Automated Determination of Reducing Sugar and Sucrose in Food Products

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ABSTRACT

This procedure provides a rapid method for quantitating the reducing sugar and sucrose contents in a variety of food products using Technicon AutoAnalyzer components. Sodium 2,4-dinitrophenolate reacts with reducing sugars to form a yellow-red azoxyphenolate compound. The nature of this reaction is discussed in detail. Absorbance at 550 nm. is directly proportional to the reducing sugar concentration. Sucrose is analyzed by the same method after automated hydrolysis. Samples are clarified and filtered manually before the automated sugar analyses. Dextrose and sucrose serve as standards. Results compare favorably with those obtained by a classical copper reduction method but require less than one-third the time.

Investigation of several classical techniques for quantitating reducing sugars led us to believe that none fulfill all the needs of a service-oriented food analytical laboratory. Both automated and manual copper reduction methods, such as those described by Munson and Walker (1), Nelson (2), Somogyi (3), and Fuller (4), provide accurate and precise reducing sugar results but are time-consuming (1,2,3), sensitive to small pH fluctuations (1,2,3,4), foul the lines of the automated system with precipitates (4), and/or produce erratic results in products containing interfering materials (1,2,3,4). Hagedorn and Jensen (5), Sawyer and Dixon (6), Bowen and Nonaka (7), Baum (8), and Ough and Lloyd (9) have published methods utilizing ferricyanide reduction by reducing sugars. The automated method (8) produces reliable quantitation of reducing sugars, but is not entirely satisfactory in determining the sucrose because a dialysis step is used which requires considerable equipment maintenance time. A buffering system which causes a loss of sensitivity is also necessary. Clinical applications of the procedures are well documented, but are not satisfactory for samples of widely varying compositions such as are encountered in food products.

Our investigation of sugar methods led us to believe that the Poe and Edson (10) and Ross (11) manual colorimetric procedures for determining reducing sugar levels in food products were the most suitable for automation. They utilize a color development between reducing sugars and sodium 2,4-dinitrophenolate (DNP). Results obtained with these methods show excellent correlation with results obtained by the widely accepted Munson-Walker procedure. Furthermore, this reaction is less sensitive to pH change than the other reduction methods and is easily adapted to sucrose determinations.

The automated DNP method, which is based on this reaction, allows the completion of approximately 25 reducing sugar analyses and 25 sucrose analyses per 8-hr. work day on most food products. The procedure has been successfully applied to cake mixes, syrups (including honey), margarine, pastry, nondairy creamers, peanut butter, dried egg, and potato products.

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MATERIALS AND METHODS

The basic Technicon AutoAnalyzer (Technicon Corporation, Chauncey, N.Y) system consists of a Sampler II, a proportioning pump, a 95°C. constant temperature bath containing two standard 40-ft. glass coils immersed in ethylene glycol, a colorimeter, a 550-nm. filter and a recorder using optical density chart paper. Pump tubing was selected from the "standard" (Technicon) types in available sizes. The sample line from the Sampler II to the proportioning pump was 0.034-in. I.D. polyethylene tubing (Technicon).

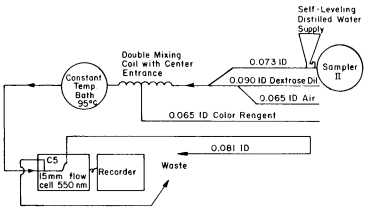
Reagents

Sodium 2,4-dinitrophenolate was purchased from K&K Laboratories, Inc., Plainview, N.Y., or Hollywood, Calif. The Brij-35 surfactant material is available from the Technicon Corporation. All other reagents are commercially available reagent grade chemicals.

Procedure

The varying physical and chemical characteristics of the food material studied here necessitate different sample preparations for each type of product. The weight of sample used and the clarification (removal of interfering substances) technique vary for different products. The proper amount of sample to be weighed prior to dilution is determined by the sugar contents of the material being analyzed. The sugar concentrations of the sample solutions must be within 0.0125 and 0.150%.

Peanut butter, mayonnaise, salad dressing, danish pastries, and some nondairy creamer products require removal of their lipid materials prior to clarification. The remainder of the applicable products can be clarified directly according to one of three techniques. Egg products are clarified using two solutions: 5.3% barium hydroxide and 5.0% zinc sulfate. Potato products use 20% lead acetate and a mixture of 13% sodium phosphate and 3% potassium oxalate. All other applicable materials use 15% potassium ferrocyanide and 23% zinc acetate solutions. All concentrations are weight per volume. The three clarification systems should always have their constituent reagents added in equal volumes. The amount of clarifying reagent added should be adjusted to afford a crystal-clear filtrate, but should never exceed 30 ml. per 1,000 ml. final volume. Concentrations of clarification reagents



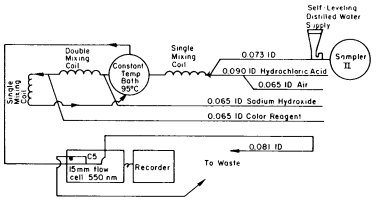


Fig. 1 (left). Automated system for reducing sugar determination.

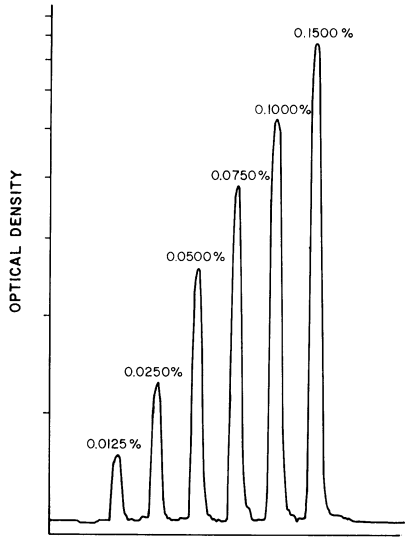
Fig. 2 (right). Automated system for sucrose determination.

above the specified maximum adversely affect the final color reaction. The clarified solutions are processed through the automated systems shown in Figs. 1 and 2.

If daily sample runs are made on materials that do not vary widely in the ratio of reducing sugar to sucrose, it may be advantageous to run both analyses simultaneously in a manner similar to that used by Ough and Lloyd (9). If, as in many laboratories, samples ranging from 0 to 80% reducing sugar and 0 to 80% sucrose are a necessity, it would be difficult to calibrate both systems over concentration ranges as wide as would be needed for simultaneous analyses.

The color reagent in both Figs. 1 and 2 contains 4% sodium 2,4-dinitrophenolate, 1% sodium hydroxide, and 10% potassium sodium tartrate plus 0.5 ml. of Brij-35 surfactant per liter of solution. The sodium hydroxide and hydrochloric acid solutions in Fig. 2 are 1.0N and 0.1N, respectively. A 0.0225% dextrose solution is used in Fig. 1 instead of a distilled water diluent stream. This dextrose solution must be added to displace the calibration curve upward to give a Beer's Law response with its origin at zero. We believe that this dextrose addition satisfies nondetectable threshold reaction and permits low reducing sugar levels to respond linearly.

Standard concentrations (weight/volume) of dextrose and sucrose at levels of 0.0125, 0.0250, 0.0500, 0.0750, 0.1000, and 0.150% are used for calibrations of the systems. Figure 3 shows a typical reducing sugar recorder chart from the AutoAnalyzer at a sampling rate of 40 samples per hour (2:1) and at the standard chart speed of a Technicon recorder. The samples are separated by a water wash in every other cup.



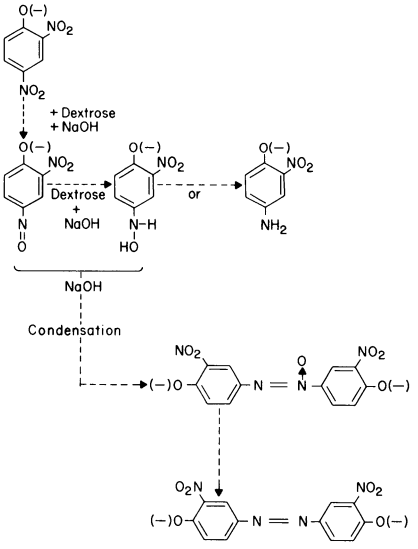


Fig. 3 (left). Recorder output of standard dextrose solutions from automated reducing sugar analysis.

Fig. 4 (right). Proposed reaction between dextrose and DNP.

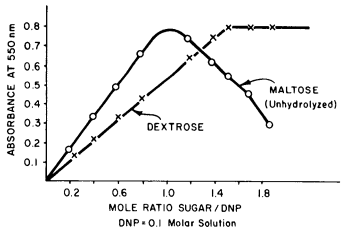


Fig. 5. Mole ratio plots of sugar-DNP reactions.

RESULTS

The absorbance maximum of the DNP-reducing sugar reaction products is at 425 nm., but the baseline of the automated system at this wave length is erratic. Measuring the absorbance at 550 nm. in this application reduces the method's sensitivity but creates a very stable automated system. The loss of sensitivity is easily overcome by adjusting sample weights. A visible absorption curve on sample solutions does now show interfering absorbances at 550 nm.

The reduction of DNP is not specific for reducing sugars. Several aldehydes, ketones, and amino acids react to varying degrees with alkaline solutions of DNP. Acetaldehyde and benzaldehyde show responses to DNP comparable to that of glucose at 95°C. (the reaction temperature of the automated procedure). Formaldehyde shows no reaction in 30 min. (reaction time of automated system is 6 min.) at 95°C. Acetone and 2-butanone show higher response to DNP than does glucose. These two ketones react completely at 27°C. in less than 3 min. These materials will interfere with the determination of both reducing sugars and sucrose if they are present at significant levels. This rarely occurs in most food products.

Proline, glutamic acid, lysine, and leucine all show slight responses to DNP at 95°C., but require approximately 20 min. to develop a color. The short reaction time of the automated system (6 min.) precludes interference of these amino acids even at concentrations up to 10% of the product.

The DNP-dextrose color reaction product was isolated by a thin-layer chromatographic technique. An infrared spectrum of the isolated material indicated the presence of azoxy, azo, and amine materials. A positive test for polynitro azoxy

TABLE I. RESPONSE OF COMMON SUGARS RELATIVE TO DEXTROSE IN THE DNP REACTION

Sugar	Ratio of Molar Absorbances in Reducing Sugar Analysis Relative to Dextrose in Reducing Sugar Analysis[a]	Ratio of Molar Absorbances in Sucrose Analysis Relative to Dextrose in Sucrose Analysis[a]
Dextrose	1.000	1.000
Sucrose	0.000	2.000
Galactose	1.253	1.200
Fructose	1.023	1.000
Maltose	1.480	1.440
Lactose	1.680	1.654

[a]All figures represent responses.

compounds was obtained by the Wallach rearrangement test (12). The production of azoxybenzene, azobenzene, and aniline from the reduction of nitrobenzene by dextrose in alkaline solution was documented by Opolonick (13). Under the conditions of our method, Opolonick's studies indicate that our major reaction product is the azoxy compound with minor acounts of azo and amine materials. This reaction, as described in Fig. 4, is consistent with the 1.5:1 dextrose:DNP mole ratio indicated in Fig. 5. Three moles of dextrose are required to convert 2 moles of DNP to the nitroso and hydroxylamine derivatives. The nitroso and hydroxylamine derivatives condense in excess sodium hydroxide to the azoxy derivative.

The dextrose-DNP reaction requires a minimum time of 5 min. for complete reaction, but additional reaction time up to a total of 30 min. is not harmful if free amino acids are not present.

Common sugars other than dextrose and sucrose vary in their responses to the DNP reaction. Table I shows the ratio of molar absorbance of four sugars to dextrose in the automated reducing sugar and sucrose analyses using the dextrose response as 1.00. The 2.0 ratio shown by sucrose indicates that complete hydrolysis of that sugar occurs during 6 min. at 95°C. in 0.1N hydrochloric acid. For maltose, the molar absorbance ratio is 1.480. This ratio agrees with the maltose:glucose ratio of molecular reducing powers published previously (14) stating that a disaccharide containing a 1→4 glycosidic linkage will exhibit about 1.4 times the molecular reducing power of glucose under specific conditions. The mole ratio plot for maltose in Fig. 5 shows that, at the mole ratio of 0.05 used in this automated analysis, maltose produces more absorbance at 550 nm. than does dextrose. This is not true once the mole ratio reaches 1.3. We expect that galactose and lactose would also have mole ratio plots whose initial slopes are greater than that of dextrose.

Lactose and maltose are not converted to their monosaccharide components by the hydrolysis conditions used in the sucrose method to produce invert sugar from sucrose. Thus, lactose and maltose give the same response compared to dextrose before and after hydrolysis, and calibration curves of these two sugars show the same responses with and without acid treatment. Since the reducing sugar content

TABLE II. STANDARD DEVIATION OF REDUCING SUGAR AND SUCROSE ANALYSIS

Product	Sugar Level %	Std. Dev. Reducing Sugar[a] %	Std. Dev. Sucrose[a] %
Cake mixes	0 – 10	0.15	0.20
Fondants	50 – 100	0.40	0.80
Salad Dressings	0 – 1.5	0.066	
Mayonnaise	0 – 0.8		0.037
Potato products	0 – 3.0	0.052	
	0 – 4.5		0.074

[a]Figures represent a minimum of ten duplicate results on separate samples.

prior to hydrolysis is subtracted from the reducing sugar content after hydrolysis, it is essential that disaccharides other than sucrose have a known response in both methods. These disaccharides or galactose could cause an error in the reducing sugar determination if they are calculated as dextrose and if they are present in significant amounts. If the reducing sugar in the sample is known to be primarily lactose, maltose, or galactose, the appropriate sugar should be used for preparing the reducing sugar calibration curve standards.

Table II shows the standard deviations of the reducing sugar and sucrose determinations, respectively, for the food products on which they have been established.

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