Volatile Components in the Vapors of Natural and Distilled Vinegars

SUMMARY

Volatile components in the vapors of natural and distilled vinegars were identified by gas chromatography by comparing their retention volumes with compounds of known composition and by functional group analysis. Twenty-five volatile components were identified from three natural vinegars (cider, 19 components; wine, 17 components; tarragon, 20 components), and 11 volatile components were identified from five samples of distilled vinegar. Four components were present in all vinegars tested: acetaldehyde, acetone, ethyl acetate, and ethyl alcohol. The natural vinegars had the largest number of volatile components. This was attributed to the nature of the fermentable carbohydrate material used for production of the alcohol subsequently used for vinegar manufacture (e.g. apples and grapes). The importance of the carbonyls, alcohols, and esters to vinegar flavor is discussed.

INTRODUCTION

Vinegar (meaning sour wine) has been used as a food ingredient for over a thousand years, and it is only in the last one hundred years that the chemical and microbiological nature has been realized (Prescott and Dunn, 1959). By definition, vinegars are condiments made from alcoholic fermentation of carbohydrates and then subsequent bacterial fermentation to acetic acid. Although most of the commercial vinegar is made by this two-step process, vinegar is also made by the acetic acid bacteria directly from ethyl alcohol.

Vinegar methods of manufacture fall into two general classes: 1) "distilled," "grain," or "spirit," which denotes that the ethyl alcohol was concentrated by distillation; and 2) "natural," which designates the carbohydrate sources for the alcoholic fermentation and is so described on the labels as "apple," "cider," "wine," "grape," and "malt." Vinegars are measured in strength by their acetic acid concentration (1 grain equals 0.1% acid), and the commercial products range from 50 to 120 grains. Aqueous solutions of glacial acetic acid have a sharp unpleasant taste, so the characteristic flavors of vinegar are dependent on substances other than acetic acid, i.e., the flavor of a vinegar depends on the constituents formed during the fermentation of the raw materials as well as the subsequent acetous fermentation. Little is known about the volatile constituents that give a particular vinegar its characteristic aroma. Earlier studies (Jacobs, 1951) on cider vinegar gave the range of volatile esters as 0.30 to 0.91 g/100 ml, measured as ethyl acetate, and of alcohol as 0.03 to 2.00 g/100 ml. In vinegar generator operations, residual alcohol concentration of 0.3–0.5% is considered satisfactory. Conner *et al.* (1959) demonstrated that as the acetic acid in the final product is increased, the cycle time lengthens, the rate at which oxygen is utilized decreases, and the residual alcohol increases (e.g., an increase from 108 to 120 grain increases cycle time from 1 to 2 weeks and residual alcohol from 0.26 to 0.56%).

Suomalainen and Kangasperko (1963), using gas chromatography, identified the volatile components of a wine vinegar as acetoin, isoamyl alcohol, optically active amyl alcohol, isobutyl alcohol, ethyl acetate, isobutyl acetate, sec. butyl alcohol, isoamyl acetate, and diacetyl. They also reported that a distilled vinegar sample contained only ethyl acetate as the volatile component.

The standards of the Food and Drug Administration define the natural vinegars as a condiment made by the alcoholic and subsequent acetous fermentation of the juices of apples, and to contain not less than 4 g acetic acid per 100 ml (20°C). The same standard applies for other vinegars except that source of carbohydrate is replaced with natural products as grape, corn, barley, rye, and malt. With distilled vinegar the standard does not indicate the carbohydrate source or method of ethyl alcohol manufacture but requires the product to be made from the ace-

tous fermentation of dilute distilled ethyl alcohol and to contain not less than 4 g acetic acid per 100 ml (20°C) (Prescott and Dunn, 1959).

MATERIALS AND METHODS

Source and preparation of vinegar samples. Five commercial vinegars, purchased from the local markets, and three vinegar samples supplied from pickle companies which operate their own vinegar generators, were analyzed for volatile flavor constituents. The description of the vinegar samples, together with their percent acetic acid, is as follows: a) apple cider (5%); b) wine (6%) manufactured from California grape wine; c) tarragon flavored (5%), a mixture of distilled and malt vinegars with tarragon spices; d) distilled vinegar, sample No. 1, labeled "Crystal Distilled" (5%); e) distilled, sample No. 2 marked "Distilled White" (5%) and manufactured from corn, barley malt, and rye; f) distilled sample No. 3 (9%) manufactured by a pickle company located in Minnesota and using the "Frings Process"; g) distilled sample No. 4 (11.6%) from a pickle company in Michigan, made by "acetator process"; h) distilled sample No. 5 (11.6%) from a pickle company in Ohio, made by "Frings process." The above five commercial vinegars were diluted by the manufacturer to the acidities indicated for uniform pickling and table strength. Ten ml of each vinegar was added to a vial containing anhydrous sodium sulfate (1.2 g/ml). The vial was sealed with a rubber septum, shaken for 5 min, and then placed in a constant-temperature water bath (70°C) for a 3-min period to equilibrate the volatile vapors. A 5-ml vapor sample was withdrawn and subjected to gas chromatographic (GLC) analysis.

Separation and identification. A Barber-Colman gas chromatograph unit, model 10, equipped with a flame ionization detector, was used for the qualitative determination of the volatile compounds. The samples were chromatographed on two different stationary phases. The U-shaped columns were heavy-walled glass tubing, 5 mm

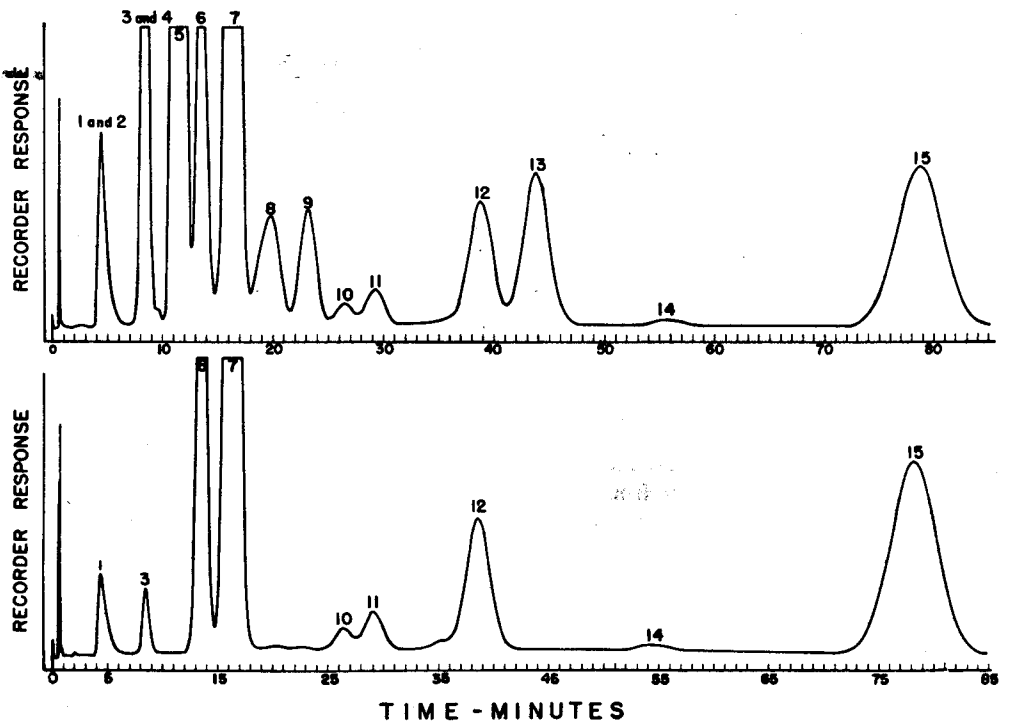

Fig. 1. Gas chromatogram of volatiles from a cider vinegar. Before (upper) and after (lower) treatment with 50% NaOH. The peaks represent 1) acetaldehyde, 2) methyl formate, 3) acetone, 4) ethyl formate, 5) ethyl acetate, 6) diacetyl, 7) ethyl alcohol, 8) sec-butyl acetate, 9) isobutyl acetate, 10) sec-butyl alcohol, 11) propyl alcohol, 12) isobutyl alcohol, 13) isoamyl acetate, 14) n-butyl alcohol, 15) pri-active amyl alcohol.

ID and 6 ft long, packed with either polyethylene glycol 600 (15%) on 60–80-mesh GC-22 firebrick or with düsodecylphthalate (15%) on 60–80-mesh celite. The operating conditions were: column temperature, 105°C; injection port, 215°C; heater bath, 145°C; carrier gas (He), 18 psig; and sensitivity, 10^{-9} amps.

The resolved components were identified by comparing their retention volumes with compounds of known composition.

Functional group analysis. The syringe technique of Hoff and Feit (1964) was used for gas chromatographic analysis of functional groups of compounds in the vapor mixtures from various vinegar samples. Under the conditions used, saturated potassium permanganate in water removed aldehydes, leaving ketones; hydroxylamine hydrochloride (4 g/50 ml) in water removed carbonyl compounds; and sodium hydroxide removed esters. However, for the latter step it was necessary to modify the above technique in order to hydrolyze the esters effectively. Two ml of 50% sodium hydroxide was added to a vial containing 10 ml of the vinegar sample and allowed to stand for 1 hr prior to withdrawing the vapor sample for GLC analysis. The alcohols were determined by difference.

RESULTS AND DISCUSSION

The fact that members of different homologous series of organic compounds may have similar retention volumes has limited the use of gas chromatography for qualitative analysis of complex mixtures. Consequently, retention volume data without a knowledge of compound type lacks sufficient specificity to permit positive identification. This problem can be readily resolved by means of functional group classification reagents (e.g. Hoff and Feit, 1964) and may be illustrated by the chromatograms in Fig. 1. The chromatogram of the cider vinegar (Fig. 1, upper) appears to be that of a mixture having 13 components. Functional group tests showed that the first peak was due to an ester and an aldehyde, and the second peak to an ester and a ketone. Thus, 15 components were present rather than the 13 indicated by the chromatogram. Furthermore, if one compares the two chromatograms (upper and lower) it will be noted that peaks 5, 8, 9, and 13 were absent (Fig. 1, lower) following treatment with the sodium hydroxide reagent, thereby indicating that the peaks represented esters. Sodium hydroxide effectively hydrolyzed the esters and produced peaks of the corresponding alcohols. However, the yield of these products was poor and it will be noted methanol was not detected (probably because of high solubility in the reagent). Following assignment of the functional group to each peak in a chromatogram, comparison with relative retention volumes of pure reference compounds permitted identification of all compounds.

Representative chromatograms of the volatiles present in several of the vinegar samples are shown in Fig. 2. Examination of the chromatograms shows qualitative differences between the vinegars made by the alcoholic and subsequent acetous fermentations (Figs. 2-A,B) as compared to the vinegars made by the acetous fermentation of dilute distilled alcohol (Figs. 2-C,D). A qualitative difference existed between the two dis-

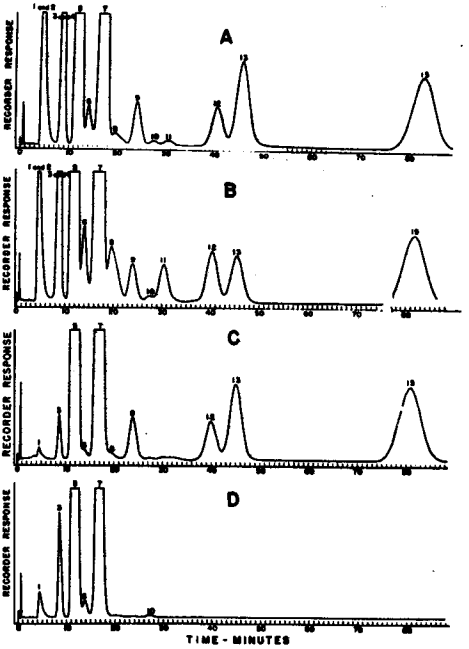

Fig. 2. Gas chromatograms of volatiles from several kinds of vinegar. Part *A*, wine vinegar; Part *B*, tarragon vinegar; Part *C*, distilled vinegar, sample No. 2; Part *D*, distilled vinegar, sample No. 1. These peaks represent: 1) acetaldehyde, 2) methyl formate, 3) acetone, 4) ethyl formate, 5) ethyl acetate, 6) diacetyl, 7) ethyl alcohol, 8) sec-butyl acetate, 9) isobutyl acetate, 10) sec-butyl alcohol, 11) propyl alcohol, 12) isobutyl alcohol, 13) isoamyl acetate, 14) n-butyl alcohol, 15) priactive amyl alcohol.

tilled vinegars (Figs. 2-C,D). This difference resulted from the acetous fermentation of an alcohol (Fig. 2-D) prepared from fermentation of a mixture of corn, barley malt, and rye (distilled vinegar No. 2), whereas the vinegar (sample 1 in Fig. 2-C) was from the acetous fermentation of an alcohol whose source was not identified on the label. Similarly, close examination of the gas chromatograms reveals that there are quantitative differences among the various samples by comparing the peak heights of some of the components.

The importance to vinegar flavor of the 25 constituents in vinegar, as listed in Table 1, will require additional quantitative data. However, studies in other fermentation areas such as dairy products, wine, and beer, indicate that the flavor characteristics of these organic compounds are very important to the acceptability of the product.

Carbonyl compounds. Five of the eight vinegar samples contained acetaldehyde, acetone, and diacetyl. Amounts of diacetyl in the distilled vinegar samples were minute as compared with the natural vinegars (cider and wine). Diacetyl is undoubtedly one of the important flavor components of vinegar. In reviews on the flavor of beer (Stevens, 1960; Lawrence, 1964), diacetyl was re-

Table 1. Volatile compounds identified in the vapor mixtures from different vinegar samples.

	Natural[a] vinegars		Distilled[b] vinegars sample no.					Special[c] "mixed" vinegar tarragon
	Cider	wine	1	2	3	4	5	
Carbonyls								
acetaldehyde	+	+	+	+	+	+	+	+
acetone	+	+	+	+	+	+	+	+
diacetyl	+	+	+	+				+
acetoin						+		+
isobutyraldehyde	+	+	+		+	+	+	+
isovaleraldehyde					+	+	+	
a-methyl valeraldehyde						+		
methyl isobutyl ketone					+	+	+	
Esters								
methyl formate	+	+						+
ethyl formate	+	+						+
ethyl acetate	+	+	+	+	+	+	+	+
ethyl propionate	+							+
isobutyl acetate	+	+		+				+
n-butyl acetate	+							+
ethyl butyrate				+				
sec-butyl acetate	+	+		+				+
isoamyl acetate	+	+		+				+
Alcohols								
ethyl alcohol	+	+	+	+	+	+	+	+
propyl alcohol	+	+						+
n-butyl alcohol	+					+		
sec-butyl alcohol	+	+	+					+
pri-active isobutyl alcohol	+	+		+				+
amyl alcohol	+	+		+				+
isoamyl alcohol	+	+						+
sec-active amyl alcohol		+						+

[a] Natural vinegars: cider (5% acetic acid); wine (6%) from California grape wine.
[b] Distilled vinegars: Sample No. 1, crystal distilled 5% acetic; No. 2, distilled white (5% acetic) and made from corn, barley and rye; No. 3, 4 and 5 distilled vinegar samples from pickle companies which manufacture their own vinegar for pickle products. Samples No. 3 and 5 made by "Frings Process" (passed through shavings) and No. 4 by "The Acetator" (submerged fermentation).
[c] Special mixed vinegar: Tarragon flavored and a mixture of distilled and malt vinegar.

ported as contributing to an objectionable off-flavor at levels of 0.5 ppm and above. In dairy products, diacetyl has been considered a desirable flavor component, with threshold levels ranging from 0.01 to 0.20 ppm (Bennett *et al.*, 1965). Diacetyl has been reported in red wine in quantities of 2–4 ppm (Prescott and Dunn, 1959). However, in the present study the possible flavor contribution of the other carbonyl compounds such as acetaldehyde, acetone, isobutyraldehyde, and acetoin has not been established. It has been suggested that acetoin may be further oxidized to diacetyl (Lawrence, 1964).

Alcohols. Seven higher alcohols were identified in addition to ethyl alcohol (Table 1). The natural fruit vinegars (cider and wine) contained six of these alcohols, and the five distilled vinegar samples contained two of these alcohols. The flavor and aroma of fruit vinegars, when compared to distilled vinegars, might be explained by the differences in their alcohol contents. Conceivably, these alcohols could form esters with the organic acids, particularly acetic acid, and thereby contribute to the aroma of the vinegars. The higher alcohols are considered the most important group of volatiles in beer flavor (Stevens, 1960; Lawrence, 1964), and they are the principal components of the fraction known as "fusel oil." The composition of fusel oil is influenced by two main factors: a) the nature of the organisms affecting fermentation; and b) the nature of the substrate. A standard fusel oil, 1 part isobutyl to 4 parts isoamyl alcohol (containing 20% of the optically active isomer), has been used for flavor studies (Hudson and Stevens, 1960). In beer, fusel oil content ranged from 39 to 323 ppm, and a sample of cider contained 98 ppm of fusel oil. In a lager beer, a panel of tasters was able to differentiate between samples containing 45 and 50 ppm of isoamyl alcohol (Hudson and Stevens, 1960). Isoamyl alcohol was identified in the natural vinegars (cider, wine and tarragon) and absent in the five distilled vinegar samples. Thus the composition and concentration of fusel oil may be partially responsible for the characteristic odor and flavor of the vinegar samples.

Esters. In the two-step fermentation (alcohol and acetic acid) of vinegars it is not surprising that esters make up the largest group of volatile flavor compounds, representing nine of the 25 identified (Table 1). Esters are known for their aromatic odors. It is plausible to assume that ethyl acetate is the predominating ester in vinegar since vinegar contains considerable amounts of acetic acid and ethyl alcohol. Also, ethyl acetate is used for denaturing industrial ethyl alcohol at the rate of 4.25 gal per 100 gal of alcohol. Suomalainen and Kangasperko (1963) showed that the ester content of a distilled vinegar (10% acid), measured as ethyl acetate, increased from 200 mg/L in a 60-day storage period to a content of 1500 mg/L and that most of the esters were formed in the first 30 days. Eight additional esters (Table 1) were identified in the volatiles from cider, wine, and tarragon vinegar samples. In four of the five distilled vinegar samples, ethyl acetate is the only ester present and may account for the contrast in odor between natural and distilled vinegars. The esters which have been identified are those of the eight alcohols and short-chain fatty acids (Table 1). They may be direct fermentation products or they may be formed during conditioning of the vinegar. The chief difference in chromatogram profiles between distilled and natural vinegars appears to be due to the higher alcohols and ester contents of the natural vinegars.

Rentschler (1942) and Jacobs (1951) reported that the presence of acetylmethylcarbinol (acetoin) can be used to distinguish a product of acetous fermentation from artificial vinegar. In the present studies, acetoin was found only in the tarragon vinegar and in distilled vinegar sample No. 4.

Toth (1941) reported that diacetyl was not detected in distilled (spirit) vinegar. In contrast, diacetyl was present in two of the five distilled vinegars used in this study.

In conclusion, it would appear that the determination of fermentation products by gas chromatography would provide an index of the genuineness of a vinegar. Similarly, it appears that the standards of identity of a particular vinegar could be determined by the relative concentration of the volatiles, especially the alcohols and esters.

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