vacuolation of the hepatic parenchymal cells that may have been related to the toxic action of the crude extract or to anorexia.

Daily feed intake of the calf given the single dose of *A. fumigatus* extract (86 g containing 3.25 g SM-1, about 48 mg of SM-1 per kg of body weight) was severely depressed and the calf failed to improve. Intake during the 4 days posttreatment was only 25% of the pretreatment level compared to 97% for the two control calves. The treated calf showed spontaneous irritability and some diarrhea. Postmortem examination revealed hemorrhagic enteritis in the small intestine and, to a lesser extent, in the large intestine. Lung fields contained mild patchy interstitial thickenings of alveolar walls, largely involving nonnuclear cells and some neutrophils. Abnormal changes were not found in other tissues. Body temperatures and calcium, magnesium, phosphorus, total protein, bilirubin, white blood cell count, packed cell volume, hemoglobin, differential leucocyte count, serum glutamic oxaloacetic transaminase activity, creatinine phosphokinase activity, and sorbitol dehydrogenase activity in all calves were within normal ranges. Chloroform extracts of the urine and feces were analyzed for SM-1 by TLC; no SM-1 was detected, but several other differences between the control and treated calves were noted.

Figure 1 shows that five daily doses of 5.7 or 11.4 g of crude extract from *A. fumigatus* cultures (approximately 2.5 and 5.5 mg of SM-1 per kg body weight) depressed feed intake. The degree of depression and time required to effect it were proportional to the dosage. The calf given 11.4 g of crude extract developed severe diarrhea on day 2 of treatment, and it persisted for 7 days posttreatment. The calf getting 5.7 g of crude extract daily developed severe diarrhea on day 4 of treatment, and it persisted until 5 days posttreatment. As with the first group of three calves, body temperatures and blood constituents were not detectably influenced by treatment. Thin-layer chromatograms of $CHCl_3$ extracts of urine and feces of the treated calves were similar to those from the calf given the single dose. Postmortem examination of the calf given the 11.4-g dose showed the small intestine had patchy areas of serous enteritis and its contents were abnormally fluid, but only normal flora were present. The lung had patchy to almost confluent areas of alveolar septal interstitial thickening by cellular elements. Significant changes were not found in other tissues. The calf given 5.7 g of crude extract was maintained on normal husbandry practices and appeared to be free of any permanent damage and grew normally.

Similarities between the clinical signs of the experimentally and naturally affected cattle were irritability, diarrhea, and malnutrition resulting from anorexia. However, the exact role of *A. fumigatus* metabolites in toxic syndromes of cattle ingesting molded silage is not known. Further studies to determine the causes of toxicity associated with ingestion of molded silage and the effects of SM-1 and SM-2 on cattle are in progress.

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# Improved Gas Chromatography Method for the Quantitation of Saccharides in Enzyme-Converted Corn Syrups

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A procedure has been developed for the quantitative analysis of sugars in enzyme-converted corn syrups, using *N*-(trimethylsilyl)imidazole to prepare the trimethylsilyl derivatives of glucose and higher glucose oligomers. The method was applied to lyophilized syrups and syrups containing 80% solids. Recoveries of spiked samples of glucose, maltose, and maltotriose were 99%.

Separation and quantitation of trimethylsilyl derivatives of sugars by gas chromatography was first described by Henglein and Scheinost (1956). Since then a number of silylating agents have been developed and their application to sugar analysis reviewed (Birch, 1973).

The standard methods used to produce trimethylsilyl derivatives of sugars in enzyme-converted corn syrups generally involve the use of either hexamethyldisilazane (HMDS) and trifluoroacetic acid (TFA) (Beadle, 1969), or HMDS and trimethylchlorosilane (TMCS) (Sweeley et al., 1963). In both cases the reaction can be exothermic and can result in splattering of the sample. Furthermore, HMDS, TMCS, and TFA reagents can produce a precipitate which interferes with subsequent sampling.

Derivatives of a number of organic compounds have been prepared with *N*-(trimethylsilyl)imidazole (TSIM). This reagent has been reported to produce trimethylsilyl derivatives of model mono- and disaccharides under mild

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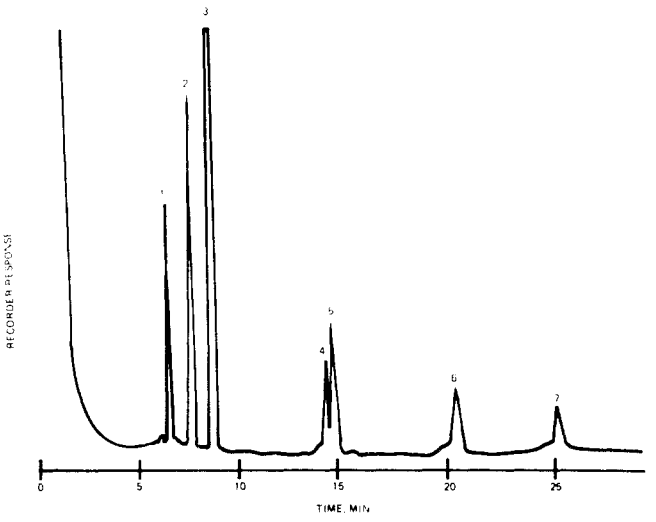


**Figure 1.** Gas chromatographic saccharide analysis of a 43 DE acid converted corn syrup.

conditions (Brittain, 1969). The application of this technique to one type of enzyme-converted corn syrup, the high fructose corn syrup, has been studied by Sennello (1971). He observed that the reaction with fructose produced multiple peaks on gas chromatography and therefore could not be used for quantitation. The problem was overcome by a combination of the reagents TSIM and TMCS (Sennello, 1971).

This report describes the application of Brittain's technique to enzyme-converted corn syrup containing the series glucose through maltotetraose. The procedure was rapid, derivatization occurred under mild conditions, heat was not evolved, and the reaction mixture was completely soluble and homogenous.

## EXPERIMENTAL SECTION

**Materials.** *N*-(Trimethylsilyl)imidazole (Tri-Sil "Z"), purchased from Pierce Chemical Co. (Rockford, Ill.), was used for the derivatization of the samples. A 43 DE acid converted corn syrup was obtained from Corn Products Co. (Argo, Ill.). Inositol was purchased from Aldrich Chemical Co., Milwaukee, Wis.

Table I. Retention Times of the Trimethylsilyl Derivatives of the Saccharides

| Peak no. | Sugar | Retention time, min | Rel retention time |
|---|---|---|---|
| 1 | α-Glucose | 6.61 | 0.75 |
| 2 | β-Glucose | 7.69 | 0.87 |
| 3 | Inositol | 8.83 | 1.00 |
| 4 | α-Maltose | 14.55 | 1.65 |
| 5 | β-Maltose | 14.93 | 1.69 |
| 6 | Maltotriose | 20.87 | 2.36 |
| 7 | Maltotetraose | 25.56 | 2.90 |

A 67 DE syrup was prepared by incubation of the 43 DE syrup with Wallerstein's Mylase ME (0.03%) at 50 °C for 72 h. An aliquot of the reaction mixture was immediately frozen prior to subsequent analysis.

**Preparation of Samples and Standards.** Aqueous mutarotated solutions of reference carbohydrates were prepared by dissolving 2.000 g of each carbohydrate in 25 mL of deionized water and heating to 65 °C for 20 min. The solutions were allowed to come to room temperature and diluted to 100 mL with deionized water. Using a microsyringe, 250-μL aliquots of each solution were placed in a test tube, and the sample was lyophilized to dryness. A 20–30-mg sample of corn syrup containing 80% solids was used as received or was diluted with water to 2% solids, and a 1.0-mL aliquot of the solution was lyophilized.

The trimethylsilyl derivatives were prepared by the addition of 3.0 mL of TSIM to a test tube containing the saccharides and 5 mg of the internal standard, inositol. After standing for 15 min at room temperature, a 1.0-μL aliquot was injected into the gas chromatograph. Derivatives of the saccharides should be prepared in a hood with appropriate protection for the hands and eyes.

**Gas-Liquid Chromatography.** Analysis was performed using a Hewlett-Packard 5830A gas chromatograph

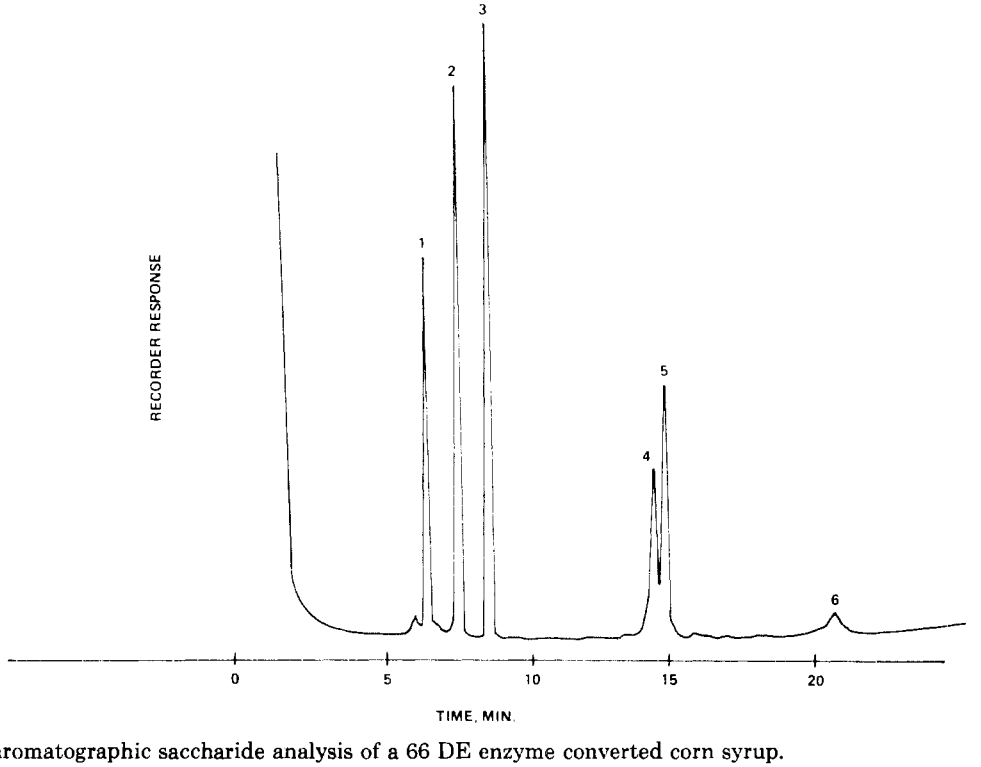


**Figure 2.** Gas chromatographic saccharide analysis of a 66 DE enzyme converted corn syrup.

Table II. Reproducibility of the Procedure Using a 43 DE Acid Converted Corn Syrup

| | Percent of total solids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Preparation number | | | | | | | |
| Sugar | I | II | III | IV | V | Mean | SD | % deviation |
| Glucose | 18.72 | 18.64 | 18.81 | 18.70 | 18.68 | 18.71 | 0.098 | 0.52 |
| Maltose | 13.00 | 13.30 | 13.32 | 13.08 | 13.24 | 13.19 | 0.257 | 1.95 |
| Maltotriose | 9.50 | 9.34 | 9.35 | 9.55 | 9.28 | 9.40 | 0.216 | 2.30 |

Table III. Analysis of an Enzyme-Converted Corn Syrup as a Lyophilized Preparation and as an 80% Solids Syrup

| | % of total solids | | | |
|---|---|---|---|---|
| Syrup | Glucose | Maltose | Maltotriose | Maltotetrose |
| Lyophilized | 36.3 | 24.0 | 4.1 | Tr |
| 80% solids | 36.0 | 24.2 | 3.8 | Tr |

equipped with dual flame ionization detectors. A 5 ft × $^1/_8$ in. i.d. stainless steel column was packed with 3% OV-1 on 100–120 mesh chromosorb W, HP, prepared according to Supina (1974). The injector and detector temperatures were 300 and 350 °C, respectively, and the oven temperature programmed from 135 to 340 °C at 8 °C per minute with a 10-min hold at 340 °C. The helium carrier gas flow was 18 mL/min. Samples were identified by comparison of their retention times with the standards (Table I) and quantitated by comparison with inositol, the internal standard, using an attached Hewlett-Packard 18850A computing processor.

## RESULTS AND DISCUSSION

The method was successfully applied to a 43 DE acid converted corn syrup (Figure 1). The $\alpha$ and $\beta$ anomers of glucose were readily separated and those of maltose were nearly so. Maltotriose and maltotetraose were readily resolved. The peaks and their retention times are listed in Table I.

The efficiency of the procedure was tested using known quantities of glucose, maltose, and maltotriose. Recovery of these sugars were better than 99%. Yields of 100% and 102% were obtained when known amounts of glucose and maltose, respectively, were added to a 43 DE acid converted corn syrup.

The reproducibility of the method is shown in Table II. The data on five separate preparations showed good agreement for the sugars tested.

The method was successfully applied to a 66 DE enzyme converted corn syrup. As indicated in Figure 2, the enzymatic hydrolysis did not affect the resolution of the peaks. The results indicated a composition of 39.1% glucose, 39.6% maltose, and 3.0% maltotriose, which was verified independently by Bedford Laboratories, Cedar Rapids, Iowa. Maltotetraose had been enzymatically converted to lower molecular weight saccharides and was not detected.

The method can be directly applied to an enzyme-converted corn syrup containing 80% solids (Table III). The results obtained are similar to the results obtained with the lyophilized sample. Samples containing more than 20% water were subject to substantial error.

## CONCLUSION

An effective method for the preparation of trimethylsilyl derivatives of sugars in enzyme-converted corn syrup employed *N*-(trimethylsilyl)imidazole. The method was rapid, accurate, and could be used with an 80% solids corn syrup and lyophilized samples.

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