

condensate before separation revealed the same series of $[M]^+$ and $[M-1]^+$ ions that were found after separation. (3) Odors of isolated components were reminiscent of the original mixture and of typical roasted peanut aroma. (4) Numerous condensates have been obtained and analyzed over a period of a year, and the occurrence and amounts of these components were surprisingly consistent.

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VINEGAR COMPOUNDS

Analysis of Vinegar by Gas-Liquid Chromatography

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Gas-liquid chromatography has been applied to the analysis of vinegars. By selecting the proper liquid phase it was possible in a single run to resolve alcohols, esters, acids, and 3-hydroxy-2-butanone. The yeast fermentation of cider yields the components of fusel oil, which in turn are partially converted to the corresponding acids. Methyl and ethyl acetates are produced during the vinegar fermentation. The vinegar fermentation of cider stock produces a large amount of 3-hydroxy-2-butanone, whereas only traces are produced by the yeast fermentation of cider.

THE SENSITIVITY of gas-liquid chromatography for detecting traces of volatile compounds has led to its extensive use for analyzing foodstuffs. A large body of information is being accumulated showing the identity of the volatile compounds in many common foods. The method is particularly well suited for the characterization of the compounds which occur in vinegar.

White or distilled vinegar is usually manufactured from ethyl alcohol denatured with ethyl acetate, formula SDA29. Such vinegars, and also those made from undenatured alcohol, contain traces of ethyl acetate and residual

alcohol. Vinegars made from fermented fruit juices such as cider and wine also contain many other compounds, including higher alcohols which collectively are called fusel oil, and oxidation products of the higher alcohols. Most of these compounds, except acetic acid and water, occur in only trace amounts. The presence of so much acid and water makes it difficult to separate and identify the other components, which may impart character and flavors to specific vinegars. Compounds responsible for the aroma of apples and cider have been identified by various workers (2, 4-8, 10-12), but little has

been done to identify the flavor components of vinegars.

A recent study of the ether-pentane extracts of wine and fruit vinegars by Suomalainen and Kangasperko (13) has shown the presence of seven or eight compounds in addition to the expected ethyl acetate: the alcohols found in fusel oil, their acetic acid esters, 2-3-butanedione (diacetyl), and 3-hydroxy-2-butanone (acetoin). They report that spirit vinegars contained only ethyl acetate. Maurel and Lafarge (9) found in fermented cider methanol and isopropyl alcohol in addition to the usual fermentation alcohols.

This paper describes the identification of volatile compounds found in cider vinegar and distilled vinegar prepared from both SDA29 and SDA29 plus fusel oil.

By selecting the proper column and detector it has been possible to show on one chromatogram the presence of esters, alcohols, acetic acid, and acids resulting from the oxidation of the alcohols in fusel oil. Concentration of the flavor components by extraction was not required.

The techniques used in this paper can be applied by vinegar manufacturers and purchasers to assess the character of both alcohol and vinegars.

Experimental

Experimental distilled vinegars were produced in the laboratory generators described by Allgeier, Wisthoff, and Hildebrandt (7), or in a laboratory model of an Acetator manufactured by Heinrich Frings, Jaegerstrasse 9, Bonn, West Germany. All the spirit vinegars were produced from synthetic alcohol. SDA29 alcohol was prepared by adding 1.0% by volume of ethyl acetate to synthetic ethyl alcohol. An alcohol of special character was prepared by adding 2.75 ml. of molasses fusel oil per liter of 190-proof SDA29 ethyl alcohol (SDA29 Special). After the alcohol was diluted to approximately 11 weight %, a nutrient composed of dextrose, inorganic salts, and calcium pantothenate was added. The generators were operated on a given feed long enough to allow the product from the 8th and 9th cycles to be combined for analysis.

Commercial cider vinegars, with one exception, were purchased locally. The experimental cider vinegar was produced from commercial pasteurized apple cider, by a pure culture yeast fermentation, followed by inoculation of the fermented cider with an acetobacter culture. This was then placed in cotton-plugged flasks and shaken for 3 days on a reciprocal shaker. Commercial samples of cider stock and finished cider vinegar were used for comparison. Table I identifies the vinegars used in this study.

Equipment

A Varian Aerograph Model 1520 gas chromatograph equipped with a hydrogen flame detector was used throughout this work. The 10-foot long column of $\frac{1}{8}$ -inch stainless steel was packed with 20% FFAP (Varian Aerograph) on 70-80 mesh DMCS-treated acid-washed Chromosorb W.

Operating conditions were: carrier gas, helium, flow 20 ml. per minute. Temperature programmed from 56° C. at 2° per minute for 4 minutes then at 5° per minute to 120° C., then held at 120° C. Injector temperature 225° C. Detector oven temperature 200° C.

Preliminary work was carried out using columns packed with the common polar liquid phases. No column tested was capable of separating free acetic acid, alcohols, and esters simultaneously.

Table I. Cider and Vinegars Studied

	Approx. % Acid (as Acetic)	Chromatogram Figure No.
1. Unfermented cider, pasteurized	Trace	7
2. Fermented cider, pure culture fermentation (made from 1)	Trace	8
3. Vinegar from fermented cider (made from 2)	4.7	11
4. Commercial cider stock, unpasteurized	1.5	9
5. Commercial cider vinegar made from 4	5.8	10
6. Distilled vinegar A	5	1
7. Distilled vinegar B	4	2
8. Distilled vinegar C	5	3
9. Vinegar made from specially denatured alcohol	12	4
10. Cider vinegar D	5	5
11. Cider vinegar E	4	6

Attention was then shifted to the use of the FFAP column, which is capable of separating alcohols, acids, and esters. However, the isopentyl and 2-methyl-1-butyl alcohols, acetates, and corresponding acids were not separated on this column. Sample sizes of 2 or 3 μ l. were used throughout this work.

Results and Discussion

The synthetic alcohol used to prepare the SDA29 alcohols contains no detectable trace materials. The analysis of specially denatured alcohol (SDA29 Special) showed the presence of propyl, isobutyl, butyl alcohols (trace), 2-methyl-1-butanol, and isopentyl alcohol in

addition to ethyl alcohol and ethyl acetate.

Comparisons of a few commercial distilled vinegars have shown wide differences in their ethyl acetate contents and, as expected, the ethyl acetate concentration varies directly with the residual alcohol concentration (Figures 1 to 3). With the exceptions noted later, these vinegars contained ethyl acetate, ethyl alcohol, and acetic acid (and water). The chromatograms showed traces of other unidentified compounds.

During the vinegar fermentation of the specially denatured alcohol the higher alcohols are partially converted to the corresponding acids and esters. Thus

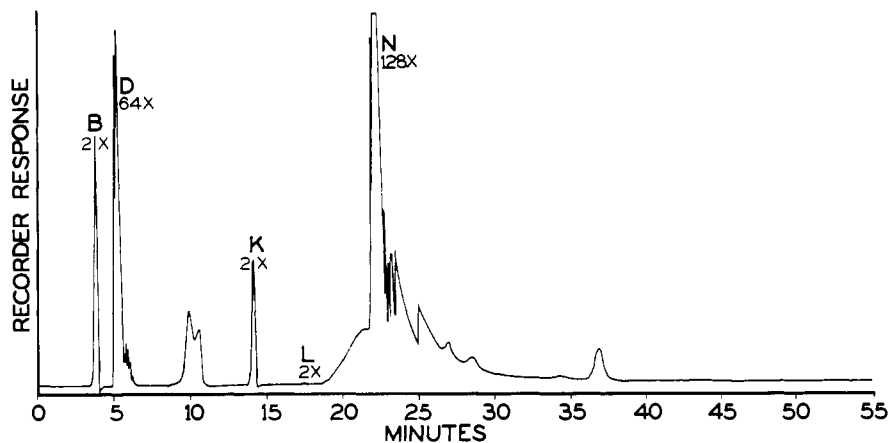


Figure 1. Chromatogram of distilled vinegar A

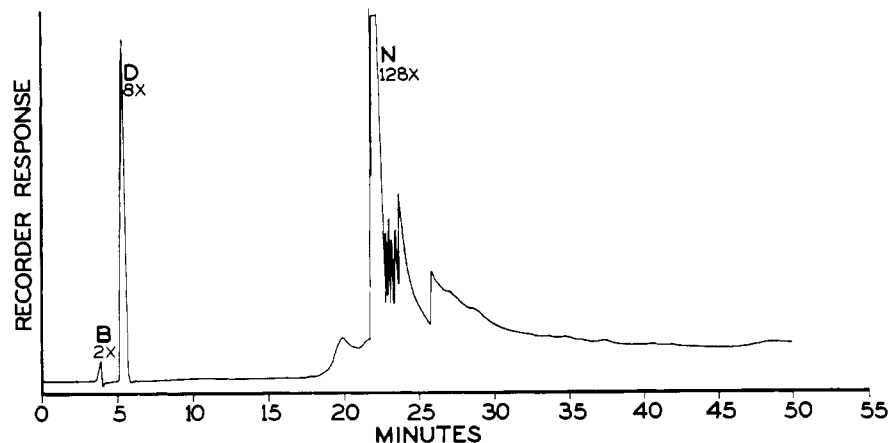


Figure 2. Chromatogram of distilled vinegar B

the vinegar contains isobutyl and C_5 acetates (this peak may represent a mixture of isopentyl and 2-methyl-1-butyl acetates), as well as propionic, isobutyric, and C_5 acids (this peak may represent a mixture of isovaleric and 2-methyl-1-butyric acids) (Figure 4).

While it is possible to have esters formed from all possible combinations of the higher alcohols and their corresponding acids, these esters would occur in extremely low concentrations. They

were not detected in the present work.

A much more complicated chromatogram is obtained when apple cider vinegar is analyzed. Many compounds occurring naturally in apple juice remain in fermented cider, and some in cider vinegar. The analyses of these vinegars are shown in Figures 5 and 6. These chromatograms show the presence of fusel oil components and some of their oxidation products—namely, propionic, isobutyric, and C_5 acids. Table II lists

the compounds found in cider, fermented cider stock, and vinegars. Thus in contrast to distilled vinegars, where one acid, one alcohol, and one ester predominate, in cider vinegar four alcohols, four acids, and three esters were detected.

As expected, some of the compounds in cider vinegar and their precursors were derived from the yeast fermentation, as shown by the analyses of unfermented and fermented cider. Chromatograms of the unfermented and fermented cider are shown in Figures 7 and 8. Figures 9 and 10 show the results obtained with a commercial fermented cider stock and the vinegar made from it, while Figure 11 is the chromatogram of a sample of cider vinegar prepared in the laboratory as described earlier. Relatively few of the volatile compounds found in cider vinegar were found in unfermented cider. Additional quantities of ethyl alcohol and higher alcohols were produced by the yeast fermentation.

The compounds given in Table III have been identified as components of cider or vinegars. More substances remain to be identified and their contribution to the aroma of the products evaluated.

During the conversion of ethyl alcohol to acetic acid many of the compounds in the fermented cider are chemically changed, either completely or in part. Thus methyl acetate, *sec*-butyl, propyl, and pentyl alcohols (the pentyl alcohol peak may represent a mixture of 2-methyl and 3-methylbutanols), and ethyl lactate disappear almost completely as a result of the action of the acetobacter. The latter organism produces the previously mentioned acids, additional amounts of 3-hydroxy-2-butanone, and several unidentified compounds which were not detected in the fermented or unfermented cider. The methyl acetate and methanol found in cider are derived from sources other than the yeast fermentation.

Apples contain pectin methylesterase (PME) which is able to hydrolyze the methoxy ester groups in the pectin to form free methanol (3). Commercial cider vinegar, which usually is produced from nonpasteurized cider, will contain the enzyme PME and thus will contain some methanol. The commercial cider which was subjected to a pure yeast fermentation in the laboratory had been pasteurized during manufacture and hence contained no PME. Therefore neither the cider fermented in the laboratory nor the vinegar derived from it contained methanol.

Both free methanol and methyl acetate occurred in nonpasteurized cider vinegar. Presumably the latter arose from the esterification of methanol. Methanol could also be converted to formic acid, which is not detected by flame ionization detectors and hence if present was not identified in this study.

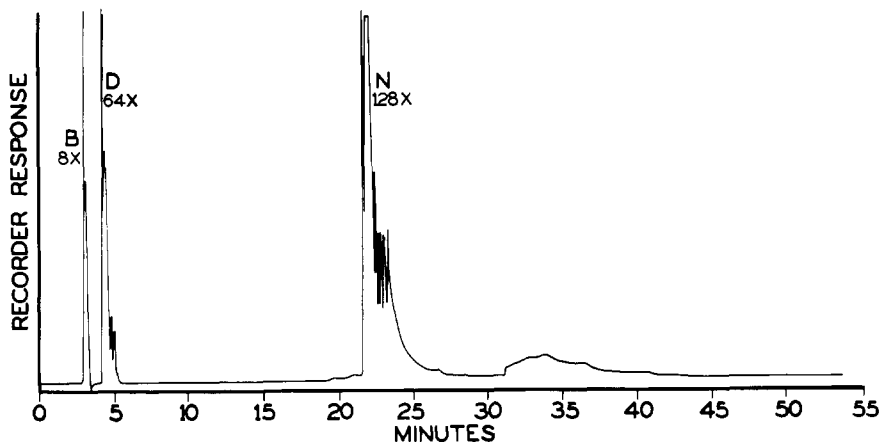


Figure 3. Chromatogram of distilled vinegar C

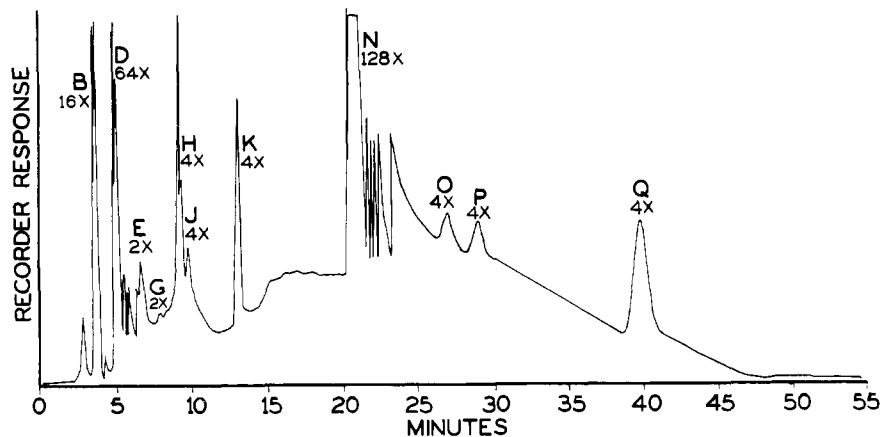


Figure 4. Chromatogram of vinegar made from specially denatured alcohol

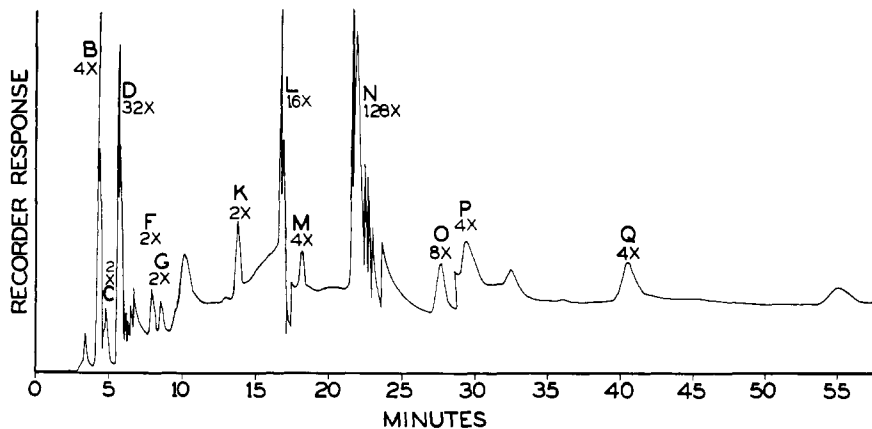


Figure 5. Chromatogram of cider vinegar D

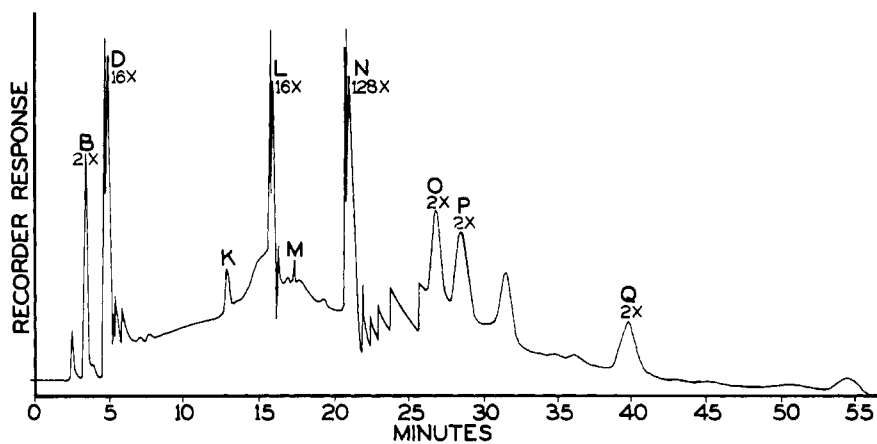


Figure 6. Chromatogram of cider vinegar E

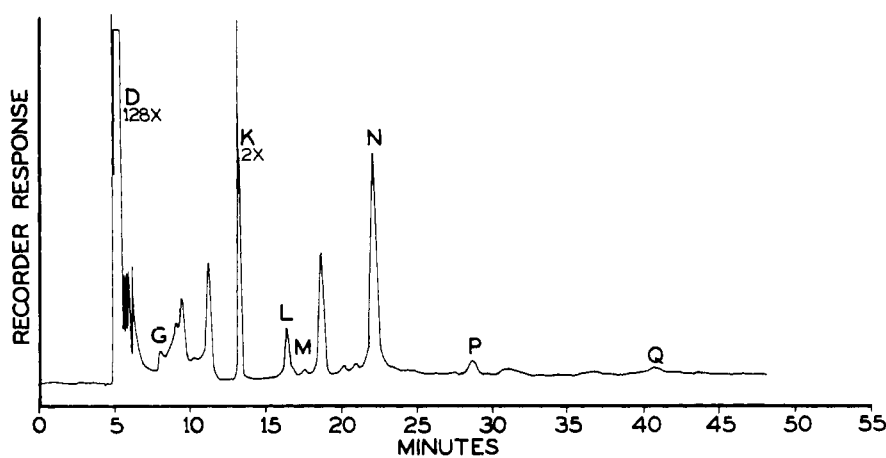


Figure 7. Chromatogram of unfermented pasteurized cider

Table II. Compounds Found in Cider, Fermented Cider, and Vinegars

Compound	Unfermented Cider	Fermented Cider	Cider Vinegar	Distilled Vinegar	Vinegar from Specially Denatured Alcohol
Methanol		+ ^a	+ ^b		
Ethyl alcohol	+	+	+		+
Propyl alcohol		+		+	+
Isobutyl alcohol					+
<i>sec</i> -Butyl alcohol		+ ^a	+ ^b		
C ₅ alcohol	+	+	+	+	+
Methyl acetate			+ ^b		
Ethyl acetate		+ ^a	+	+	+
Isobutyl acetate					+
C ₅ acetate					+
Ethyl lactate	+	+	+		
3-Hydroxy-2-butanone		+	+	+	
Acetic acid	+			+	+
Propionic acid		+ ^a	+ ^b		+
Isobutyric acid		+	+		+
C ₅ acid	+	+	+		+

^a In nonpasteurized commercial cider stock.

^b In vinegar made from commercial cider stock.

Table III. Gas Chromatographic Identification of Components

GC Peak	Identity
A	Methyl acetate
B	Ethyl acetate
C	Methanol
D	Ethyl alcohol
E	Isobutyl acetate
F	<i>sec</i> -Butyl alcohol
G	Propyl alcohol
H	Isobutyl alcohol
J	Isopentyl and/or 2-methyl-1-butyl acetate
K	Pentyl alcohol (2-methyl and/or 3-methyl-butanol)
L	3-Hydroxy-2-butanone
M	Ethyl lactate
N	Acetic acid
O	Propionic acid
P	Isobutyric acid
Q	Isovaleric and/or 2-methyl-1-butyric acid

Figure 8. Chromatogram of fermented cider (pure culture fermentation)

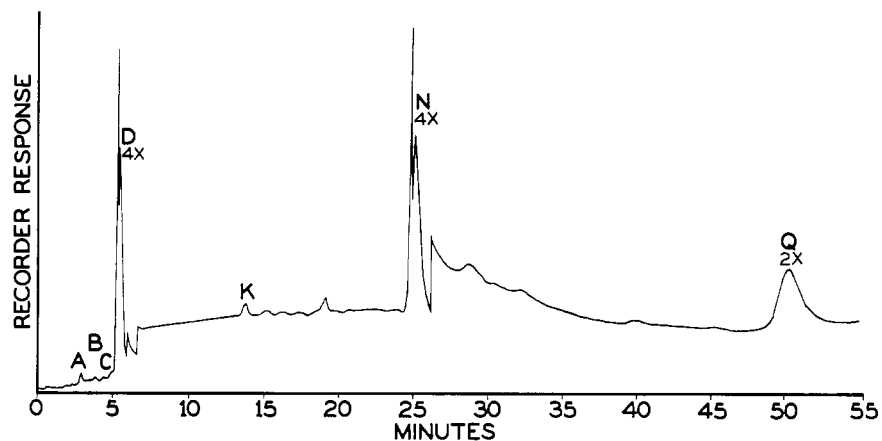


Figure 9. Chromatogram of commercial unpasteurized cider stock

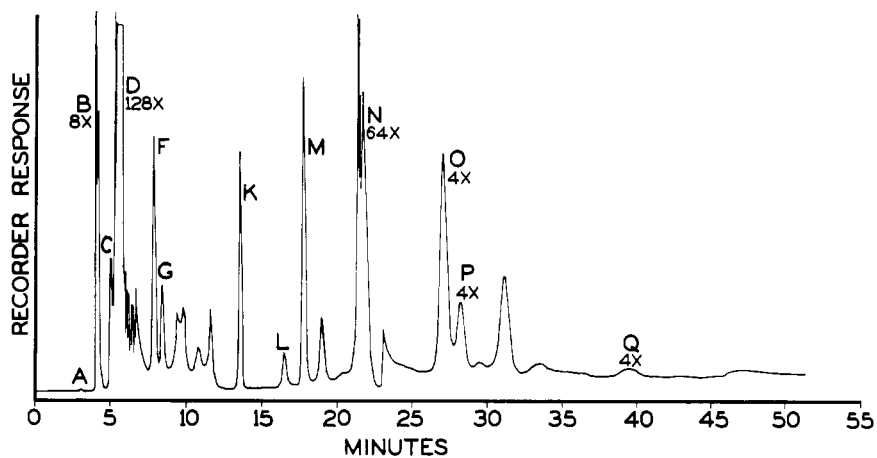


Figure 10. Chromatogram of commercial cider vinegar made from unpasteurized cider stock

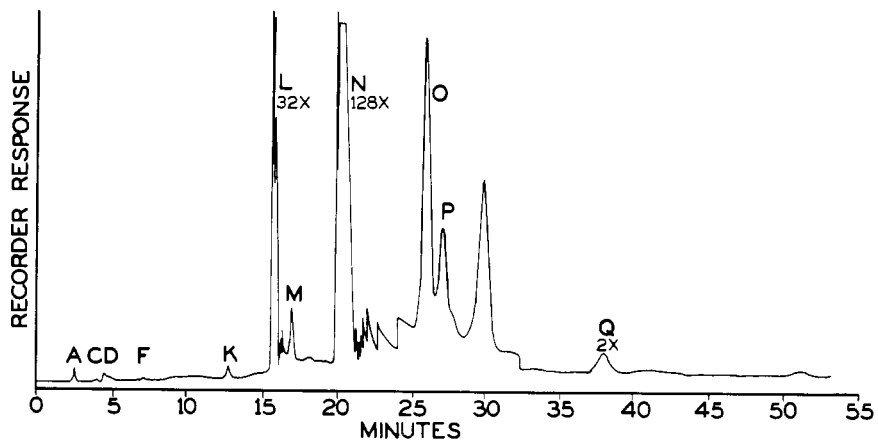
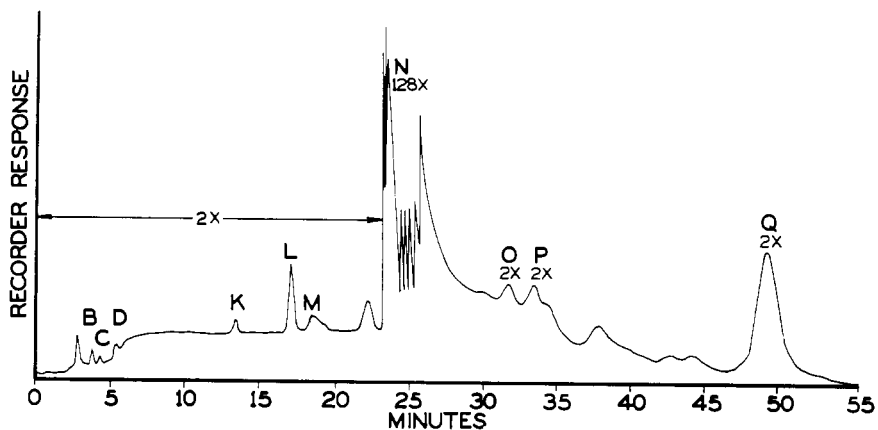


Figure 11. Chromatogram of vinegar made from a pure culture fermented cider



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IRRADIATION COMPOUNDS

Gas Chromatographic and Mass Spectral Identification of Some Volatile Components of Gamma-Irradiated Milk Fat

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Milk fat, sealed in cans under vacuum, was irradiated at 4.5 Mrad with cobalt-60. The small amount of oxygen present in the headspace of the cans was consumed, while H₂, CO, CO₂, and CH₄ were produced. The irradiated fat was analyzed for monocarbonyls by forming their 2,4-dinitrophenylhydrazone derivatives. The volatiles of the irradiated milk fat were obtained by vacuum-steam distillation, and the components in the distillate were extracted with ethyl ether and separated by gas-liquid chromatography (GLC). GLC in conjunction with rapid-scan mass spectrometry was used for identification. *n*-Alkanes and 1-alkenes from C₅ to C₁₇, some alkadienes, *n*-alkanals, short chain fatty acids, and certain γ - and δ -lactones were among the compounds identified. Possible mechanisms involved in the formation of these compounds are presented.

VARIOUS changes take place when milk fat is irradiated. In the presence of air or oxygen, hydroperoxides and secondary products of autoxidation are formed. The mechanism for the formation of these products is similar to that of autoxidation (23, 30). In the absence of air, hydroperoxides are not formed, but off-flavors are still produced along with carbonyl formation and the destruction of carotenoids (7, 17). Day and Papaioannou (7) reported that the flavor of irradiated milk fat was

due to hydrolytic rancidity, oxidative rancidity, and the presence of components with a candle-like odor. It was suggested that carbonyls with medium to long carbon chains were responsible for the candle-like odor. Since gas-liquid chromatography (GLC) of volatiles from irradiated milk fat showed many components other than carbonyls, it was of interest to characterize these compounds to understand the effect of irradiation better.

Experimental Methods

Preparation and Irradiation of Milk Fat. The milk fat was obtained from

raw sweet cream prepared from mixed herd milk in the Oregon State University creamery. The milk fat was washed free of phospholipid and clarified by centrifuging at 30,000 \times G for 20 minutes. The clear milk fat was then sealed in C-enamelled 307 \times 409 (No. 2) cans under 29 inches of vacuum. The cans were irradiated with gamma rays from a 3600-curie cobalt-60 source at 0.4 Mrad per hour to a total dose of 4.5 Mrad. Although irradiation was carried out at room temperature, the temperature of the fat rose to approximately 40° C. during the irradiation period. The irradiated fat was stored at -18° C. until analyzed.

Analysis of Headspace Gases. The cans containing irradiated milk fat and

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