# Detection and Gas-Chromatographic Determination of Propionic Acid Added as a Preservative to Corn[1]

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**ABSTRACT**

A simple, rapid gas-chromatographic procedure was developed for quantitatively determining the low levels of propionic acid in treated corn. The sample to be analyzed was homogenized in water containing *n*-butyric acid as an internal standard, and the resulting aqueous solution was analyzed directly by gas chromatography, thus eliminating any distillation or solvent extraction step. Homogenization required 15 min; less than 4 min was needed for chromatographic separation. Over the range of 0.2-1.0%, the method was accurate to within a few hundredths of a percent, and it was sufficiently sensitive to allow analysis on a single kernel. Propionic acid at a concentration of less than 1 ppm could be detected in the aqueous extract. A procedure employing methyl red was also devised as a screening method for detecting the acid in treated kernels.

Because of its speed, accuracy, sensitivity, and ease of quantitation, gas chromatography offers advantages over other methods for analyzing volatile fatty acids. In setting forth the principles of gas-liquid chromatography, James and Martin (1952) reported the separation and "micro-estimation" of volatile fatty acids, from formic to dodecanoic, in a specially constructed gas chromatograph with an automatic titrator as detector. The procedure was later modified (Gehrke and Lamkin 1961) for use with a commercial instrument having a thermal conductivity detector. Methods for the determination of volatile fatty acids by gas chromatography have since undergone considerable evolution.

The development of a system that allowed direct injection of an aqueous solution of the free acids onto the chromatographic column (Emery and Koerner 1961) was an important advance. Tween 80 was employed as the liquid phase in conjunction with a flame ionization detector, which is relatively insensitive to the presence of water. More recently, Hollis (1966) described the unique gas-chromatographic properties of porous polymers of styrene and its derivatives. Separation of an aqueous solution of formic, acetic, and propionic acids was accomplished without the use of a liquid phase on a column packed with porous polymer beads synthesized from ethylvinylbenzene and divinylbenzene. Comparison of the chromatographic properties of various porous polymers (Dave 1969) showed that Chromosorb 101, a copolymer of styrene and divinylbenzene, gives good separation of the free volatile fatty acids with minimal tailing. This column has been employed to determine propionic acid added as a preservative to bakery products (Petró-Turza et al 1980), but only after steam distillation and removal of water. Ottenstein and Bartley (1971) also studied the separation of the free acids, from acetic through valeric, in dilute aqueous solution and found that the best separations are given by Chromosorb 101 and by 10% SP-1200, a low polarity ester, on Chromosorb W with 1% phosphoric acid added.

Inclusion of formic acid vapor in the carrier gas effectively blankets adsorption sites with columns used for free fatty acid analysis (Ackman and Burgher 1963, Geddes and Gilmour 1970). In the case of the porous polymer columns, the formic acid vapor reduces tailing and other adsorption effects without seriously modifying retention times or affecting column life (Ackman 1972). Retention characteristics of the porous polymers can be modified by inclusion of a liquid phase to facilitate difficult separations, without greatly decreasing column efficiency (Jansson et al 1970).

A significant innovation was the use of columns containing graphitized carbon black, which separates mainly according to differences in geometric structure and polarizability (Kiselev 1967). Columns packed with the graphitized carbon, modified by coating it with a liquid phase, give good separation of the volatile fatty acids and make possible analysis of trace amounts in aqueous solution (Di Corcia 1973, Di Corcia and Samperi 1974). Best results were obtained with polyethylene glycol 20M containing phosphoric acid to block adsorption sites.

The present study was undertaken in response to a need for a rapid method for quantitative determination of propionic acid added as a preservative to prevent fungal growth (Milward 1976, Sauer and Burroughs 1974) in high-moisture corn. In the United States, corn so treated is permitted only for use as animal feed and must be graded "sample grade." The concentration of propionic acid needs to be sufficient to prevent fungal growth but, at the same time, within safe limits. Detailed information on the use of propionic acid and other chemicals as preservatives for grain is available through the bibliography by Pomeranz (1982). Our approach was to try to make the procedure as simple as possible. We attempted to devise a method that would allow direct analysis of an aliquot taken from an aqueous extract, thereby eliminating any preliminary steam distillation or solvent extraction step. To make possible the analysis of mixtures containing kernels with different levels of propionic acid, a special effort was made to give the method sufficient sensitivity for analysis of single kernels containing as little as 0.1%. Although the present study was limited to propionic acid, which is the most commonly employed antifungal additive for corn, the procedure we developed could, with only minor modifications, also be used to determine other volatile fatty acids.

## MATERIALS AND METHODS

### Corn Samples

Corn samples of a kilogram or more were obtained for preparing the smaller samples used in the study. One sample was a composite obtained from F. W. Bakker-Arkema (Michigan State University, East Lansing). All others were hybrids (Pioneer 3300 and Ferry-Morse 3020) grown in fields near Manhattan, KS. Broken kernels and foreign material were removed. Except for one sample to which aqueous sodium hydroxide was added for neutralization, samples were harvested while still wet (>20% moisture) and were used without further drying. Propionic acid was added to the samples in the storage studies as soon after harvest as possible.

### Apparatus and Reagents

Propionic acid was a Mallinckrodt analytical reagent, 99.0%

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minimum purity, and the *n*-butyric acid employed as an internal standard was an Aldrich "gold label" product, 99+%. Isobutyric acid used as an internal standard in some of the earlier studies was 99+% purity, also from Aldrich Chemical Co. Formic acid was an ACS reagent chemical. Chromosorb 101 and Carbopack C with 0.3% Carbowax 20M and 0.1% phosphoric acid used for the preparation of chromatographic columns were obtained from Analabs and Supelco, respectively. Methyl red hydrochloride was a Baker analyzed reagent, and sodium hydroxide was a carbonate-free reagent, from J. T. Baker Chemical Co. Ethyl alcohol, 95%, was a denatured product from Mallinckrodt.

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 $1 \times 10^{-12}$ 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. Inlet of the system was modified with a Supelco N-1 septum nut to prevent excessive fragmentation of the septum. Nitrogen was used as the carrier gas, and a cylinder packed with Linde molecular sieve, type 5A, was placed in the carrrier gas line to remove water and impurities. Chromatographic peaks were integrated with an Autolab Minigrator (Spectra-Physics).

Samples were introduced for chromatography by means of a Hamilton 1801 syringe with specially made spacers, which allowed reproducible injection of 1.0-$\mu$l samples. Corn samples were homogenized in a Virtis model 45 homogenizer having both "macro" and "micro ultra shear" assemblies. A variable speed control allowed the conditions of homogenization to be adjusted precisely.

**Samples with Added Propionic Acid**

Corn samples containing known concentrations of propionic acid (0.2–1.0%) were prepared by weighing on an analytical balance. To a weighed sample of corn (about 50 g) in a 250-ml borosilicate Erlenmeyer flask with a rubber-lined screw cap was added a measured volume of propionic acid, and the amount added was determined by reweighing the flask. The flask was then inverted 100 times to mix the sample, and 6 or more days was allowed for equilibration. Moisture content of the corn before addition of the propionic acid was determined by drying in a forced air oven at 103 ± 1°C for 72 hr as recommended by the American Association of Cereal Chemists (1980). Triplicate analyses were made. For storage studies, 250-ml linear polyethylene bottles with tightly fitting linear polyethylene screw caps were used in place of the Erlenmeyer flasks, and the samples (about 100 g each) were stored at 4°C until needed.

With three samples, after addition of the propionic acid and equilibration, sufficient 0.500*N* sodium hydroxide was added to neutralize the added acid. To ensure uniform application, the flask was shaken immediately after addition of the sodium hydroxide, again after several hours, and a final time after 24 hr. In this case, the corn used to prepare the samples had been dried to about 12% moisture, and the water introduced with the sodium hydroxide was taken into consideration in calculating the final moisture content.

**Homogenization and Analysis**

A 4-g sample of treated corn to be analyzed was weighed on an analytical balance into a 250-ml beaker containing 100.00 ml of a known concentration of *n*-butyric acid (about 100 ppm) in deionized water. The sample was then homogenized for 15.0 min at a speed setting of 40 in a Virtis model 45 homogenizer with a "macro ultra shear" assembly. The resulting solution was filtered through Whatman no. 2 filter paper, and the filtrate was diluted with the 100 ppm *n*-butyric acid to a propionic acid concentration of 50 ppm or less. Propionic acid was then determined by injecting 1.0 $\mu$l of diluted filtrate into the gas chromatograph, with the *n*-butyric acid serving as an internal standard. In a variation of the procedure for samples with propionic acid in the salt form, the *n*-butyric acid solution used for homogenization and dilution was made 0.050*M* with respect to formic acid. For analysis of single kernels, homogenization was in 10.00 ml of solution in a test tube with the "micro ultra shear" assembly.

A 75 cm × 4 mm i.d. borosilicate-glass column containing 0.3% (w/w) Carbowax 20M and 0.1% (w/w) phosphoric acid on 60-80 mesh Carbopack C was used for chromatographic separation of propionic acid. To reduce adsorption, the usual plug at the inlet end of the column was omitted, and a phosphoric acid-treated glass wool plug (Supelco) was used at the outlet end. Conditioning of the column was at 205°C for 15 hr at a nitrogen flow rate of 32 ml/min. Before use, 32 4.0-$\mu$l samples of water were injected onto the column to reduce extraneous peaks resulting from decomposition products generated when a new column is initially exposed to water. The chromatographic conditions we used are given in the legend to Fig. 1; the results illustrated in Figs. 1–5 were obtained under the same conditions.

By injecting 1.0-$\mu$l samples of aqueous standards containing known concentrations of propionic acid plus *n*-butyric acid (about 100 ppm) as an internal standard, a calibration curve was constructed for the relative peak area of propionic acid (propionic acid peak area/*n*-butyric acid peak area) as a function of concentration over the range of 0–50 ppm (Fig. 2), which corresponded to the concentration of the unknowns after dilution. From the slope and intercept of the resulting straight line, the concentration of propionic acid in an unknown was then easily calculated from its relative peak area. Correction for ghosting was made by running a standard, containing approximately the same concentration of propionic acid as the unknown, immediately after each sample. The known concentration of the standard was then subtracted from the amount found, and the result, which served as an estimate of the error due to ghosting, was used to correct the unknown.

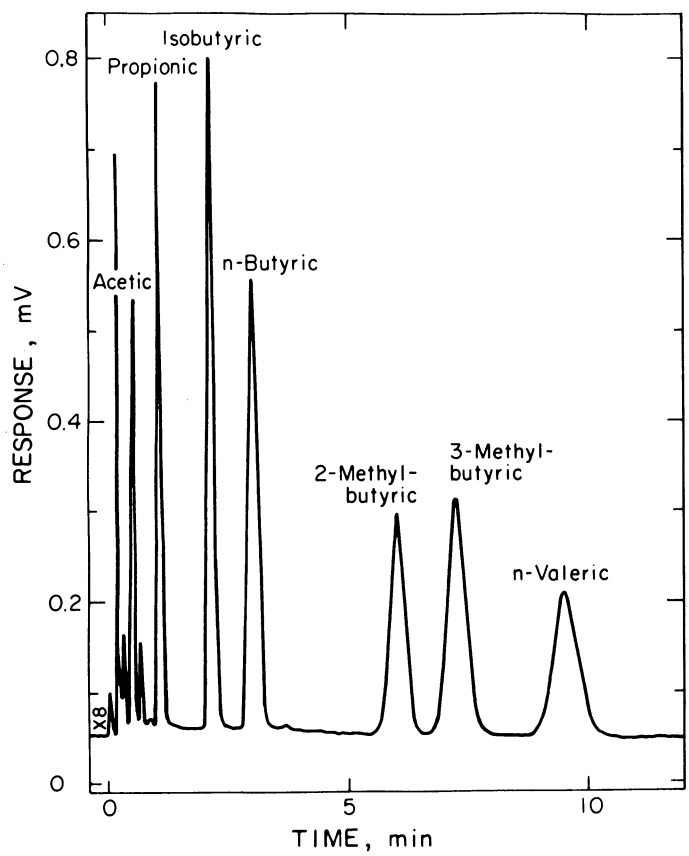


**Fig. 1.** Gas-chromatographic separation of an aqueous solution of volatile fatty acids on a coated Carbopack C column conditioned for 15 hr at 205°C. Column: 75 cm × 4 mm i.d. borosilicate-glass packed with 0.3% (w/w) Carbowax 20M and 0.1% phosphoric acid on 60-80 mesh Carbopack C. Flow rate: $N_2$, 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: 1.0 $\mu$l containing 100 ppm of each acid.

### Color Test for Propionic Acid

A kernel of corn was placed in a test tube containing 2.00 ml of deionized water, a drop of 0.10% (w/v) methyl red hydrochloride in 95% denatured ethyl alcohol was added, and the tube was shaken. After 2.0 min, the solution in a tube containing a kernel treated with propionic acid became pink; it remained yellow to orange if the kernel was not treated. In a variation of the test, the kernel was sliced into six pieces and mixed for 15 sec on a Thermolyne Maxi Mix (Sybron Corp.) before adding the methyl red. In this case, color development was almost immediate.

## RESULTS AND DISCUSSION

### Chromatographic Separation

Water in sample extracts can cause problems in gas-chromatographic analyses. With packed columns, it tends to be adsorbed by the support material, resulting in peak tailing of sample components. Use of Chromosorb 101, a copolymer of styrene and divinylbenzene, was investigated because it was reported to be effective in separating aqueous solutions of the volatile fatty acids (Ackman 1972, Dave 1969). A 166 cm × 4 mm i.d. borosilicate-glass column packed with 80-100 mesh Chromosorb 101 gave satisfactory separation of an aqueous solution of propionic acid from the other volatile fatty acids, but with less sensitivity and poorer resolution than the column selected.

The column chosen was Carbopack C coated with 0.3% (w/w) Carbowax 20M and 0.1% (w/w) phosphoric acid (Fig. 1). This packing has rather unique properties due to the presence of Carbopack C, which is a graphitized carbon support. As might be expected, the support has adsorptive properties and plays an active role in the separation. Carbowax 20M, a polyethylene glycol, has been used in various combinations as a liquid phase for separating volatile fatty acids, and the phosphoric acid acts to reduce tailing by neutralizing adsorption sites. As shown in Fig. 1, the column gave a good separation of the volatile fatty acids from acetic through *n*-valeric in only 11 min. Formic acid, which gives low response in the flame ionization detector, is not shown but was also well resolved from the propionic peak. Propionic acid was well separated from the other acids and had a retention time of only about a minute.

The temperature used for conditioning the column proved to be very important. A column conditioned at the relatively low temperature of 175° C for 15 hr gave excellent separation of the volatile fatty acids. When the column was used to determine propionic acid at high sensitivity, however, there were a number of peaks in the vicinity of the propionic acid peak, which caused difficulty in accurately measuring the area with an electronic integrator. With a column conditioned at 240° C, as recommended by Di Corcia and Samperi (1974), the separation was adequate, but the peaks tailed appreciably, probably due to loss of phosphoric acid at the high conditioning temperature. A temperature of 205° C proved to be a satisfactory compromise. The separation on a column conditioned at this temperature (Fig. 1) was comparable to that given by the column conditioned at 175° C, and although it may not be obvious from the chromatogram, extraneous peaks caused no problems in accurately integrating the area of the propionic peak. Any loss of phosphoric acid from the column during conditioning was minimal.

To correct for variations in injected sample volume, *n*-butyric acid was used as an internal standard. Samples and standards contained 100 ppm of internal standard, and the ratio of the area of the propionic peak to that of the internal standard was used as a measure of the propionic acid concentration. *n*-Butyric acid was used because it could be obtained in high purity, was well resolved from propionic acid, and had a retention time of only about 3 min. Isobutyric acid also was sufficiently well resolved from the propionic peak for use as an internal standard, as was cyclohexanone, which can be obtained in high purity.

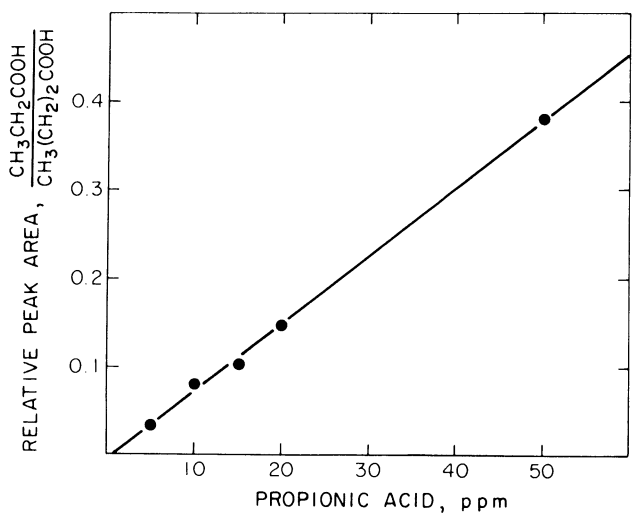


**Fig. 2.** Relative peak area of propionic acid as a function of concentration over the range of 0–50 ppm. Sample size was 1.0 μl with 100 ppm *n*-butyric acid included as an internal standard.

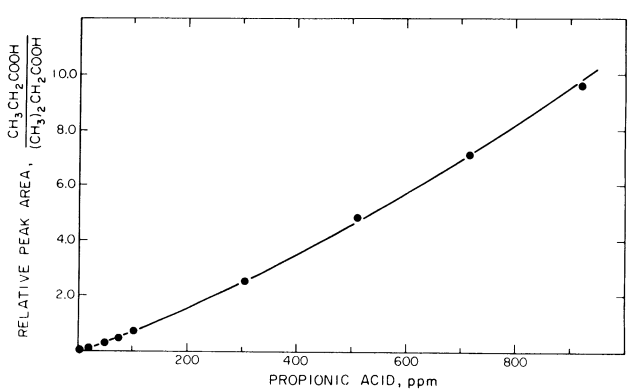


**Fig. 3.** Relative peak area of propionic acid as a function of concentration over the range of 0–950 ppm. Sample size was 1.0 μl with 102 ppm isobutyric acid included as an internal standard.

### Quantitation and Ghosting

Application of the Carbopack C column coated with Carbowax 20M to the analysis of propionic acid required a more thorough study of the quantitative aspects of its use for this purpose than had previously been reported. A calibration curve for relative peak area vs. propionic acid concentration over the range of 0 to about 900 ppm is shown in Fig. 3. In this case, isobutyric acid was the internal standard. Unexpectedly, the curve was nonlinear with the more concentrated samples showing enhanced responses. When the calibration was done, the samples were chromatographed in the order of increasing concentration, and the shape of the curve was influenced by a phenomenon known as ghosting, which is commonly encountered when aqueous solutions are analyzed by gas chromatography. An example of ghosting is shown in Fig. 4, a chromatogram obtained by injecting 1.0 μl of water only. Although

**TABLE I**
**Precision and Accuracy for Gas-Chromatographic Analysis of Propionic Acid in Corn**

| Propionic Acid (%, w/w) | | Relative Error (%) | Coefficient of Variation (%) |
|---|---|---|---|
| Theory[a] | Found[b] | | |
| 0.201 | 0.195 ± 0.003 | −3.14 | 1.45 |
| 0.302 | 0.304 ± 0.010 | +0.55 | 3.31 |
| 0.405 | 0.391 ± 0.007 | −3.46 | 1.68 |
| 0.489 | 0.472 ± 0.004 | −3.51 | 0.75 |
| 0.591 | 0.579 ± 0.008 | −2.02 | 1.41 |
| 0.809 | 0.780 ± 0.005 | −3.62 | 0.62 |

[a] Moisture content was 22.76% (w/w) before addition of the propionic acid.
[b] Average ± standard deviation for triplicate analyses of five 4-g samples.

the sample contained neither propionic nor *n*-butyric acid, peaks were obtained for both. The acids were adsorbed on the column from previous samples, and the ghost peaks were a result of desorption when the highly polar water was injected. The peaks for a given sample can thus be larger than they should be, depending on the concentration of previous samples.

Although not always mentioned in published procedures, ghosting is not an uncommon phenomenon when aqueous solutions of volatile fatty acids are subjected to gas chromatography. It has been reported with silicone, polyester, and Tween 80 substrates (Ackman and Burgher 1963, Geddes and Gilmour 1970) and even occurs with polystyrene bead packings (Mahadevan and Stenroos 1967) and capillary columns (Kaderavek and Volonterio 1966). With packed columns, it is primarily due to adsorption on the support material, on glass wool column plugs, and on charred deposits at the injection site (Geddes and Gilmour 1970).

With the Carbopack C column, ghosting is not a serious problem if the analyst is aware of its presence. It is greatly reduced if the concentration of propionic acid in the samples is 50 ppm or less. As shown in Fig. 2, a calibration curve over the range of 0–50 ppm was completely linear, and samples to be analyzed were therefore diluted to 50 ppm or less before chromatography. In this concentration range, ghosting on the Carbopack C column was minimal compared to other columns that might be employed.

Even without correction for ghosting, analyses for propionic acid could be made in the 0–50 ppm range with a relative error of only a few precent. All results were corrected for ghosting, however, because accuracy was considerably improved by the correction, which was both simple and rapid. The correction procedure was devised as a result of careful experiments with samples containing known amounts of propionic acid and was based on the observation that ghosting remains relatively constant for consecutive samples having approximately equal concentrations. Inclusion of formic acid vapor in the carrier gas to minimize ghosting (Ackman 1972, Ackman and Burgher 1963, Geddes and Gilmour 1970) was considered but was thought to be unwise due to possible adverse effects on the column packing and detector. Formic acid is corrosive toward stainless steel and, over a period of time, would cause problems with the detector.

**TABLE II**
**Gas-Chromatographic Analysis of Propionic Acid in Stored Corn Samples**

| Storage Time (months)[a] | Propionic Acid (%, w/w) | | Relative Error (%) |
|---|---|---|---|
| | Theory | Found[b] | |
| 17 | 0.202 | 0.193 ± 0.004 | −4.24 |
| 17 | 0.303 | 0.283 ± 0.005 | −6.64 |
| 17 | 0.394 | 0.381 ± 0.004 | −3.46 |
| 17 | 0.499 | 0.478 ± 0.009 | −4.26 |
| 17 | 0.598 | 0.579 ± 0.005 | −3.17 |
| 17 | 0.790 | 0.754 ± 0.009 | −4.45 |
| 24 | 0.304 | 0.286 ± 0.005 | −6.01 |
| 24 | 0.400 | 0.380 ± 0.007 | −4.89 |
| 24 | 0.489 | 0.480 ± 0.013 | −1.84 |
| 24 | 0.597 | 0.562 ± 0.012 | −5.82 |
| 24 | 0.795 | 0.754 ± 0.011 | −5.14 |

[a] Before addition of the propionic acid, moisture contents were 21.06 and 22.61% (w/w) for the samples stored for 17 and 24 months, respectively.
[b] Average ± standard deviation for triplicate analyses of five 4-g samples.

**TABLE III**
**Propionic Acid in Corn by Gas-Chromatographic Analysis with Added Formic Acid[a]**

| Pretreatment | Propionic Acid (%, w/w) | | Relative Error (%) |
|---|---|---|---|
| | Theory | Found[b] | |
| None[c] | 0.306 | 0.304 ± 0.010 | −0.80 |
| None[c] | 0.491 | 0.473 ± 0.008 | −3.67 |
| None[c] | 0.790 | 0.760 ± 0.011 | −3.89 |
| None[c] | 0.982 | 0.944 ± 0.004 | −3.91 |
| NaOH[d] | 0.298 | 0.290 ± 0.017 | −2.88 |
| NaOH[d] | 0.506 | 0.497 ± 0.030 | −1.76 |
| NaOH[d] | 0.703 | 0.679[e] ± 0.012 | −3.47 |

[a] A 0.050 *M* formic acid solution was used for extraction and dilution.
[b] Average ± standard deviation for triplicate analyses of five 4-g samples.
[c] Moisture content was 22.76% (w/w) before addition of the propionic acid.
[d] Sufficient sodium hydroxide was added to neutralize the propionic acid completely. Final moisture contents were 18.77, 23.69, and 28.35% (w/w) for the three samples, respectively.
[e] Four 4-g samples.

**Accuracy and Precision**

Gas-chromatographic analyses of corn samples treated with 0.2–0.8% (w/w) propionic acid (Table I) gave results in close agreement with the percentages calculated from the propionic acid added. Average deviation of an analytical result from the theoretical percentage was only −0.013%. This represents an average relative error of −2.53%, which probably reflects a slight loss of propionic acid due to volatility in the flasks used to prepare the samples. Precision was excellent with an average standard deviation of only ± 0.006% or, expressed as coefficient of variation, 1.54%. Each value in Table I is an average of five analyses, each of which was the average of three replicate gas-chromatographic determinations on the same solution. Comparable results were obtained when only the first of the three replicate analyses by gas chromatography was used. The 4-g samples were obviously adequate for the purpose of the present study. For large lots likely to be heterogeneous, a larger sample size would be required to ensure that the sample is representative of the lot as a whole.

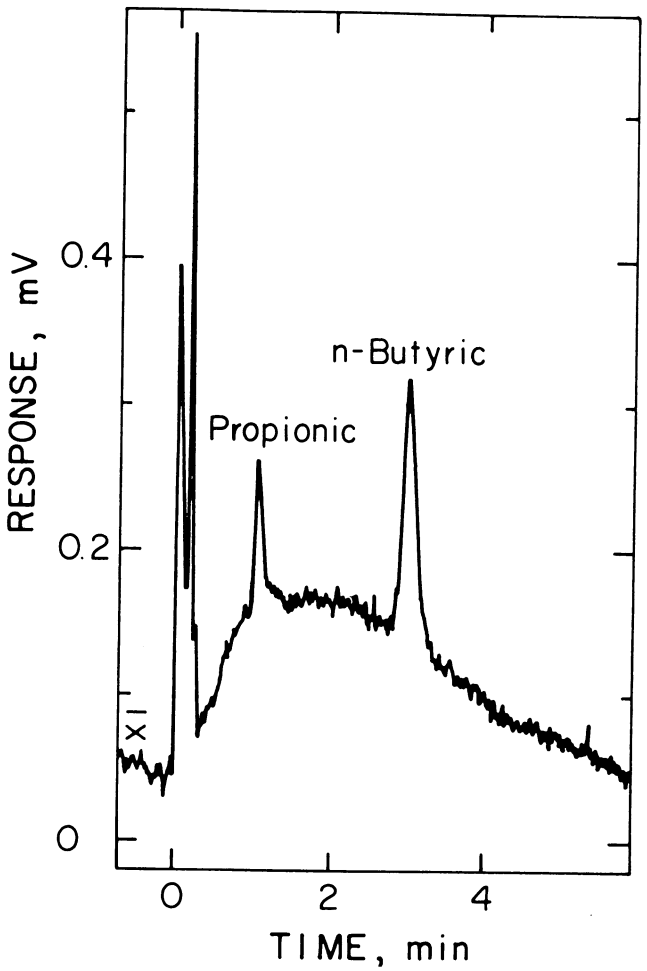


**Fig. 4.** Chromatogram showing ghost peaks for propionic and *n*-butyric acids when 1.0 µl of deionized water was injected.

### Stored Samples

Because of the possibility that compounds producing extraneous peaks or other interferences might be present in corn samples that were not freshly prepared, samples stored under laboratory conditions (4° C) both for 1 year and 5 months and for 2 years after addition of propionic acid were also examined. Analyses for propionic acid (Table II) were again in close agreement with the calculated percentages. The average deviation of −0.022% of an analysis from the theoretical percentage was only slightly greater than that for analyses of the freshly prepared samples, perhaps indicating a slight loss of propionic acid under the storage conditions. Among samples stored for 2 years, no propionic acid was found in one sample (not shown in Table II), which originally contained 0.209% propionic acid. Presumably, the acid was metabolized by microorganisms. The other samples in the group apparently had a sufficiently high concentration of propionic acid to prevent microbial action under the storage conditions. A sample in the group stored for 1 year and 5 months, which also contained about 0.2% propionic acid (Table II), showed little or no loss during storage. This sample was obtained earlier in the harvest season than the sample stored for 2 years and apparently was more nearly free of viable microorganisms when the propionic acid was added. Other than the loss of propionic acid from the sample with an insufficient amount to prevent microbial action, no unusual problems were encountered in analyzing the stored samples.

### Addition of Formic Acid

In analyses for propionic acid, corn samples in which the acid is partially neutralized might occasionally be encountered. If the analyst wishes to determine total propionic acid including the salt form, the solution used for homogenization and dilution of the sample may be made 0.050*M* with respect to formic acid. As shown in Table III, results by this procedure for samples with free propionic acid only were essentially the same as results when no formic acid was employed (Table I). For three of the samples (Table III), sufficient sodium hydroxide had been added to completely neutralize the propionic acid, and for these, accuracy was comparable to that for samples containing unneutralized propionic acid (Tables I and III). The higher standard deviations probably reflect slight heterogeneity of the samples due to uneven distribution of the added sodium hydroxide. Because the formic acid in the solution injected onto the chromatographic column will cause the peaks to become sharper and consequently change the slope of the calibration curve for propionic acid, the standards must also contain 0.050*M* formic acid when the acid is present at this concentration in the samples being analyzed. Because formic acid is only rarely used as a mold inhibitor for corn, its addition to the samples during analysis would not ordinarily cause problems. The formic acid in the samples and standards caused a reduction in ghosting but did not eliminate it.

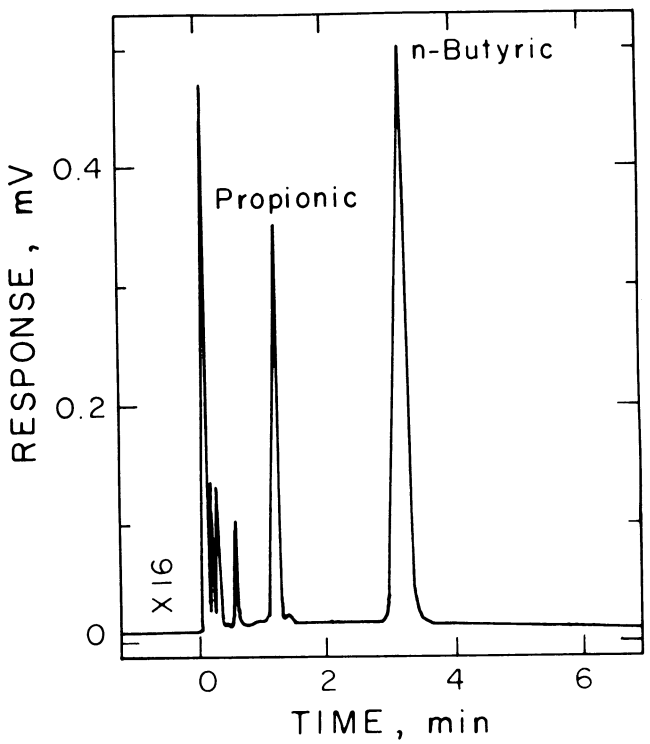


**Fig. 5.** Chromatogram for the analysis of a single kernel of corn containing only 0.3% propionic acid. Sample size was 1.0 μl with 100 ppm *n*-butyric acid included as an internal standard.

### Sensitivity

In addition to simplicity, sensitivity of the method was one of its principal advantages. A concentration of 1 ppm or less in the aqueous extract could easily be determined. If one assumes that the minimum detectable amount must give a peak height equal to twice the random noise level, then the lower limit of detection under the chromatographic conditions employed would be about 0.2 ppm. We are not aware of any published procedure for the gas-chromatographic analysis of propionic acid in treated corn affording comparable sensitivity.

Although the actual analysis of mixtures was considered to be beyond the scope of the present study, providing sufficient sensitivity for the analysis of single kernels was an important criterion in developing the procedure. We felt that only by analyzing individual kernels could one check the possibility that a corn sample might be a mixture of components with different levels of propionic acid.

As shown by the chromatogram of Fig. 5, sensitivity of the system was well in excess of that required for an analysis to be performed on a single kernel. In this case, the kernel, which contained only 0.3% propionic acid, was homogenized in 10.00 ml of 0.050*M* formic acid in deionized water, and the solution was filtered and diluted 3.25:10. Analyses of randomly selected kernels from a mixture with different levels of propionic acid should allow the composition of each component to be determined. It would, of course, be necessary to select a sufficient number of kernels of each component to constitute a representative sample. Our experience has shown that the percent propionic acid in the individual kernels of a sample will vary slightly from the average for the sample. In a typical experiment, the average of six analyses on separate kernels from a sample with 0.306% propionic acid showed a relative error of only −2.70% with a standard deviation of 0.019%, which probably reflects real differences among the kernels analyzed.

### Color Test

In addition to an analytical procedure for determining propionic acid, a method for detecting its presence in a corn sample was needed for use as a screening procedure. An attempt to use an indicator in the form of a spray reagent for detection was not successful. With a spray reagent containing either methyl red or bromcresol green, the treated kernels could not be distinguished from the untreated ones. Presence of the acid could easily be detected, however, by merely placing a kernel in a test tube containing water with a drop or two of methyl red solution. If a whole kernel is used, a few minutes are required for the distinct pink color to develop, but the color develops within 15 sec if the kernel is sliced into several sections before placing it in the tube. The test should allow rapid screening of samples to determine whether they contain propionic acid.

## CONCLUSIONS

The gas-chromatographic method described makes possible the analysis of propionic acid in treated corn by a simple procedure affording high sensitivity. The salt form of the acid can also be determined by acidification of the extraction solution with formic acid. The filtration step described could possibly be eliminated but was included to prolong column life. Due to ghosting, the linear range of the chromatographic system is limited to 0–50 ppm, where the effect is minimal, and sample extracts more concentrated than

50 ppm require dilution before chromatography. High sensitivity of the system allows single kernels to be analyzed and may make possible the analysis of mixtures. Because the column separates all the common isomers from acetic through valeric acid, the procedure could, with only slight modification, also be used to determine other volatile fatty acids that might be used as preservatives for high-moisture corn.

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