Improved Gas–Liquid Chromatography for Simultaneous Determination of Volatile Fatty Acids and Lactic Acid in Silage

A gas–liquid chromatography method for simultaneous determination of volatile fatty acids and lactic acid was improved by the use of a new packing material, poly(ethylene glycol phthalic acid ester) coated on a solid terephthalic acid support. The method appears to be accurate, simple, and quick enough for routine silage analysis.

For the quantitation of mixtures of volatile fatty acids (VFA) and lactic acid by one gas–liquid chromatography (GLC) system, derivative formation of lactic acid may be mandatory (Schwarze and Gilmour, 1969; Jones and Kay, 1976), but quantitative derivatization is very difficult to achieve satisfactorily (Wilson and Terry, 1977).

A solid terephthalic acid (TPA) support treated with a stationary liquid containing hydroxyl groups can be an excellent column packing material for GLC of free lower fatty acids (Miyake et al., 1968). Kageyama et al. (1973) used polyethylene glycol 6000 (PEG 6000) coated on TPA for simultaneous determination of VFA and lactic acid in silage. Any pretreatment of samples was unnecessary in this method because derivative formation of lactic acid occurred on the surface of TPA in the column as detected by GLC–mass spectrometry (Kageyama, 1979). In the present paper an improved method using poly(ethylene glycol phthalic acid ester) (PEGPE 3000) on TPA is reported.

MATERIALS AND METHODS

Apparatus and Reagents. The instrumentation consisted of a Hewlett-Packard 5700A gas chromatograph equipped with dual flame ionization detectors, a Recordall recorder, and a Spectra-Physics Autolab Minigrator integrator. Standard VFA solutions were prepared from certified or reagent grade acetic acid, propionic acid, *n*-butyric acid, isobutyric acid, *n*-valeric acid, and isovaleric acid (Fisher, NJ). Either certified grade (Fisher, NJ) or analytical reagent grade (BDH, Canada) was used to prepare standard lactic acid solutions.

Column Packings. Either commercial 10% PEG 6000 on TPA (60–80 mesh) or a trial product sample of 3% PEGPE 3000 on TPA (60–80 mesh) was packed in dual 1.5 m × 2 mm i.d. glass columns up to 10 cm from the top ends. To protect the packing material from the high temperature of the injection port, we placed a 3-cm layer of quartz powder (Shimalite-Q, 100–180 mesh) on top of the packing material such that, when the columns were in place, the upper portion of quartz powder was in the injection block, while the lower portion was in the oven. The above three materials were obtained from Chromato Packings Center of Shinwakako Co., Kyoto, Japan. (Commercial PEGPE 3000 on TPA, the trade name being Thermon 3000 on TPA, is now available from this firm.) A 0.5-cm layer of granular aluminum (Fisher, NJ) was placed on top of the quartz powder to achieve rapid vaporization of liquid samples.

Preparation of Silage Juice Sample. One hundred grams of chopped timothy (*Phleum pratense*, L.) silage plus 200 mL of doubly distilled water were placed in a 800-mL glass jar. The jar was capped and allowed to sit for 24 h at 3 °C in a refrigerator, with occasional shaking. The mixture was squeezed through two layers of cheese cloth and filtered through Whatman No. 42 paper with suction. When PEG 6000 on TPA was used, 0.2 mL of concentrated H_2SO_4 was added to the filtrate before injection into the column as Kageyama et al. suggested (1973). For the column packed with PEGPE 3000 on TPA, no H_2SO_4 was added to the filtrate.

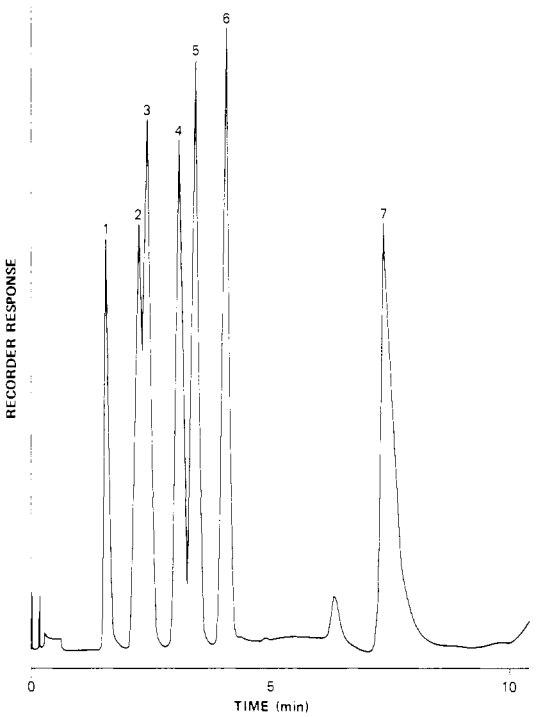

Figure 1. Gas chromatogram of a mixture of 0.06% VFA and 0.30% lactic acid separated with 3% poly(ethylene glycol phthalic acid ester) 3000 coated on terephthalic acid: (1) acetic acid, (2) propionic acid, (3) isobutyric acid, (4) *n*-butyric acid, (5) isovaleric acid, (6) *n*-valeric acid, and (7) lactic acid.

GLC Analysis. All columns were conditioned at 190 °C for 24 h. Five microliters of standard acid or silage filtrate was injected directly into the column.

Operating parameters when using columns packed with PEG 6000 on TPA were similar to those used by Kageyama et al. (1973), except the oven temperature was increased at a rate of 8 °C/min and the carrier gas flow rate was 40 mL/min.

When columns packed with PEGPE 3000 on TPA were used, the oven temperature was held at 120 °C for 2 min and increased to 190 °C at a rate of 16 °C/min, then held at 190 °C for 3 min. Other parameters were as follows: nitrogen carrier gas flow rate, 40 mL/min; injector temperature, 200 °C; detector temperature, 250 °C. To prevent broadening of the lactic acid peak, we flushed the columns after every five analyses by injecting 10 μL of doubly distilled water and holding the oven temperature at 190 °C for 3 min.

RESULTS AND DISCUSSION

The response of PEG 6000 on TPA to lactic acid was

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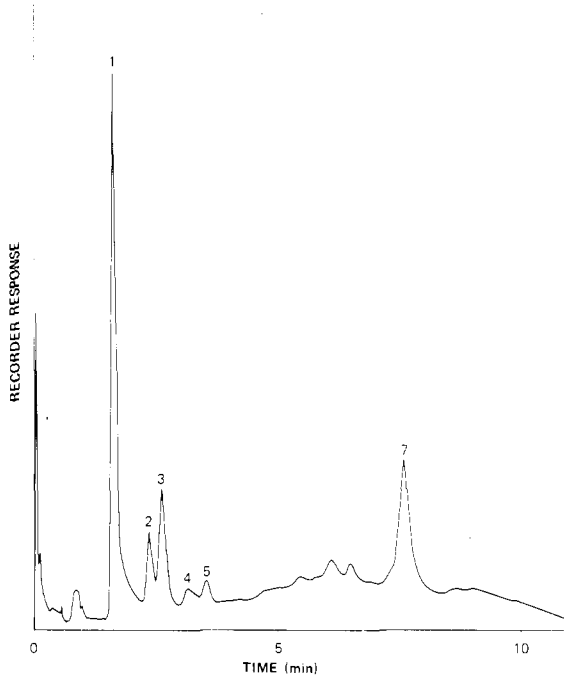

Figure 2. Gas chromatogram of a timothy silage extract separated with 3% poly(ethylene glycol phthalic acid ester) 3000 coated on terephthalic acid: (1) acetic acid, (2) propionic acid, (3) isobutyric acid, (4) *n*-butyric acid, (5) isovaleric acid, (6) *n*-valeric acid, and (7) lactic acid.

inconsistent, depending on manufacturer's production lots, and in some cases no peak appeared even when a high concentration of lactic acid was injected. To activate derivative formation and improve the response, Kageyama et al. (1973) recommended the use of concentrated H_2SO_4. In the present study this technique did not improve the response when a commercial lot of PEG 6000 on TPA was used. A sharp peak of lactic acid always appeared when a mixture of VFA and lactic acid was analyzed with PEGPE 3000 on TPA (Figure 1).

The analysis with PEGPE 3000 on TPA was completed within 10 min, and the detection limits of VFA and lactic acid were 0.010 and 1.0 μg, respectively. The coefficient of variability based on 10 determinations of 0.30% lactic acid was 1.36%. Straight line standard curves were obtained between 0.01 and 0.10% of each VFA and 0.05 and 0.50% of lactic acid. The chromatogram of VFA obtained with PEGPE 3000 on TPA was very similar to that with PEG 6000 on TPA.

A typical example of a chromatogram of a silage sample with PEGPE 3000 on TPA is shown in Figure 2.

The peak shape of lactic acid was almost symmetrical and was sharp enough for quantitative determination. No deterioration of the packing material was noticed after 50 silage analyses, as long as the columns were flushed with doubly distilled water as previously described.

Large columns, such as 5 mm i.d. (Miyake et al., 1968) and 3 mm i.d. (Kageyama et al., 1973), have been recommended for packing a TPA support. It was not easy to pack PEG 6000 on TPA into a smaller column. This material was apt to shrink after heating at 190 °C or higher temperatures, causing air spaces in the column. PEGPE 3000 on TPA was more temperature resistant (up to 280 °C, according to the manufacturer's specifications), and there was no difficulty in packing this material into a standard 2-mm column.

The results of this study suggest that the GLC method with PEGPE 3000 on TPA is more reproducible, accurate, simple, and rapid than other GLC methods as previously reported by Schwartz and Gilmour (1969), Kageyama et al. (1973), Jones and Kay (1976), and Wilson and Terry (1977), and it is applicable to routine determination of VFA and lactic acid in silage.

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