The Determination of Sucrose in Concentrated Steffen Filtrate by G.L.C.

JAN KARR and LLOYD W. NORMAN

Received for publication October 30, 1973

Introduction

The polarimetric quantitation of sucrose in the presence of other optically active compounds has been recognized as a significant problem in the sugar industry (1, 3, 12, 13)[1]. As the ratio of sucrose to other optically active components of the system decreases the accuracy of the polarimetric analysis may be severely impaired.

Henglein et al. (7) described a procedure for the preparation of the octa trimethylsilyl ether of sucrose and other carbohydrate silyl ethers. These ethers were thermally stable and had a higher vapor pressure than the parent compounds. Using this procedure, Sweeley et al. (16) developed a qualitative gas chromatographic method for the detection of mono and disaccharides. A modification of this method was used by Walker (17) for qualitative investigation of molasses. Using sorbitol as an internal standard and the silylation procedure of Sweeley, Dowling and Libert (6) reported a gas chromatographic method for mono and disaccharides that had an accuracy comparable to that of the standard polarimetric method for low purity samples. Brobst and Lott (2) developed a similar technique for the analysis of mono, di, tri, and tetra saccharide components of corn syrups. Using the disaccharide trehalose as an internal standard, Rumpf (15) published a technique for the analysis of carboxyllic acids and carbohydrates found in potato extracts.

The silylating reagent commonly used is a mixture of hexamethyldisilazane and trimethylchlorosilane. In addition to silylating hydroxyl groups, amino and carboxyl moieties are partially silylated (16). N-trimethylsilylimidazole was shown by Horning (8) to be selective for hydroxyl groups. The use of such a derivatizing reagent eliminates possible interference from amino acids and polypeptides.

The primary objective of this work was to develop a method for the determination of sucrose in C.S.F. that was accurate, precise and specific for sucrose. A secondary objective was to determine whether there was any correlation between the polarimetric result and the gas chromatographic result and develop an equation for the correction of the polarimetric result.

---

[1]Numbers in parentheses refer to literature cited.

Experimental

Reagents and standards

Trehalose, the internal standard used, was obtained from Nutritional Biochemical Company, Cleveland, Ohio and recrystallized twice from an alcohol-water system. The silylating reagent, N-trimethylsilylimidazole, was purchased from Ohio Valley Specialty Chemical Company, Marietta, Ohio. Analytical reagent grade N N dimethylformamide was used as obtained from J. T. Baker.

Equipment

A Hewlett Packard Research Gas Chromotograph, Model 7620A, equipped with a dual flame ionization detector, a digital integrator, Model 3370A, and an automatic injector, Model 7670A, was used in the course of this study. Dual stainless steel columns, one-fourth inch diameter, six feet in length were packed with ten percent OV-17 on Chromsorb W, 80/100 mesh, (Applied Science Laboratories, Inc., State College, Pa.) and coiled. Preconditioning of the columns at 290°C overnight with a helium flow rate of 20 ml per minute was necessary for base line stability. The columns were operated isothermally at 265°C with a helium flow rate of 60 ml per minute. Temperatures of the injection port and dual flame ionization detector were maintained at 280°C and 325°C respectively. Flow rates of hydrogen and compressed air were 40 and 600 ml per minute. The samples were injected into the gas chromotograph using a 25 ul Hamilton Syringe, Model 702. All weighings were performed on a Mettler M-5 micro balance.

Infrared spectroscopy

A stream splitter was introduced into the gas chromatograph just prior to the detector. The peaks of the sucrose and trehalose derivatives were collected in dry glass tubing. Several injections were required to obtain a sufficient amount of material for infrared analysis. After the samples were collected, the tubes were sealed prior to analysis. The infrared analysis was performed using a Perkin-Elmer Model 621 spectrophotometer with a sodium chloride micro cell. The sample was dissolved in 30 ml of tetrachloromethane and injected into the micro cell. Each sample was run against tetrachloromethane blank.

Mass spectroscopy

A stream splitter was introduced in the gas chromatograph just prior to the detector. The TMS sucrose and trehalose peaks were collected in dry glass tubing and sealed. Mass spectra of sucrose and trehalose derivatives were run on a CEC 21-110 mass spectrometer using electron multiplier detection and 70 ev ionizing voltage. The samples were introduced directly into the ion source. The temperature of volatization was 130°C.

Methods

Gas chromatographic procedure

Approximately 15 mg of trehalose and 100 mg of a well mixed C.S.F. sample were accurately weighed into a tared serum bottle. The container was capped, 3 ml of N-trimethylsilylimidazole and 3 ml of N N dimethylformamide added. The bottle was heated at 60°C for a period of 15 minutes and cooled to room temperature. A seven microliter aliquot was injected into the gas chromatograph. The areas under the sucrose and trehalose peaks were integrated. The following equation was used to calculate the percent sucrose.

$$\% \text{ Sucrose (W/W)} = \frac{\text{Wt. Trehalose} \times \text{Area Sucrose} \times K}{\text{Wt. Sample} \times \text{Area Trehalose}}$$

$$K = \frac{\text{Response Per Unit Mass of Trehalose} \times 100}{\text{Response Per Unit Mass of Sucrose}}$$

Polarimetric procedure

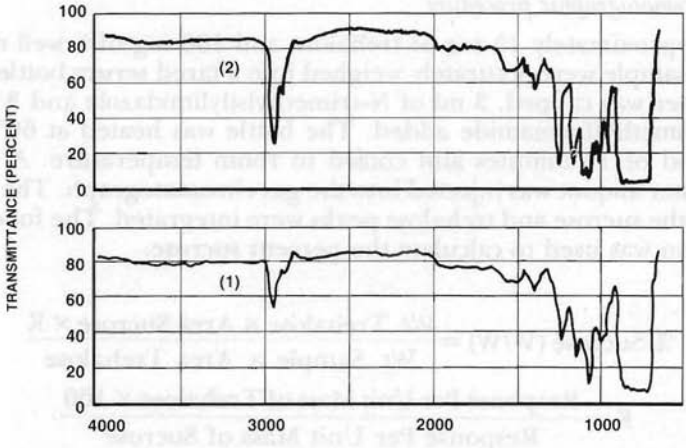
A 13.00 g aliquot of a well mixed C.S.F. sample was quantitatively transferred to a 200 ml volumetric flask. The sample was neutralized to a phenolphthalein end point with 20% acetic acid. After the addition of 20 ml of 55 brix basic lead acetate solution and thorough mixing, the volume was adjusted to 200 ml with distilled water. The resultant solution was thoroughly mixed and filtered. The filtrate was polarized in a 200 mm tube. The resultant reading was multiplied by four to determine the percent sucrose in the sample.

Results and Discussion

The kinetics of the silylation of the disaccharides has previously been reported (9). Derivatization was shown to be complete in fifteen minutes at room temperature. The pure octa trimethylsilyl ethers of sucrose and trehalose were isolated gas chromatographically and subjected to infrared and mass spectrographic analysis, Figures 1 and 2. Similarly the sucrose and trehalose peaks from C.S.F. samples were isolated and subjected to infrared and mass spectrographic analysis.

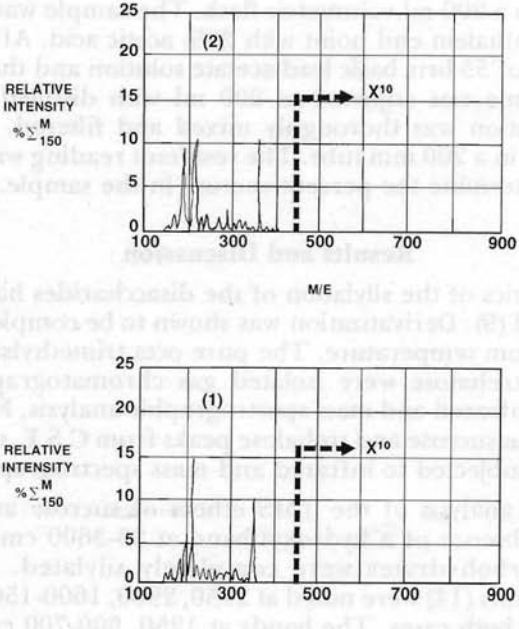
Infrared analysis of the TMS ethers of sucrose and trehalose showed the absence of a hydroxyl band at 33-3600 cm^{-1} indicating that both carbohydrates were completely silylated. Methyl and methylene bands (14) were noted at 2950, 2900, 1600-1500 and 1400-1420 cm^{-1} in both cases. The bands at 1250, 900-700 cm^{-1} coincide with the $SiCH_3$ absorption bands reported by Liu and Yahg (11). Also the Si-O-C absorption bands at 1080 and 1060 cm^{-1} reported by Liu and Yahg (11) were noted in both spectras. The infrared spectras of

Figure 1



WAVENUMBER(Cm^{-1}) I.R. SPECTRA OF OCTA TMS SUCROSE (1) & OCTA TMS TREHALOSE (2)

Figure 2



PARTIAL MASS SPECTRA OF OCTA TMS SUCROSE (1) & OCTA TMS TREHALOSE (2)

Figure 3

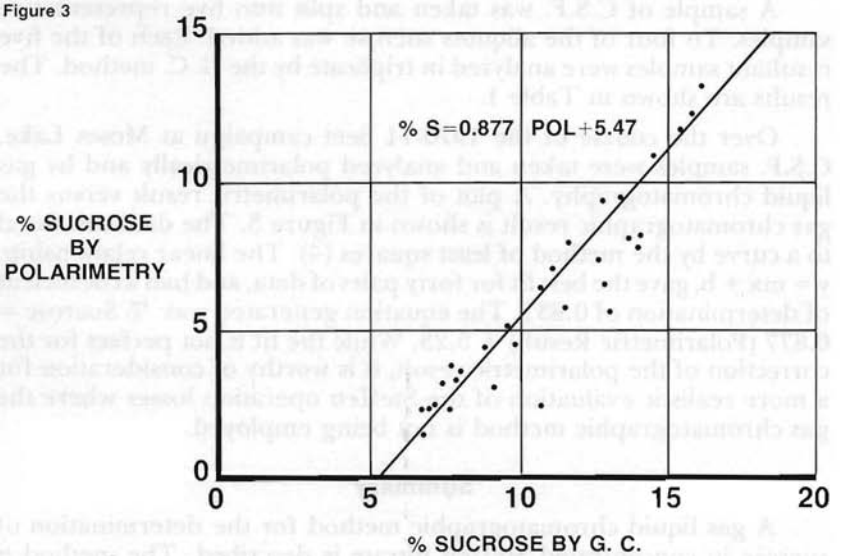


Table 1.—Mg sucrose per 100 Mg sample.

Added	Total	Found ± Std. Dev.
0.00		9.31 ± 0.04
0.90	10.21	10.18 ± 0.01
1.84	11.15	11.15 ± 0.03
2.64	11.95	11.96 ± 0.06
3.58	12.89	12.94 ± 0.06

sucrose and trehalose were significantly different in the 900-1100 cm^{-1} range so that they are readily differentiated from each other. Infrared analysis of similar fractions prepared from C.S.F. were in agreement with the respective standards.

Partial mass spectra recorded for pure samples of sucrose and trehalose trimethylsilyl ethers above *m/e* 150 are shown in Figure 2. Additional intense peaks occurred at *m/e* 73, 89, 117, 129, 133 and 147 in both compounds. Molecular ions are absent from the spectra, but the M-CH_3 ion (*m/e* 903) provides indirect verification of molecular weight, as reported for similar compounds (15, 16). The sucrose TMS ether produces an intense rearrangement ion at *m/e* 437 which is of low intensity in the trehalose spectrum, so that the compounds are easily differentiated. Significant differences in the spectra also occur in the 500-600 *m/e* region, a region reported (5, 10) to be of value in the assessment of glycosidic linkages. Mass spectra of the chromatographic effluents of TMS sucrose and trehalose from C.S.F. were comparable to that of the pure standards. No impurities were detected in those samples, which indicates that the sucrose and trehalose TMS peaks contain single components.

A sample of C.S.F. was taken and split into five representative samples. To four of the aliquots sucrose was added. Each of the five resultant samples were analyzed in triplicate by the G. C. method. The results are shown in Table 1.

Over the course of the 1970-71 beet campaign at Moses Lake, C.S.F. samples were taken and analyzed polarimetrically and by gas liquid chromatography. A plot of the polarimetric result versus the gas chromatographic result is shown in Figure 3. The data was fitted to a curve by the method of least squares (4). The linear relationship, $y = mx + b$, gave the best fit for forty pairs of data, and had a coefficient of determination of 0.931. The equation generated was: % Sucrose = 0.877 (Polarimetric Result) + 5.23. While the fit is not perfect for the correction of the polarimetric result, it is worthy of consideration for a more realistic evaluation of the Steffen operation losses where the gas chromatographic method is not being employed.

Summary

A gas liquid chromatographic method for the determination of sucrose in concentrated Steffen filtrate is described. The method is accurate, precise and selective for sucrose. Comparative analyses, polarimetric vs. gas chromatographic, demonstrated a significant error in the polarimetric procedure. The error was consistent. Using the method of least squares, an equation was generated for the correction of the polarimetric results at the Columbia Basin refinery. Utilization of the equation has permitted a more realistic evaluation of Steffen operation at the Columbia Basin refinery.

Acknowledgments

Grateful acknowledgment is given to Mrs. Pamela McBroom and Mr. Fouad Shaker for their technical assistance. The mass spectrographic analytical support by Dr. William Haddon of the Western Regional U.S.D.A. Laboratory is deeply appreciated. Infrared spectral support work by Dr. Bruce Ettling, Washington State University is duly acknowledged.

Literature Cited

(1) BATES, F.J. & ASSOICATES, 1942, *Polarimetry, Saccharimetry and the Sugars,* Circular of the National Bureau of Standards, C440, U.S. Department of Commerce, United States Government Printing Office, Washington, D.C.

(2) BROBST, K.M., AND C.E. LOTT, JR., 1966, Determination Of Some Components In Corn Syrup By Gas-Liquid Chromatography Of The Trimethylsilyl Derivatives, Cereal Chem., *43,* 35.

(3) BROWNE, C.A. AND F.W. ZERBAN, 1941, *Physical and Chemical Methods of Sugar Analysis,* 3rd Ed., Wiley & Sons Inc., New York, 372-373.

(4) DANIELS, F., J. MATHEWS, J. WILLIAMS, P. BENDER AND R. ALBERTY, 1956, *Experimental Physical Chemistry,* McGraw-Hill Book Co., Inc., New York, p. 339.

(5) DEJOUGH, D.C., T. RADFORD, J.D. HRIBAR, S. HAUESSIAN, M. BIEBER, G. DAWSON, AND C.C. SWEELEY, 1969, Analysis of Trimethylsilyl Derivatives of Carbohydrates by Gas Chromatography and Mass Spectrometry, J. Am. Chem. Soc., *91,* 1728.

(6) DOWLING, J.F. AND J.F. LIBERT, 1966, Applications of Gas-Liquid Chromatography in the Sugar Industry, Proc. 1966 Tech. Session Cane Sugar Refinery Research, 113-125.

(7) HENGLEIN, F.A., G. ABELSNES, H. HENEKA, K. LIEHARD, O. NAKHRE AND K. SCHEINOST, 1957, Organosilyl Derivatives of Dicarboxyllic Acids, Hydroxy Carboxyllic Acids and Sugars, Makromol. Chem., *24,* 1.

(8) HORNING, M.G., A.M. MOSS, E.A. BOUCHER AND E.C. HORNING, 1968, The GLC Separation of Hydroxyl-Substituted Amines of Biological Importance Including The Catecholamines. Preparation of Derivatives For Electron Capture Detection. Anal. Letters, *1,* (5), 311.

(9) KARR, J. AND L.W. NORMAN, 1970, A Gas Liquid Chromatographic Method for the Determination of Sucrose in Molasses, presented at the Sixteenth General Meeting of the Am. Soc. Sugar Beet Technologists, Denver, Colorado.

(10) KOCHETKOV, N.N., O. CHIZHOV, AND N. MOLODTSOV, 1968, Mass Spectrometry of Oligosaccharides, Tetrahedron, *24,* 5587.

(11) LIV, S. AND M. YAHG, 1964, Some Bis (trimethylsiloxy) Compounds, J. of Chinese Chem. Soc. (Taiwan), *11,* 202-204

(12) MCGINNIS, R.A., ED., 1971, *Beet Sugar Technology,* 2nd Ed., Beet Sugar Development Foundation, Fort Collins, Colorado, Chapter 2.

(13) MEADE, G.P., ED., 1963, *Cane Sugar Handbook,* 9th Ed., Wiley & Sons, Inc., New York, 434-40.

(14) NAKANISHI, K., 1964, *Infrared Absorption Spectroscopy,* Holder-Day, San Francisco.

(15) RUMPF, G., 1969, The Silylation of Substances Occurring in Natural Products and Detectable by Gas Chromatography, J. Chromatography, *43,* 247.

(16) SWELLEY, C.C., R. BENTLEY, M. MAKITA AND W.W. WELLS, 1963, Gas-Liquid Chromatography of Trimethylsilyl Derivatives of Sugars and Related Substances, J. of Am. Chem. Soc., *85,* 2497.

(17) WALKER, H.G., JR., 1965, GLC Examination of Molasses Carbohydrates, Internal. Sugar J., *67,* 237.