Identification of Volatile Components in Vinegars by Gas Chromatography–Mass Spectrometry

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Sixty-one compounds, many not previously reported, were identified in six different concentrates prepared by neutralizing vinegars with magnesium oxide and extracting with ether–pentane. The compounds include 16 esters, 11 alcohols, 11 halogenated compounds, 8 hydrocarbons, 7 carbonyls, and 5 ethers and acetals. Chromatograms are presented of extracts of distilled and cider vinegars produced by the trickling and submerged culture processes, respectively. Few differences in volatile compounds were found between products of the two manufacturing processes, but large differences were noted between cider, distilled, malt, and wine vinegars.

In a previous paper (Kahn *et al.*, 1966), about 16 vinegar components were separated by gas chromatography and identified by comparing their retention times with those of pure reagents. Alcohols, acids, and esters were separated in a single run using a column packed with FFAP as the stationary phase. It was obvious that many more volatile components are present in vinegar (Yamaguchi and Arima, 1968) and that their elucidation would not be possible without a prior concentration procedure. The high proportions of acetic acid and water relative to the amounts of other components in vinegar interfere with the separation of these components, present only in trace quantities. Elimination of the acetic acid and water would facilitate the concentration of the aroma components.

Suomalainen and Kangasperko (1963), using ether–pentane extracts of wine and fruit vinegars, showed the presence of approximately ten compounds. Jones and Greenshields (1969, 1970) employed a preliminary treatment with sodium hydroxide solution to neutralize the acid in vinegar, followed by either a potassium permanganate treatment to remove aldehydes or a strong sodium hydroxide treatment to remove esters. Approximately 13 compounds were identified by these means.

This paper reports the separation and identification of 61 compounds from concentrates prepared by neutralizing and extracting six different vinegars.

EXPERIMENTAL

The use of sodium hydroxide for the neutralization of the acids was rejected because of possible saponification of the esters (Aurand *et al.*, 1966). Sodium bicarbonate and sodium carbonate solutions were found to be good neutralizing agents but it was believed that many low-boiling components would be lost by entrainment with the evolution of released carbon dioxide. It was found that by adding solid magnesium oxide to vinegar, the acids could be neutralized successfully without carbon dioxide evolution and, hopefully, without appreciable loss of low-boiling compounds.

The neutralized material then was filtered to remove precipitates of magnesium salts and extracted with ether–pentane according to the procedure employed earlier in the identification of whiskey components (Kahn *et al.*, 1968, 1969). Specifically, a 200-ml aliquot of vinegar was neutralized to approximately pH 7–8, with solid magnesium oxide employing a pH meter and a magnetic stirrer. After removing the precipitate by filtration, the clear filtrate was extracted with two 40-ml portions of 2:1 ether–pentane solution, and the extract was dried over anhydrous sodium sulfate. The solvents were removed with the use of a rotary evaporator, leaving less than 0.5 ml of an oily residue in the vial. 2-μl aliquots were taken for analysis.

EQUIPMENT

For the preliminary separation of components, a 10-ft long column of $^1/_8$-in. o.d. stainless steel packed with 30% Igepal CO-880 on 100/120 mesh firebrick was used in a Varian Aerograph Model 1740 gas chromatograph equipped with a hydrogen flame detector.

Operating conditions were: helium carrier gas flow, 25 ml per min; injector and detector heaters, 250°C, column oven temperature programmed from 60°C at 4°C per min to 180°C, and then held isothermally; the 1 mV–1 sec Westronics recorder was operated at a speed of 24 in. per hr.

Identification of compounds by coupled gc–mass spectrometry involved the use of the same column (and a SE-30 column for reference) but employing an F&M Model 700 gas chromatograph with WX filaments (operated at 190 mA) connected to an Atlas CH-4 mass spectrometer equipped with an EC-1 splitting valve for monitoring the chromatographic effluent. Fast-scan spectra were obtained using a Honeywell oscillographic recorder. The column oven was temperature programmed from 45°C at 4°C/min to 180°C to obtain resolution and peak shapes more conducive to identification by the mass spectrometer.

SAMPLES

Samples of commercial distilled and cider vinegars from trickling and submerged culture generators were analyzed, as well as samples of commercial domestic malt and imported red wine vinegars. The acidity and geographical source of each sample is shown in Table I.

RESULTS AND DISCUSSION

Figures 1 and 2 show chromatograms of the distilled (trickling) and cider (submerged culture) vinegar extracts, respectively, using the Varian instrument with flame detector. Because of space limitations, chromatograms are presented only for the two vinegars of major importance, distilled (trickling) and cider (submerged culture).

All compounds identified in the various samples are shown

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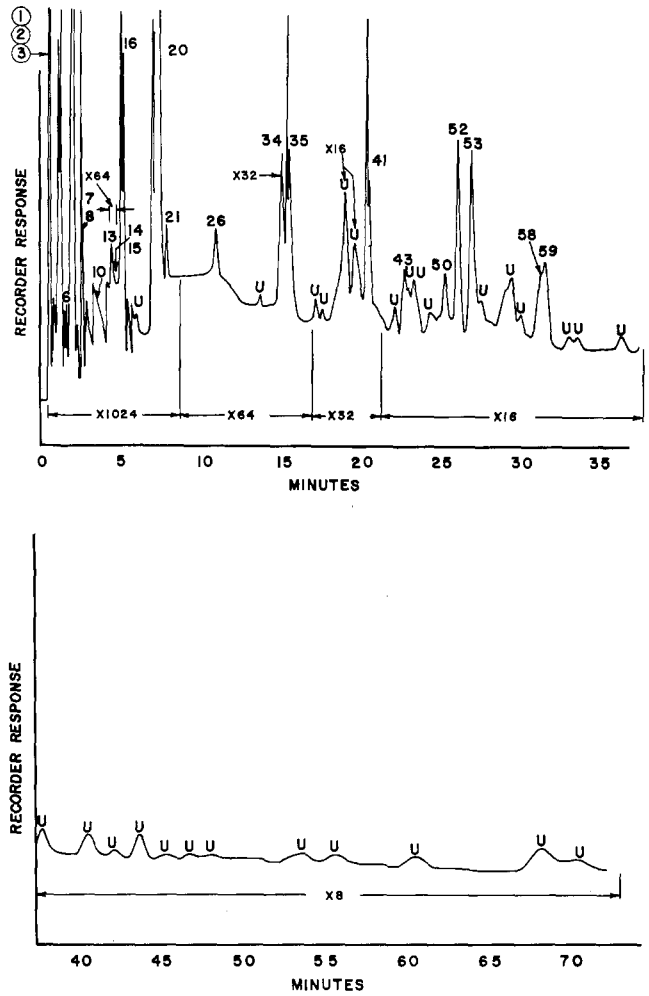


Figure 1. Chromatogram of extract of distilled vinegar (trickling)

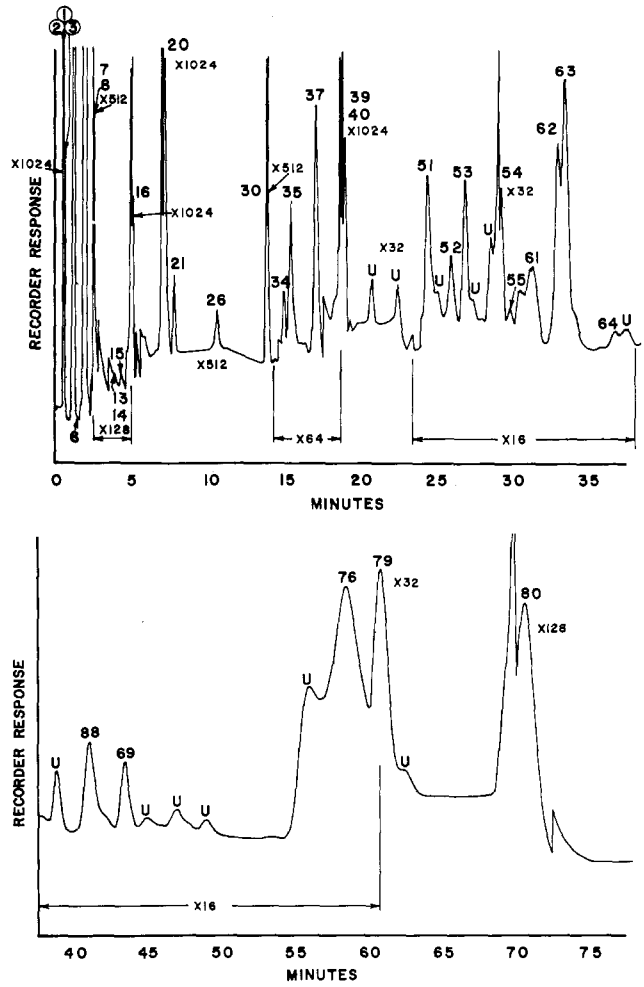


Figure 2. Chromatogram of extract of cider vinegar (submerged culture)

in Table II and are listed in the order of increasing retention times. The identities of the compounds were confirmed by the comparison of their mass spectra and glc retention times with those of authentic compounds. The chromatograms show many relatively large and small peaks which could not be identified because of the lack of necessary reference mass spectra or because insufficient quantities were present. Some compounds sensed by the flame detector were not "seen" by the filament detector of the glc–ms equipment and hence could not be identified. Several compounds which are known to be present in vinegars have not been detected in this study because they probably have been lost or changed chemically by the sample preparation treatments such as neutralization, extraction, or solvent evaporation. These include low-boiling compounds, some alcohols, and other hydroxy compounds. Conversely, it is feasible that a few compounds may have been formed during the sample preparation procedure.

The relative heights of the peaks on the chromatograms are indicated in this table by the number of + marks. It must be stressed that there may be no direct correlation between the size of a peak on the chromatogram from an extract and the actual concentration of the component, represented by the peak, in the untreated vinegars. Peak size is affected not only by the extractability or preferential solubility of each compound in the extracting solvents, but also by the compounds' glc responses, which are known to vary according to chemical structure, number, and size of side chains and chemical classes.

Many of the compounds listed in Table II have not been reported previously in vinegars, to the best of our knowledge. These compounds include 16 esters, 11 alcohols, 8 hydrocarbons, 7 carbonyls, and 5 ethers and acetals which have been positively identified. Of considerable interest are the 11 confirmed halogenated compounds, especially the bromo compounds. Their origin in the samples remains the subject of much speculation at this time.

No halides were detected in the ether and pentane solvents and only methyl chloride and a trace of methyl bromide were found to originate in the magnesium oxide reagent (Matheson Coleman and Bell; stated chloride analysis

Table I. Vinegars Studied

	% acid, as acetic	Geographical source
Distilled, trickling process	12.6	Midwest[a]
Cider, trickling process	5.0	New England
Distilled, submerged culture	11.2	Texas
Cider, submerged culture	5.8	Midwest[a]
Domestic malt[b]	4.0	Unknown
Imported red wine[b]	5.6	Italy

[a] The samples came from plants in different areas of the Midwest. [b] Manufacturing process unknown.

Table II. Compounds Identified in Various Vinegars

No.		Distilled (submerged)	Distilled (trickling)	Cider (trickling)	Cider (submerged)	Malt (domestic)	Wine (imported)
1	Carbon monoxide	+++	+++	+++	+++		
2	Methane	+++	+++	+++	+++		
3	Carbon dioxide	+++	+++	+++	+++	++++	++++
4	Ethane					++++	++++
5	Dimethyl ether			+			
6	Chloromethane	++	++	++	++	++	++
7	Acetaldehyde	++++	++++	++++	++++	+++	++++
8	Bromomethane	++	++	++	++	++	++
9	Ethyl isopropyl ether (tent.)					++	++
10	Methyl formate	++	+				+
11	Bromoethane					++	+
12	Ethyl *sec*-butyl ether					++	+
13	Propionaldehyde	++	+	++	++	+	+
14	Acetone		+		++	++	++
15	Iodomethane	+	+	+	+	+	+
16	Ethyl formate	++++	++++	++++	++++	++++	++++
17	1,1-Methoxyethoxyethane	++					
18	1-Bromopropane					+	+
19	Methanol	++					
20	Ethyl acetate	++++	++++	++++	++++	++++	++++
21	Ethanol	++++	+++	++++	+++	+++	+++
22	Acetal	++		++			
23	Possible C_8 aliphatic H.C., mol wt 114					+	
24	Isovaleraldehyde					+	+++
25	Isobutyl formate					++	++
26	2-Pentanone	+++	++	+++	++	++	++
27	Possible Br cpd, mol wt 130					++	
28	2-Methyl-3-buten-2-ol					++	
29	Isobutyl acetate					+++	+++
30	Isobutyl alcohol			++++	++++	++++	++++
31	Toluene					+	+
32	Isopentyl formate					+++	++
33	2,2-Dimethyl-1-propanol					++	+
34	3-Pentanol	+++	+++	+++	+++		
35	2-Pentanol	+++	+++	++++	+++	++	+
36	1,1-Dibromoethane					+	
37	Isopentyl acetate			+	+++	+++	++++
38	Ethylbenzene						+
39	2-Methylbutanol	++		++++	++++	++++	++++
40	Isopentyl alcohol	++		++++	++++	++++	++++
41	2-Bromo-4-chloropentane (tent.)	++++	+++			+++	
42	Br–Cl cpd					++	
43	Dodecane		+				
44	Branched alcohol (tert or sec)	++++					
45	Styrene					+	+
46	Br cpd, mol wt 122	++				+	
47	Br cpd, mol wt 136	++				+	
48	Br cpd	++				+	
49	Br cpd					++	
50	Br cpd		+				
51	Ethyl lactate			++	++		
52	Bromoacetaldehyde diethyl acetal	++++	++	++	++	++	++
53	Tridecane		++	++	++	++	++
54	An acetate ester			++	+++	+++	+++
55	Oxygen + Br cpd	++			+		
56	A hydrocarbon					++	
57	Unknown cpd, mol wt 186			+			
58	1,1,2,2-Tetrachloroethane		+				
59	Tetradecane		+				
60	An ethyl ester					++	++++
61	Br cpd				+		
62	Oxygen cpd possible acetate			++	++		
63	Benzaldehyde			+++	+++	+++	+++
64	Br cpd				+		
65	γ-Butyrolactone						+++
66	An acetate					++	+++
67	Methyl benzoate						++
68	Phenylacetaldehyde			++	++	++	++
69	Diethyl succinate			++	++	++	++
70	An acetate						++
71	An ethyl ester, mol wt 108			+			
72	Ethyl benzoate			+		+	+

Table II. *(Continued)*

No.		Distilled (submerged)	Distilled (trickling)	Cider (trickling)	Cider (submerged)	Malt (domestic)	Wine (imported)
73	1,2,4-Trichlorobenzene					+	+
74	Benzyl acetate						++
75	An alcohol (isomeric to No. 76)			++			++++
76	*o*-Methyl benzyl alcohol			++	+++		
77	β-Phenethyl bromide					+	
78	β-Phenethyl formate					+++	
79	β-Phenethyl acetate			+++	+++	++++	++++
80	β-Phenethyl alcohol			++++	++++	++++	++++
81	Isopentyl benzoate					+	++
82	Diethyl phthalate						+

Size of peak: + = trace; ++ = small peak; +++ = moderate peak; ++++ = major peak.

0.010%) when the latter was employed in a blank neutralization of synthetic acetic acid containing added methyl, ethyl, and propyl alcohols, as well as acetaldehyde.

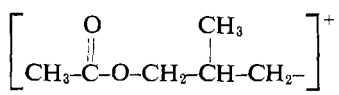
Bromine and other halogens in undetermined form are normal constituents of all plant tissues. Guyot and Balatre (1966) determined the total bromine content of cider and wines, while several workers found halogens in wines from various areas (Jaulmes *et al.*, 1962; Vassiliou and Danilatos, 1965; Brochon, 1967; Martin and Vayreda, 1967). Limits for the bromine content in grains used in the production of fermented malt beverages have been set by the U.S. Government (Federal Register, 1967). Presumably, some of the halo compounds found in the cider, malt, and wine vinegars may be traced back to the halogens known to be present in their respective starting materials. No appreciable amounts of halogen compounds in apples have been found and reported according to information obtained from the U.S. Department of Agriculture (Teranishi, 1970). The derivation of these compounds in distilled vinegars is unknown at this time; however, a possible contamination from municipal water systems cannot be ruled out.

It is a possibility that unknown bromo compounds in vinegars or their raw materials are converted to bromoacetaldehyde or its diethyl acetal under the oxidizing conditions of the generators. The latter compound, represented by peak 52, has been identified in all of the samples in appreciable concentration. This is a reasonable speculation as to the origin of the bromoacetaldehyde diethyl acetal, but sheds no light on the nature of the bromo compounds present originally.

Peak 41 probably represents an aliphatic C_5 hydrocarbon containing one atom each of bromine and chlorine. Judging from its mass spectrum, the best fit is 2-bromo-4-chloropentane, but this interpretation is very tentative. An isomer with the halogens in other than the 1,5 positions is also possible. Further information on this compound cannot be obtained because no reference mass spectra are available. It is known that the structure of the compound in question is not the 1,5 isomer because the latter's retention time is much longer than that of the former. Many of the other halo compounds detected could not be identified because of a lack of reference mass spectra.

The occurrence of higher hydrocarbons as well as several aromatic compounds was totally unexpected. Peak 44 represents a highly branched secondary or tertiary alcohol consisting of at least 7 or 8 carbon atoms for which no absolute identification was possible. The presence of an unsaturated C_5 alcohol (peak 28) is worth mentioning.

The mass spectrum of peak 54 was interpreted as representing an acetate ester of unknown structure, a basic part of which is thought to be



The length of the chain cannot be determined since the mass spectrum does not give a molecular weight peak. Similar difficulties exist with peak nos. 60, 66, 70, and 71, which represent either ethyl or acetate esters of unknown structure.

The mass spectrum of peak 75 suggests an isomer of, or a compound very similar to *o*-methyl benzyl alcohol; however, the peak probably does not represent the meta or para isomers, and certainly not *sec*-phenethyl alcohol, even though these compounds' retention times match quite well with that of the unknown compound.

Various processes and types of vinegars were evaluated in order to determine if any differences exist in their volatile aroma constituents. However, the compositions of the various vinegars are somewhat different, as would be expected.

Naturally, the distilled vinegar extracts produce much less complicated chromatograms than those of the cider, malt, or wine vinegars. There appear to be very few differences between distilled vinegars produced by the submerged culture and trickling processes; one major difference is the branched alcohol (peak 44) detected only in the distilled vinegar produced by the submerged culture process. Since only one vinegar from each process was analyzed, the finding of the branched alcohol cannot be taken as a reliable indication of a difference resulting from the two processes.

Minor differences were found also between cider vinegars produced by both processes. The higher fusel alcohols were more prevalent in both ciders and in the malt and wine vinegars than in the distilled vinegar.

The two latter products yielded less complex chromatograms than the other four samples. It should be pointed out that the three halomethanes (peaks 6, 8, 15) and bromoacetaldehyde diethyl acetal (peak 52) are the only halo compounds which have been definitely identified in all six samples.

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The Influence of Water Content and Water Activity on the Sugar–Amino Browning Reaction in Model Systems Under Various Conditions

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Nonenzymatic browning due to the Maillard reaction between reducing sugars and glycine was studied in systems containing varying amounts of water, glycerol, and hydrophilic polymers. We observed that the browning rate decreased with increasing water content, except in systems in which mobility of reactants became substantially impeded at low water contents. The effect of water was complex and depended on the presence of various water-binding agents among other factors. The inhibitory effect of high water contents could be due to the fact that water is a product of several condensation steps in the browning reaction.

Sugar–amino browning reactions in foods and model systems of low moisture content occur over a wide range of water activities (Karel, 1960; Heiss, 1968). A maximum browning reaction occurs in most foods between water activities (a_w) 0.3 and 0.7. The position of this maximum depends on the type of food. Therefore, water activity, though it reflects the effect of water being bound to specific polar groups in the food and other factors limiting the availability of water molecules for chemical reaction, cannot be used to predict optimum browning conditions. Furthermore, in some studies the browning rate increased with increasing water *without a maximum* (Jones, 1954, 1956), and in others increased with *decreasing* moisture content (Rosen *et al.*, 1953; Loncin *et al.*, 1965).

Water's influence on the rate of the sugar–amino browning reaction in a food system is unclear. At higher water activities the reaction rate decrease has generally been attributed to dilution of the reaction partners. The decreased reaction rate at low water activities when the amount of mobile water lowers has been ascribed to an *increasing diffusion resistance* which lowers the mobility of the reaction partners (Labuza *et al.*, 1970).

We performed this study to elucidate the influence of water content, water activity, dilution of the reactants, and of viscosity on the browning rate of reducing sugars and amino acids. By varying the glycerol content in a sugar–amino acid–glycerol–water system, we changed the water content of this system while maintaining a constant water activity and *vice versa*. In this way we investigated the action of water content and water activity on the browning rate separately. We demonstrated the influence of dilution of the reactants by adding increasing amounts of glycerol to the above mentioned system. In order to show the influence of the diffusion resistance on the browning rate, we increased the viscosity of the system by adding water-soluble polymers and lowering the water activity. The viscosity was decreased by the plasticizing effect of glycerol.

MODEL SYSTEMS

The model systems studied contained glucose (glucose and fructose) and glycine as the reactants, as well as additives allowing water activity and water content control.

Materials. Microcrystalline cellulose (Avicel-PH-105), FMC Corp. Methylcellulose (Methocel), visc. 4000 cps, Type MC, Dow Chemical Co. Gum arabic (Hallmark), Stein, Hall & Co., Inc. Polyvinyl alcohol (Elvanol), grade 50-42, and grade 51-05, E. I. du Pont de Nemours & Co., Inc.

Preparation of Sugar–Amino Acid Solutions. 1. 10 g (55.5 mmol) of glucose were dissolved in: a. 10 ml of water; b. 10 ml of a glycerol–water mixture containing 20% glycerol (w/w); c. 10 ml of a glycerol–water mixture containing 40% glycerol (w/w); d. 10 ml of a glycerol–water mixture containing 60% glycerol (w/w); and e. 10 ml of a glycerol–water mixture containing 80% glycerol (w/w). In each solution 2.085 g (27.8 mmol) of glycine were dissolved; in solution e, part of the glycine remained undissolved.

2. Same as solutions 1a–1e, only 1.043 g (13.9 mmol) of glycine were used.

3. Same as solutions 2a–2e, only 5 g of glucose plus 5 g of fructose were used instead of 10 g of glucose.

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