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Note

Gas chromatographic separation of α-lactose and sucrose as the trimethylsilyl derivatives

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Gas chromatography of the trimethylsilyl (TMS) derivatives of sugars has found wide application in the food and related industries[1–7]. The derivatives are easy to prepare, and have excellent chromatographic properties[8]. Polar liquid phases such as ethylene glycol succinate (EGS) and Carbowax 20M have provided good resolution of the monosaccharides[8,12]. These phases are generally unsuitable for analyses of disaccharides due to the excessive retention times for disaccharides at the temperature limits for these phases. A retention time of nearly 70 min was reported for α-glucose on Carbowax 20M at 170°. We are not aware of any separations of disaccharides reported on these highly polar phases.

Relatively non-polar high temperature phases such as SE-52 and SE-30 permit elution of disaccharides in a reasonable time, usually within 70 min. However, these phases do not provide an adequate separation of α-lactose and sucrose. Conversion to oximes and then TMS ethers has been applied to the separation of lactose and sucrose, using an SE-30 column[13]. Conversion to oximes resulted in fewer anomers, and a single peak was obtained for lactose. Support coated open tubular (SCOT), columns have been successfully applied to the separation of the TMS derivatives of lactose and sucrose, using OV-17 as the stationary phase[9].

Both of these alternatives are undesirable for many food chemistry laboratory applications. The SCOT columns are difficult to prepare and expensive to purchase. The conversion to the oxime prior to formation of TMS derivatives is time-consuming, and we feel it is advantageous to be able to measure individual anomers of sugars in foods routinely analyzed.

This paper describes a packed column utilizing the moderately polar XE-60, a cyanoethylmethylpolysiloxane as the stationary phase to successfully separate α-lactose from sucrose as the TMS ethers. No prior conversion to oximes or alditols is necessary. The column is inexpensive and easy to prepare. In addition to yielding a specific separation this column also resolves the normal mono- and disaccharides and sugar alcohols that occur in food products.

EXPERIMENTAL

Reagents

Hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) were obtained from the Pierce Chemical Company, Rockford, Ill., U.S.A. Reagent

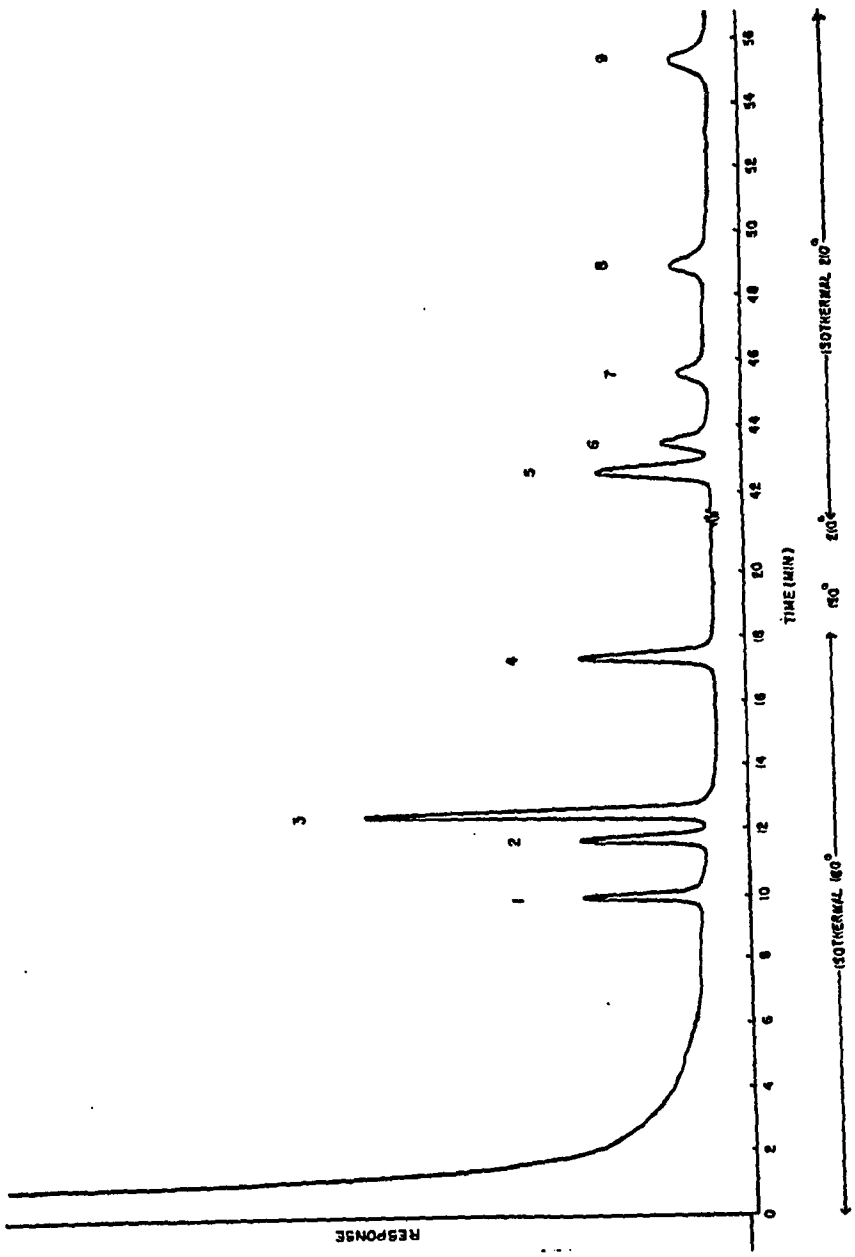

Fig. 1. Chromatogram of a standard sugar mixture. 1=β-Fructose-TMS, 2=α-glucose-TMS, 3=sorbitol-TMS, 4=β-glucose-TMS, 5=sucrose-TMS, 6=α-lactose-TMS, 7=α-maltose-TMS, 8=β-maltose-TMS, 9=β-lactose-TMS.

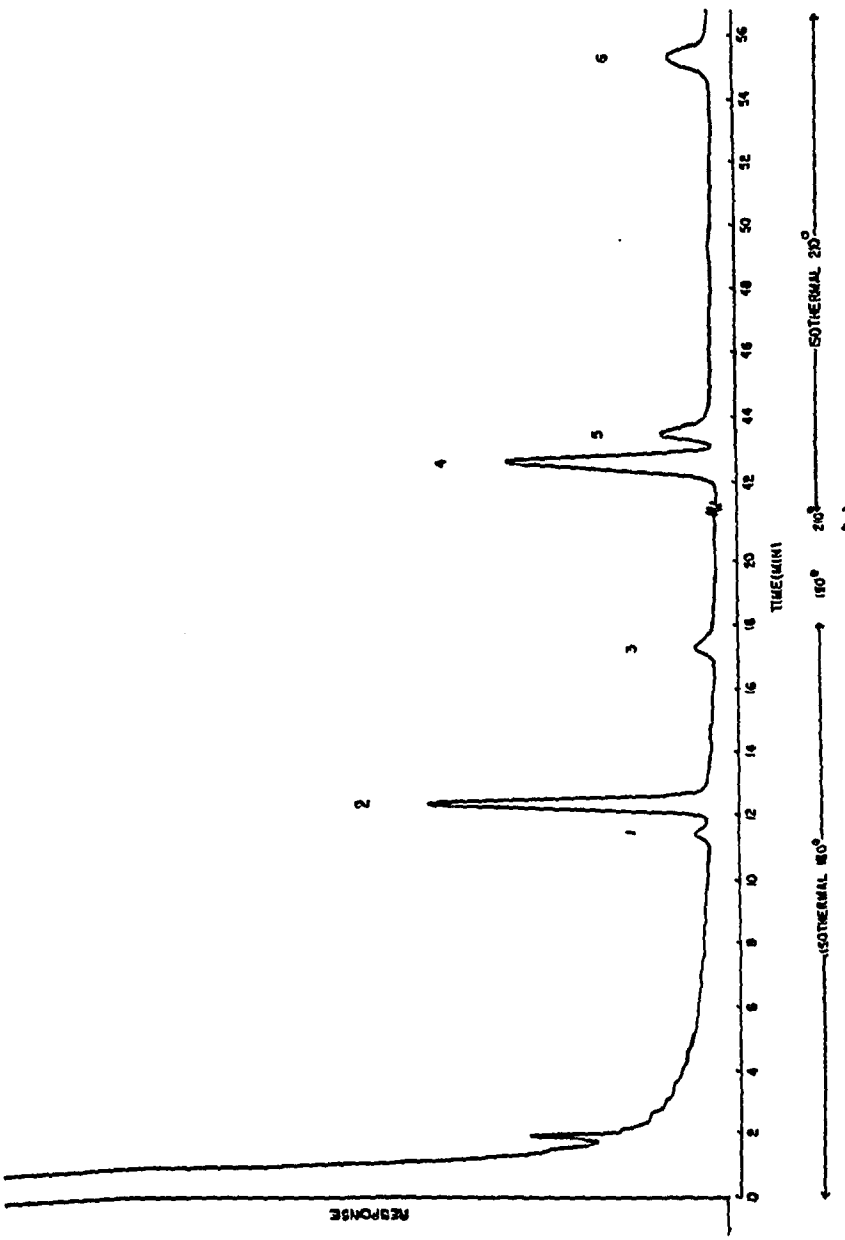

Fig. 2. Chromatogram of sugars in a commercial ice cream. 1=α-Glucose-TMS, 2=sorbitol-TMS, 3=β-glucose-TMS, 4=sucrose-TMS, 5=α-lactose-TMS, 6=β-lactose-TMS.

grade pyridine (Mallinckrodt, St. Louis, Mo., U.S.A.) was used without further treatment. XE-60, SE-52, 70/80 mesh Anakrom ABS, and 100/120 mesh Chromosorb G, acid washed, dimethyldichlorosilane treated, were obtained from Analabs, Inc., North Haven, Conn., U.S.A.

Apparatus

A Research Specialties Model 1670 gas chromatograph equipped with dual flame ionization detectors was employed. The column consisted of two sections, connected with a Swagelok union. The first section was a 20 ft. (1/8 in. O.D.) aluminum column packed with 1.5% XE-60 on 100/120 mesh Chromosorb G. The second section was a 7 ft. (1/8 in. O.D.) aluminum column packed with 3% SE-52 on 70/80 mesh Anakrom ABS. In both cases, the packing was prepared simply by dissolving a preweighed amount of stationary phase in methylene chloride, pouring it over the preweighed solid support (immersed in methylene chloride), and evaporating the solvent under vacuum while agitating the suspension.

The column temperature was held isothermally at 175° until all of the monosaccharides were eluted, and then programmed at 10°/min to 220° to elute the disaccharides. Injection port temperature was 260°, and the detector temperature was 255°. The helium carrier gas flow-rate was 20 ml/min.

The TMS derivatives were prepared by a modification of the method of Sweeley *et al.*[8]. An amount of sample containing 10–20 mg of sugars and not more than 25 mg of water was weighed into a small vial with a Teflon®-lined screw cap. One millilitre of pyridine, 0.6 ml of HMDS, and 0.3 ml of TMCS were added in that order. The vial was capped and placed in an ultrasonic bath for 2 min to facilitate dissolution of the sugars. After an additional 1/2 h, the sample was ready for analysis by GLC without further treatment.

RESULTS AND DISCUSSION

Sorbitol was chosen as an internal standard for quantitative analysis of the various sugars since it is absent in most food product samples. Sorbitol and α-glucose do not separate on XE-60, but good separation is obtained using the XE-60/SE-52 combination, without impairment of the sucrose–α-lactose separation. Fig. 1 illustrates the separation of the common sugars encountered in food products.

The addition of excess HMDS and TMCS permits the direct analysis of samples containing large amounts of water[5,11]. Shown in Fig. 2 is the chromatogram obtained from the direct trimethylsilylation of a 40 mg sample of ice cream which contained about 25 mg of water. No spurious peaks due to incomplete derivatization are present. The sucrose and α-lactose peaks are well separated, which is essential for a complete analysis of the sugars present in this sample.

We suggest that OV-225 would make another excellent phase for sugar determinations, since its Rohrschneider constants are close to those of XE-60[10], and it is somewhat more stable at high temperatures than XE-60. We should indicate, however, that the stability of XE-60 at our operating temperatures is excellent. In five years of intermittent use in our laboratory, a column as described in this paper has shown no deterioration in separation efficiency.

REFERENCES

1 J. M. Slanski and R. J. Mosby, *J. Chromatogr.*, 35 (1968) 94.
2 T. Cayle, F. Viebrook and J. Schiaffino, *Cereal Chem.*, 45 (1968) 154.
3 J. Blum and W. R. Kochler, *J. Gas Chromatogr.*, 6 (1968) 120.
4 J. F. Clapperton and A. G. Holliday, *J. Inst. Brew., London*, 74 (1968) 164.
5 K. M. Brolost and C. E. Lott, *Cereal Chem.*, 43 (1966) 35.
6 W. C. Ellis, *J. Chromatogr.*, 41 (1969) 325.
7 J. B. Bedle, *J. Agr. Food Chem.*, 17 (1969) 904.
8 C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
9 W. Averill, *Perkin-Elmer Instrum. News*, 18 (1967) 10.
10 W. E. Supina and L. P. Rose, *J. Chromatogr. Sci.*, 8 (1970) 214.
11 P. K. Davison and R. Young, *J. Chromatogr.*, 41 (1969) 12.
12 J. S. Sawardeker and J. H. Sloneker, *Anal. Chem.*, 37 (1965) 1602.
13 B. S. Mason and H. T. Slover, *J. Agr. Food Chem.*, 19 (1971) 551.