Gas-Chromatographic Determination of Low Concentrations of Propionic Acid in Grain Sorghum[1]

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ABSTRACT

A simple gas-chromatographic procedure was developed for analysis of low concentrations of propionic acid added to prevent fungal growth in high-moisture grain sorghum. After extraction of the propionic acid from the grain into an aqueous solution by using an homogenizer having a shearing action, an aliquot of filtrate was analyzed directly by gas chromatography. Chromatography required only 4 min, and this time could be further reduced if necessary. Over the range of 0.2–0.8% propionic acid, average relative error was less than 1.5% with an average coefficient of variation of less than 1.0%. The method was sufficiently sensitive for the analysis of a single kernel of grain sorghum containing less than 0.03% propionic acid.

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Gas chromatography has been employed for analysis of volatile fatty acids since 1952, when their separation and microestimation was reported by James and Martin in the original paper setting forth the principles of gas-liquid chromatography. As a result of its speed, accuracy, sensitivity, and ease of quantitation, this technique offers advantages over other methods for the analysis of volatile fatty acids.

A simple and rapid gas-chromatographic method for determining the low levels of propionic acid added as a preservative to prevent fungal growth in high-moisture corn was recently reported by Lamkin et al (1985). The method allows for direct injection onto the chromatographic column of an aliquot taken from an aqueous extract, thereby eliminating any steam distillation or solvent extraction step. The chromatographic procedure employed a column containing graphitized carbon black, which separates mainly according to differences in geometric structure and polarizability (Kiselev 1967), modified by a coating of Carbowax 20M (Di Corcia 1973, Di Corcia and Samperi 1974). Phosphoric acid was added to block adsorption sites. The column gave good separation of propionic acid from the other volatile fatty acids and provided sufficient sensitivity for the analysis of a single kernel of corn.

The present study was undertaken in response to a need for a rapid method for quantitative determination of low levels of propionic acid employed as a preservative for high-moisture grain sorghum used for animal feed. As with corn, the concentration of propionic acid must be high enough to prevent fungal growth but sufficiently low for safety. Early experiments showed that the method developed for corn could not be applied directly to sorghum without modification. A more thorough review of the literature is given in the previous paper by Lamkin et al (1985).

MATERIALS AND METHODS

Apparatus and Reagents

Samples were chromatographed in a Bendix 2600 gas chromatograph equipped with a Bendix Mark III electrometer, a flame ionization detector, and a Hewlett-Packard model 7123 recorder with a 1-mV span. At maximum sensitivity, a signal of 1×10^{-12} A gave full-scale response. The instrument was designed to accept U-shaped glass columns and allowed direct injection of the sample onto the chromatographic column. The nitrogen carrier gas, hydrogen, and air were purified by passing them through cylinders packed with Union Carbide type 13X molecular sieve containing type 4A indicating molecular sieve.

Samples were introduced for chromatography with a Hamilton 1801 syringe as previously described, and peak areas were integrated with an Autolab Minigrator (Spectra-Physics). Grain sorghum samples were homogenized in a Virtis model 45 homogenizer having both "macro" and "micro ultra shear" assemblies. The conditions of homogenization could be adjusted precisely with the variable speed control provided.

Propionic acid was a Mallinckrodt analytical reagent, 99.0% minimum purity, and *n*-butyric acid used as an internal standard was an Aldrich "gold label" product, 99+%. Phosphoric acid-treated glass wool for column plugs and Carbopack C with 0.3% Carbowax 20M and 0.1% phosphoric acid used for the preparation of chromatographic columns were obtained from Supelco. Formic acid was an ACS reagent chemical.

Grain Sorghum Samples

Grain sorghum samples were hybrids grown in fields near Manhattan, KS: Funks G-623GBR, a bronze hybrid; Funk's G-550, a white hybrid; and Mustang, a bronze hybrid from Asgrow Growers. All were harvested while still wet (> 19.8% moisture) and were used without further drying. Glumes, foreign material, and

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broken kernels were removed before use. Moisture content was determined by measuring the weight loss of 20 g of grain on drying for 20 hr in a forced-air oven at 120 ±1° C (Seitz et al 1975).

Grain sorghum samples containing known concentrations of propionic acid were prepared. Measured volumes of propionic acid were added to weighed samples of sorghum (approximately 50 g) in 125-ml borosilicate Erlenmeyer flasks having rubber-lined screw caps, and the added amounts were determined by reweighing the flasks. Samples containing propionic acid (approximately 100 g) for use in the storage study were prepared in 250-ml linear polyethylene bottles with tightly fitting linear polyethylene screw caps and stored (29 months) at 4° C until analyzed.

Analytical Procedure

A 4-g sample of grain sorghum was weighed to the nearest 0.1 mg into a 250-ml beaker containing 100.00 ml of 0.050*M* formic acid and a known concentration of *n*-butyric acid as an internal standard. The sample was then homogenized for 15.0 min in a Virtis model 45 homogenizer with a "macro ultra shear" assembly at a speed setting of 40. After the slurry was allowed to settle, a few milliliters of the supernatant was filtered through a Millipore Millex-SR (0.5 μm) membrane filter unit with the aid of a syringe, and the filtrate was diluted with 0.050*M* formic acid to a propionic acid concentration of 50 ppm or less. For the analysis of single kernels, homogenization was in 3.00 ml of solution in a test tube. The "micro ultra shear" assembly was used, and to facilitate the homogenization, a kernel was placed in the test tube and cut into four pieces with a scalpel.

Propionic acid was determined by injecting 1.0 μl of diluted filtrate into the gas chromatograph, with the *n*-butyric acid (approximately 100 ppm) serving as an internal standard. The chromatographic column, described in the legend to Figure 1, was prepared and conditioned for use as previously described (Lamkin et al 1985). A standard curve was prepared by chromatographing standards containing 0–50 ppm propionic acid in 0.050*M* formic acid, with 100 ppm *n*-butyric acid again included as an internal standard. Chromatographic conditions employed for all samples and standards are shown in the legend to Figure 1. Correction for ghosting was as described previously (Lamkin et al 1985). A standard containing approximately the same concentration of propionic acid as the unknown was run immediately after each sample, and the error due to ghosting was calculated and used to correct the analytical result.

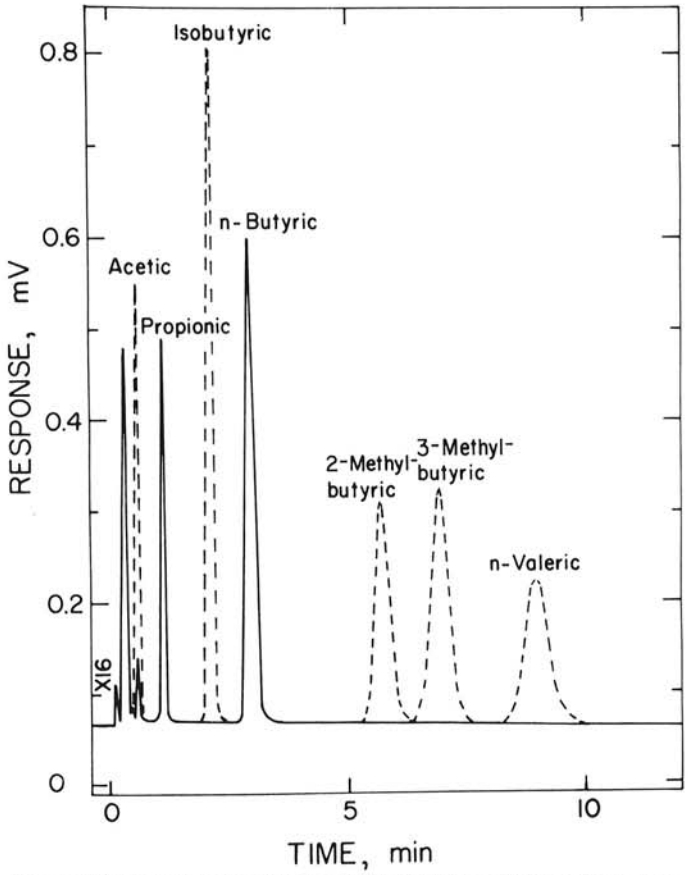

Fig. 1. Chromatogram for the analysis of propionic acid in grain sorghum with 100 ppm *n*-butyric acid included as an internal standard. For comparison, the positions of peaks for volatile fatty acids not present are indicated by a broken line (– – –). Column: 75 cm × 4 mm i.d. borosilicate-glass packed with 0.3% (w/w) Carbowax 20M and 0.1% (w/w) phosphoric acid on 60-80 mesh Carbopack C. Flow rate: N_2, 41 ml/min; H_2, 63 ml/min; air, 333 ml/min. Temperature: column, 119° C; flash heater, 121° C; flame ionization detector, 208° C. Sample size: 1.0 μl.

RESULTS AND DISCUSSION

Homogenization and Analysis

Preparation of a sample for analysis involves a simple, straightforward procedure. Because of its high solubility, extraction of the propionic acid into the aqueous phase was rapid. As shown in Figure 2, under the homogenization conditions employed, extraction was virtually complete after 10 min, and after 15 min, no more propionic acid was extracted. The procedure previously developed for determining propionic acid in corn gave unsatisfactory results when applied to grain sorghum. Values obtained for propionic acid were low because of adsorption of the acid by the insoluble residue remaining after the extraction. Filtration through Whatman No. 2 filter paper under vacuum in a Büchner funnel followed by washing improved recoveries, but results were still low. Recoveries were essentially quantitative, however, when several milliliters of the supernatant was withdrawn with a hypodermic syringe and filtered through a 0.5-μm membrane filter.

A typical analysis of a grain sorghum sample, containing 0.4% propionic acid, is shown in the chromatogram of Figure 1. Under the chromatographic conditions employed, propionic acid had a retention time of only about 1 min, and the *n*-butyric acid internal standard had a retention time of about 3 min. Analysis time could be reduced slightly by substituting isobutyric for *n*-butyric acid as the internal standard, but *n*-butyric was preferred because of the better resolution between it and the propionic acid.

The ratio of integrated area of the propionic peak to that of the 100 ppm *n*-butyric acid internal standard served as a measure of the propionic acid concentration. Response was linear over the range of 0–50 ppm. As a result of ghosting, the response deviated from linearity at higher concentrations, so samples were diluted to 50 ppm or less before chromatography. Ghosting occurs when a component is adsorbed on the column from previous samples and

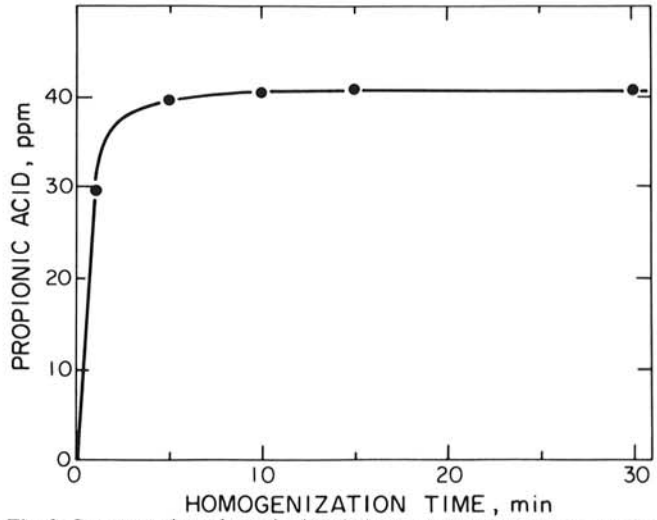

Fig. 2. Concentration of propionic acid in the aqueous phase as a function of homogenization time when 4 g of grain sorghum is extracted with 100 ml of 0.050*M* formic acid in a Virtis model 45 homogenizer at a speed setting of 40.

desorbed upon injection of a sample dissolved in a highly polar solvent such as water. It can produce extraneous peaks or cause a peak representing a sample component to be larger than it should be. Adsorption on the column, and thus ghosting, was minimized by making all samples and standards chromatographed to 0.050*M* with respect to formic acid. A more thorough discussion of ghosting is presented in an earlier paper by Lamkin et al (1985).

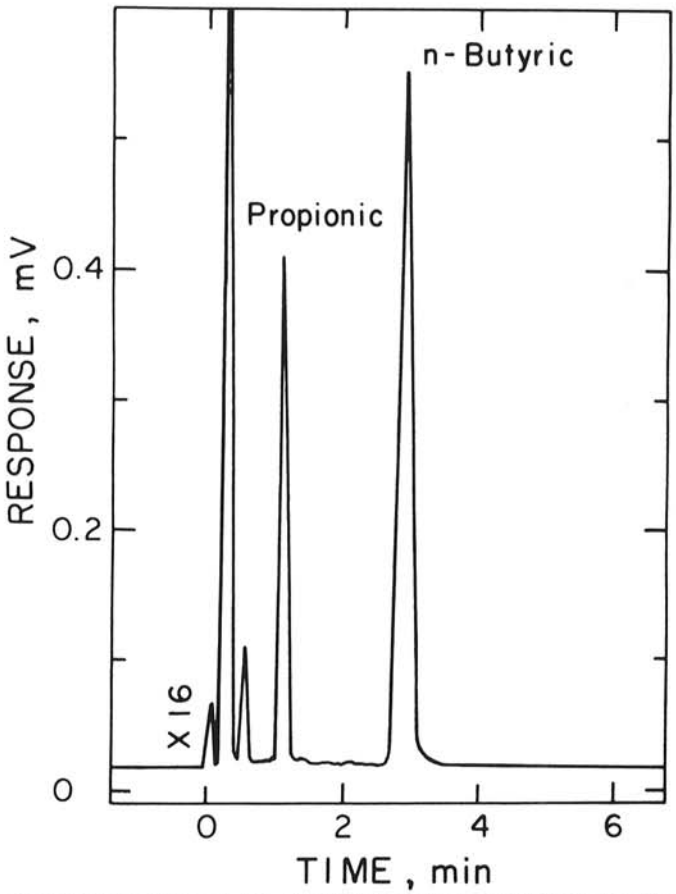

Fig. 3. Chromatogram for the analysis of a single kernel of grain sorghum containing only 0.3% propionic acid. Volume of aqueous extract injected was 1.0 μl, with 100 ppm *n*-butyric acid included as an internal standard.

TABLE I
Precision and Accuracy in the Gas-Chromatographic Analysis of Propionic Acid in Grain Sorghum

Sample	Propionic Acid (%, w/w) Theory	Propionic Acid (%, w/w) Found[a]	Relative Error (%)	Coefficient of Variation (%)
Bronze[b]				
	0.211	0.206 ± 0.001	−2.04	0.66
	0.302	0.300 ± 0.002	−0.65	0.71
	0.406	0.410 ± 0.004	+1.06	0.98
	0.505	0.504 ± 0.007	−0.27	1.33
	0.605	0.587 ± 0.004	−3.05	0.71
	0.802	0.778 ± 0.005	−2.91	0.62
White[c]				
	0.193	0.188 ± 0.001	−2.93	0.55
	0.299	0.298 ± 0.001	−0.40	0.43
	0.407	0.401 ± 0.004	−1.50	1.05
	0.507	0.500 ± 0.011	−1.35	2.23
	0.586	0.578 ± 0.004	−1.37	0.62
	0.789	0.772 ± 0.009	−2.13	0.97

[a] Average ± standard deviation of five 4-g sample analyses, each of which was an average of triplicate determinations on the same extract.
[b] Funk's G-623GBR hybrid. Moisture content was 19.84% (w/w) before addition of propionic acid.
[c] Funk's G-550 hybrid. Moisture content was 22.49% (w/w) before addition of propionic acid.

Accuracy and Precision

Concentrations of proprionic acid determined from analysis of a group of Funk's G-623GBR grain sorghum samples with 0.2–0.8% (w/w) added propionic acid (Table I) were in close agreement with the theoretical values. Average relative error was only −1.31%, which probably reflects a slight loss of propionic acid in the flasks used to prepare the samples. Precision was very good, with an average standard deviation of only ±0.004% or, expressed as coefficient of variation, 0.84%.

Analyses of samples of Funk's G-550 sorghum, a white hybrid (Table I), were essentially in agreement with the analyses of Funk's G-623GBR, a bronze hybrid. The analytical results again agree closely with the theoretical values. Average relative error was only −1.61%, and average coefficient of variation was 0.98%.

Stored Samples

To determine whether any unusual problems such as extraneous peaks might be encountered in the chromatography of sorghum samples containing propionic acid that were not freshly prepared,

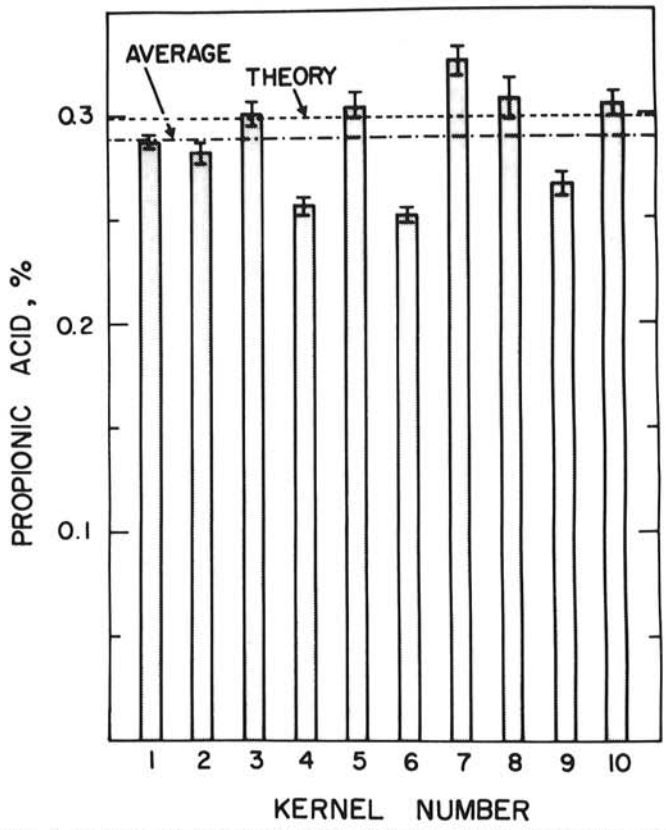

Fig. 4. Analyses of 10 individual kernels from a grain sorghum sample containing 0.299% propionic acid. Each bar represents an average of triplicate analyses of the same extract.

TABLE II
Gas-Chromatographic Analysis of Propionic Acid in Stored Samples of Grain Sorghum[a]

Propionic Acid (%, w/w) Theory	Propionic Acid (%, w/w) Found[b]	Relative Error (%)	Coefficient of Variation (%)
0.221	0.127 ± 0.002	−42.63	1.35
0.316	0.281 ± 0.006	−11.05	2.06
0.436	0.411 ± 0.004	−5.76	1.00
0.501	0.488 ± 0.006	−2.60	1.13
0.614	0.591 ± 0.004	−3.75	0.73
0.786	0.765 ± 0.007	−2.66	0.82

[a] Samples were stored at 4° C for 29 months. Mositure content was 22.39% (w/w) before addition of the propionic acid.
[b] Average ± standard deviation of five 4-g sample analyses, each of which was an average of triplicate determinations on the saame extract.

samples stored under laboratory conditions (4° C) for 29 months also were analyzed (Table II). Low results were obtained for the two samples containing the lowest concentrations of propionic acid (0.2 and 0.3%), probably because an insufficient amount was present to stop microbial growth. For the remaining four samples, which contained a sufficiently high concentration of propionic acid to prevent microbial action, analytical results agreed closely with the calculated percentages. Average deviation from the theoretical concentration was only −0.021%, which probably reflects a slight loss of propionic acid under the storage conditions: precision was about the same as for the freshly prepared samples, with an average coefficient of variation of 1.18%. Although some minor extraneous peaks were observed, analysis of the stored samples indicated no special problems.

Single Kernels

Under the chromatographic conditions employed, the lower limit of detection of propionic acid in the aqueous phase injected into the gas chromatograph was about 0.2 ppm. This provided sufficient sensitivity for the analysis of a single kernel of sorghum (Fig. 3). If homogenization is in 3.00 ml of solution, a good analysis can be obtained on a kernel containing only 0.1% propionic acid, and by reducing this volume, an analysis can be performed on a kernel containing less than 0.03%. Single-kernel analyses of 10 kernels from a grain sorghum sample containing 0.299% propionic acid (Fig. 4) were in close agreement with the amount present in the sample. The result for one kernel was about 16% low, whereas that for another was almost 9% high, but the average of 0.288% for the 10 analyses deviated from the theoretical value by only −0.011%. The rather high coefficient of variation of 8.43% for the analyses of all 10 kernels together is not surprising, because a fairly large variation would be expected among kernels in the concentration of the added propionic acid due to differences in kernel size, composition, etc.

In developing the procedure, we wanted to be able to check the possibility that a grain sorghum sample might be a mixture of sorghums with different levels of propionic acid. We felt that this could be done only by analyzing individual kernels, and so we attempted to develop a method with sufficient sensitivity to analyze single kernels. By analyzing randomly selected kernels from a mixture, it should be possible to approximate the propionic acid concentration in each component of the mixture.

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