

listed in Table IV. The activities of 62, 69, 70, and 71 again probably reflect loss of formaldehyde. The other compounds were active in the range of 2-7  $\mu\text{g/mL}$  as expected.

The percent changes in the VFA compositions listed in Table V show a marked decrease in acetate, a range of -20 to -59%, associated with increases in propionate (+6 to +27%), butyrate (0 to +117%), and valerate (+25 to +175%). The acetic/propionic ratio was reduced from 1.55 for the control to a range of 0.50-1.11. Combined with the high potency of these compounds in the reduction of methane,  $\text{CH}_4$   $\text{ID}_{50}$  in the range of 0.50-2.0  $\mu\text{g/mL}$ , these results have prompted additional testing of the diimides on the ruminal parameters in sheep. The sheep tests will be reported separately (Baylis et al., 1982).

From all of these data, and also taking into account relative cost and ease of synthesis, pyromellitic diimide (1) and pyromellitic *N*-(2-hydroxyethyl)diimide (43) were judged to be the most promising compounds for further study and are now undergoing investigation for enhancement of feed efficiency in ruminants.

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## COMMUNICATIONS

### Analysis of Sugars in Foods Containing Sodium Chloride by High-Performance Liquid Chromatography

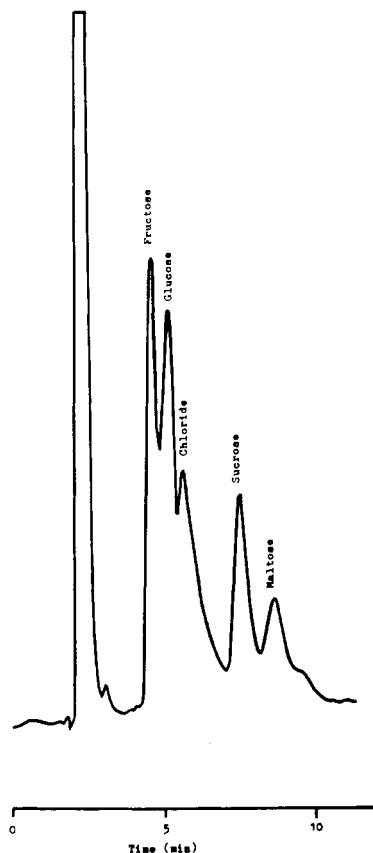
The presence of salt in foods was found to interfere with the analysis of sugars by high-performance liquid chromatography due to chloride ion producing a response with the refractive index detector that was close to that of glucose. The addition of a PIC reagent (tetrabutylammonium phosphate) to the acetonitrile/water mobile phase markedly reduced the retention time of chloride so that it eluted well before any sugar. The PIC reagent also improved the resolution of the sugars.

Analysis of individual sugars by high-performance liquid chromatography (HPLC) using a silica-based weak anion exchange column with acetonitrile/water as the mobile phase and a refractive index detector has been reported for a wide range of foods [e.g., De Vries et al. (1979), Hurst et al. (1979), and Warthesen and Kramer (1979)], has been adopted as an official AOAC method (No. 31.138-145; AOAC, 1980) and is routinely used in many research and quality control laboratories. We have found that for many processed foods an extra peak that did not correspond to any sugar was present and interfered with the estimation of glucose (Figure 1). In this paper we report on a method for removing this interference.

#### MATERIALS AND METHODS

Sugars were extracted from food with hot 85% v/v

methanol, and the volume of the solution was reduced by evaporation and made up to 10 mL with water (Wills et al., 1980). After filtration through a membrane ultrafilter, an aliquot (20  $\mu\text{L}$ ) was injected onto a  $\mu\text{Bondapak}$ /carbohydrate column (Waters Associates) installed in a Waters liquid chromatograph (Model No. ALC/GPC 244) equipped with a 41-MPa pump and U6K injector. The mobile phase was acetonitrile/water (80/20) at 2 mL/min, and column effluents were monitored with a refractive index detector (Waters Associates Model No. R401) at  $\times 8$  attenuation. Individual sugars were identified on the basis of their retention times. A range of salts and organic acids was injected onto the column to establish the identity of the nonsugar compound. The addition of the ion pairing reagent, tetrabutylammonium phosphate (PIC A, Waters Associates) to the mobile phase was examined to remove



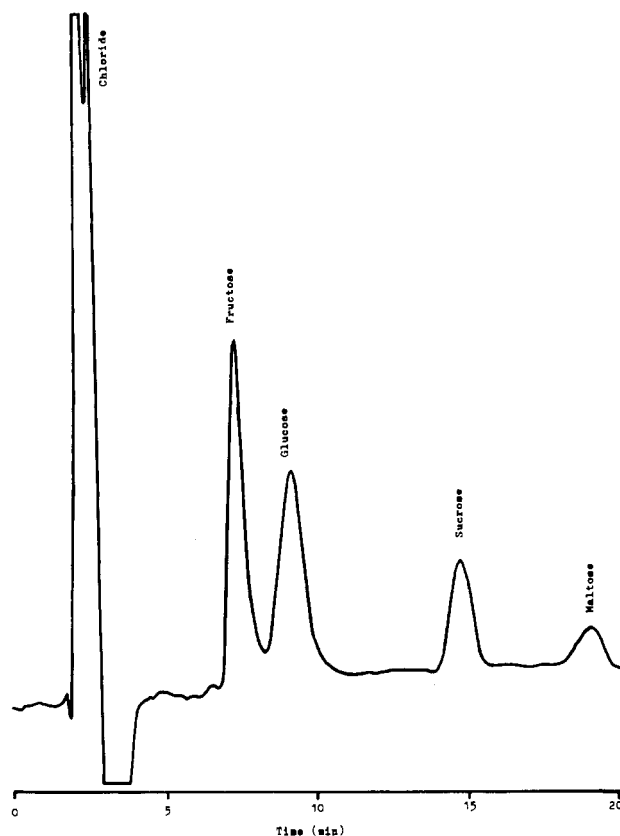
**Figure 1.** Analysis for sugars of pizza extract by HPLC with acetonitrile/water as the mobile phase. Amounts of sugars in the injection volume (20  $\mu$ L) were 0.1 mg of glucose and fructose, 0.05 mg of sucrose, and 0.02 mg of maltose.

the interfering peak.

#### RESULTS AND DISCUSSION

The interfering substance was found to have a retention time that corresponded to that of chloride ion. A response was detected when 10  $\mu$ g of chloride was injected onto the column. Under the experimental conditions used for the analysis of sugars, the presence of >100 mg of chloride/100 g of food would be detected. Since most of the chloride in processed foods would be derived from sodium chloride, the presence of salt at >160 mg/100 g would constitute an interference. An examination of foods which produced the additional peak showed that all contained salt well in excess of 160 mg/100 g. No response was recorded that could be attributed to sodium.

The addition of PIC A to the mobile phase markedly reduced the retention time of chloride ion so that it eluted on the tail of the solvent. The amount of PIC A required depended on the amount of chloride present; with insufficient PIC A, excess chloride appeared as a peak in the original position near glucose. A negative ghost peak was also produced just after the elution of chloride, but baseline conditions were restored before fructose was eluted. The ghost peak would be caused by a temporary interruption of the PIC A equilibrium in the column system altering the refractive index of the mobile phase. The addition of PIC A at the rate of 2.5 mM, which is half the manufacturer's recommendation for normal use, was found to be satisfactory for the levels of chloride likely to be



**Figure 2.** Analysis for sugars of pizza extract by HPLC with a PIC reagent (2.5 mM) added to the acetonitrile/water mobile phase.

found in most processed foods (<3.5 g of NaCl/100 g). PIC A also improved the resolution of sugars but had no significant effect on base-line stability or on detector response factors. These effects are shown in Figure 2. The same results were obtained by using a new column and a well-used column. If the amount of chloride present is extremely high, then the amount of PIC A added to the solvent could be increased. The addition of 5 mM PIC A, the highest concentration examined, had no adverse effect on column stability or performance.

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