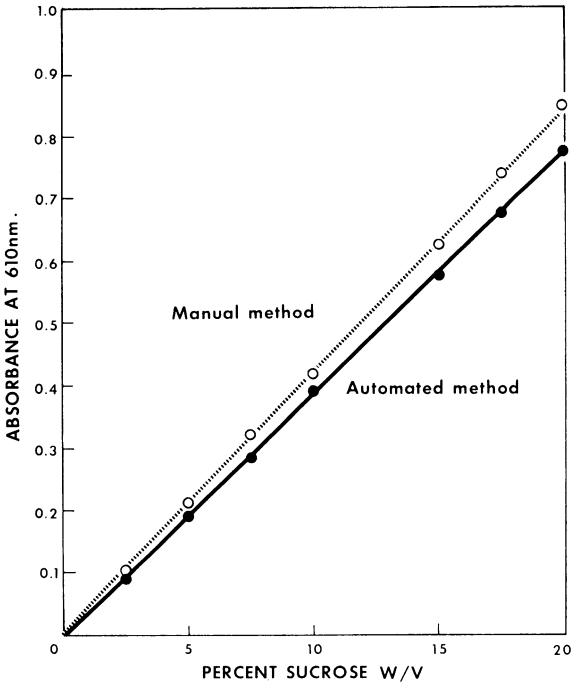

Fig. 2. Absorbance at 610 nm. of various concentrations of sucrose after reaction with anthrone.

TABLE II. THE EFFECT OF VARIOUS SUGARS ON THE DETERMINATION OF SUCROSE BY THE AUTOMATED METHOD

Sample Composition	Sucrose Found
15% sucrose	15.0
15% sucrose + 5% glucose	15.1
15% sucrose + 10% glucose	14.9
15% sucrose + 5% fructose	15.1
15% sucrose + 10% fructose	15.0
15% sucrose + 5% maltose	14.9
15% sucrose + 10% maltose	15.1

In order to check for interference of other sugars, levels up to 10% of glucose, fructose, maltose, and combinations of these sugars were added to 15% sucrose solutions, and sucrose was determined by both the manual and automated methods. Results indicated no significant differences in the sucrose values due to the presence of reducing sugars (Table II). Maltose does interfere at higher temperatures, but at 40°C. no color develops.

Saunders et al. (8) have reported the presence of sucrose and several other fructose-containing sugars in wheat. Soy is also known to contain sucrose, raffinose, and other fructose-containing sugars (9). This native sucrose is detected by the cold anthrone reaction as is raffinose (3) and other sugars which contain a fructose

TABLE III. DETERMINATION OF TOTAL APPARENT AND ADDED SUCROSE BY THE MANUAL AND AUTOMATED ANTHRONE METHODS

Product	Percent Total Apparent Sucrose		Percent Added Sucrose[a]	
	Manual method	Automated method	Manual method	Automated method
WSB + 13.0% sucrose	16.20	16.46	12.89	13.16
WSB + 14.0% sucrose	17.11	17.40	13.83	14.13
WSB + 14.5% sucrose	17.71	17.85	14.46	14.60
WSB + 15.0% sucrose	18.06	18.31	14.82	15.08
WSB + 17.5% sucrose	20.50	20.72	17.38	17.58
CSM + 13.0% sucrose	15.70	15.41	13.29	12.99
CSM + 14.0% sucrose	16.77	16.42	14.39	14.03
CSM + 14.5% sucrose	17.19	16.90	14.82	14.52
CSM + 15.0% sucrose	17.65	17.45	15.29	15.09
CSM + 17.5% sucrose	19.80	19.93	17.50	17.64

[a]Value of 3.78% sucrose used for WSB and 2.77% for CSM. (Table I): See Materials and Methods Section, equation 2, for method of calculation.

moiety not at the reducing end. Since the levels of apparent native sucrose (sucrose plus other low-temperature anthrone-positive sugars not destroyed by Fehling solution) in WSBs and CSMs must be known before added sucrose can be determined in the sweetened WSBs or CSMs, several samples were analyzed by both the manual and automated anthrone methods. Results are given in Table I. The apparent native sucrose in WSBs averaged 3.78% and in CSMs 2.77%, while the automated method reproducibly (but unexplained) gave slightly higher sucrose values (for simplicity we have averaged the values from the manual and automated methods).

Sucrose was added to WSB and CSM at 13, 14, 14.5, 15, and 17.5%. Total and added sucrose were determined according to the procedures described in the Materials and Methods Section. Results are shown in Table III.

Using the automated anthrone procedure, added sucrose was determined on more than 300 commercial samples of sweetened CSM (on the order of 15% added sucrose). The standard deviation of 50 of these samples analyzed in duplicate was $\sigma = 0.12$. Such precision is sufficient for routine quality assurance on such products.

Acknowledgments

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