

daKoven for valuable observations.

LITERATURE CITED

- Amerine, M. A., *Adv. Food Res.* **5**, 353 (1954).
 Brander, C. F., *Am. J. Enol. Viticult.* **25**, 13 (1974).
 Cover, T. M., Hart, P. E., *IEEE Trans. Inform. Theory*, **21** (1967).
 Harper, A. M., Duewer, D. L., Kowalski, B. R., Fasching, J. L., "ARTHUR and Experimental Data Analysis: The Heuristic Use of a Polyalgorithm", ACS Symposium Ser. No. 52, American Chemical Society, Washington, DC, 1977.
 Kowalski, B. R., Bender, C. F., *J. Am. Chem. Soc.* **94**, 5632 (1972).
 Kowalski, B. R., Bender, C. F., *J. Pattern Recogn.* **8**, 1 (1976).
 Kwan, W. O., Kowalski, B. R., *J. Food Sci.* **43**, 1320 (1978).
 Kwan, W. O., Kowalski, B. R., Skogerboe, R. K., *J. Agric. Food Chem.* **27**, 1321 (1979).

- Sakato, K. H., Hoekman, M., Kepner, R. E., Webb, A. D., Muller, C. J., *Am. J. Enol. Viticult.* **26**, 70 (1975).
 Stern, D. J., Lee, A., McFadden, W. H., Stevens, K. L., *J. Agric. Food Chem.* **15**, 1100 (1967).
 Van Wyk, C. J., Webb, A. D., Kepner, R. E., *J. Food Sci.* **32**, 660 (1967).
 Webb, A. D., Kepner, R. E., Maggiora, L., *Am. J. Enol. Viticult.* **18**, 190 (1967).
 Webb, A. D., Kepner, R. E., Maggiora, L., *Am. J. Enol. Viticult.* **20**, 16 (1969).
 Webb, A. D., Muller, C. J., *Adv. Appl. Microbiol.* **15**, 75 (1972).
 Webb, A. D., Noble, A. C., *Biotech. Bioeng.* **18**, 939 (1976).
 Wold, S., *J. Pattern Recogn.* **8**, 127 (1976).

Received for review June 18, 1979. Accepted October 9, 1979.

Determination of Thiolacetates and Some Other Volatile Sulfur Compounds in Alcoholic Beverages

Olavi A. Leppänen,* John Denslow, and Pentti P. Ronkainen

Methyl and ethyl thiolacetates (*S*-methyl and *S*-ethyl esters of ethanethioic acid) were identified and determined for the first time in beers and wines. The average content of methyl thiolacetate was 17 $\mu\text{g/L}$ in Finnish medium strength beer and 25 $\mu\text{g/L}$ in Finnish strong beer. The respective average contents for white and red wines were 7 and 11 $\mu\text{g/L}$. In most samples the concentration of ethyl thiolacetate was about 10% that of methyl thiolacetate. The strong beers contained significantly more methyl and ethyl thiolacetates than the medium beers from the same brewery, but the content of methyl sulfide was not significantly different. Carbon disulfide was identified for the first time in wines, in concentrations of up to 10 $\mu\text{g/L}$. The contents of thiolacetates were quantified down to 1 $\mu\text{g/L}$ by adsorbing the head-space volatiles of samples on porous polymer. Because thiolacetates can be hydrolyzed to give free thiols, these esters are a potential source of off-flavors in alcoholic beverages despite their own high sensory thresholds.

Direct head-space methods are rapid and simple but are often not sensitive enough for the analysis of low concentrations of sulfur compounds in alcoholic beverages. The methyl sulfide in beer samples has been determined from the head-space down to 2–10 $\mu\text{g/L}$ (Jansen et al., 1971; Anderson and Howard, 1974; Takahashi et al., 1978), but the concentrations of other sulfur compounds in the head-space of beers and wines are commonly near or below the detection limits of the flame photometric detector.

Liquid-liquid extraction has been used to concentrate the less volatile sulfur compounds, notably 3-(methylthio)-1-propanol and 3-(methylthio)propyl acetate, in beers and wines (Schreier et al., 1974a,b; Nakajima and Narziss, 1978).

Pickett et al. (1976) developed a method of trapping head-space volatiles at low temperatures (below -46°C) under reduced pressure. With this method they identified some volatile sulfur compounds in British lagers and ales and determined the methyl sulfide contents.

Schreier et al. (1976) used on-line enrichment to concentrate the more volatile sulfur compounds of beer. The method involves sweeping the volatiles from the sample in a stream of nitrogen and condensing them in a cold trap connected directly to the chromatographic column. They determined the contents of methyl sulfide and disulfide

in several types of beer and found no characteristic differences between them.

Activated carbon (Clark and Cronin, 1975) and porous polymers (Jennings et al., 1972; Bertuccioli and Montedoro, 1974; Williams et al., 1978) have proved suitable for concentrating the volatiles from foodstuffs and alcoholic beverages. An advantage of the polymers is their relative indifference to polar compounds; they selectively adsorb relatively more of the less polar compounds than the more abundant water and alcohols in the head-space of alcoholic beverages.

We here report the content of volatile sulfur compounds, some of which have not previously been identified, in various beers and wines. The volatiles are first adsorbed from the head-space in a small tube of Chromosorb 101 and then desorbed directly onto the chromatographic column.

EXPERIMENTAL SECTION

Apparatus and Materials. A Varian 1400 gas chromatograph fitted with a flame photometric detector (Melpar Inc.) was used for analysis. It was connected to a Hewlett-Packard 3352B laboratory computer, which integrated and named the peaks. A 4 m \times 2 mm FEP (fluorinated ethylene propylene copolymer) column packed with 12% DC-200 on Chromosorb W (AWS, 60–80 mesh) was used for the quantitative work. The nitrogen carrier gas flow was 15 mL/min and the oven temperature was programmed from 40 to 150 $^\circ\text{C}$ at 8 $^\circ\text{C}/\text{min}$. The 120 \times

Research Laboratories of the State Alcohol Monopoly (Alko), Box 350, SF-00101 Helsinki 10, Finland.

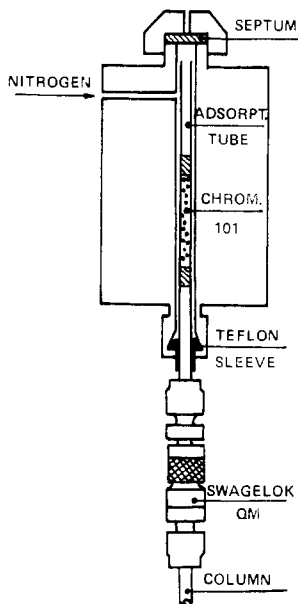


Figure 1. The injection block modified to receive the adsorption tube.

2 mm PTFE adsorption tube used to concentrate the volatiles contained 20 mg of Chromosorb 101.

The mass spectra were run on a JEOL JMS-D 100 combined GC-MS at the Technical Research Center of Finland (Otaniemi, Finland).

The following reference compounds were used. Methyl sulfide (Sigma Chemical Co., St. Louis, MO), methyl ethyl sulfide (Pract., Fluka AG., Busch, Switzerland), methyl disulfide (Pract., Fluka AG.), and carbon disulfide (PA, E. Merck, Darmstadt, West Germany). Methyl and ethyl thiolacetates (*S*-methyl and *S*-ethyl esters of ethanethioic acid) were synthesized by the anhydride method of Wenzel and Reid (1937).

Methods. Methyl ethyl sulfide (2 μg), an internal standard with a volatility close to that of methyl sulfide, was added to each sample (200 mL in a 350-mL sample bottle). Wines were first neutralized to pH 6.0 with sodium hydroxide to bind free sulfur dioxide, and beers received a drop of Antifoam RD (Dow-Corning) to prevent foaming. A nitrogen gas stream (50 mL/min) was bubbled through the sample held at 20 °C to carry the volatiles to an adsorption tube kept at 20 °C. After a collection time of 10 min, the tube was connected to the chromatographic column with a Swagelok QM Quick-connect and pushed directly into the injection block (200 °C) from the oven side (Figure 1). The block was fitted with a PTFE sleeve to form a gas-tight joint and was already pressurized with carrier gas when the adsorption tube was slid in. The trapped volatiles were desorbed into the hot carrier gas and so carried directly to the column. When the analysis was complete, the laboratory computer integrated and named the peaks of interest. The total analysis time for each sample was 25 min and the coefficient of variation for replicated analyses was 5%. The detection limits for a 10-min collection from beer samples at 20 °C were 2 $\mu\text{g}/\text{L}$ for methyl sulfide (Me_2S), 0.1 $\mu\text{g}/\text{L}$ for methyl disulfide (Me_2S_2), 1 $\mu\text{g}/\text{L}$ for methyl and ethyl thiolacetates (MeSAC and EtSAC), and 0.5 $\mu\text{g}/\text{L}$ for carbon disulfide (CS_2).

Calibration curves were obtained by adding internal standard and known amounts of authentic compounds to samples of beer and wine. Approximately linear curves were obtained by plotting concentration against (peak area/internal standard area) to the power 0.6. The curves were linearized so as to facilitate extrapolation to lower

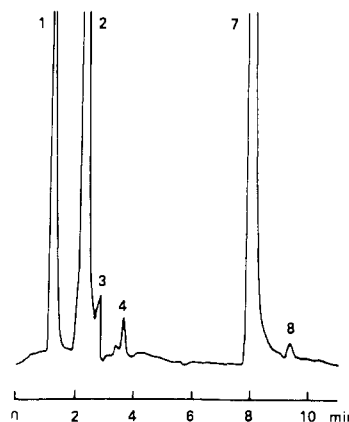


Figure 2. Chromatogram of volatile sulfur compounds from synthetic medium metabolized by *Saccharomyces cerevisiae*; DC-200 column: (1) hydrogen sulfide, (2) methanethiol, (3) ethanol, (4) methyl sulfide, (7) methyl thiolacetate, (8) methyl disulfide.

concentrations. This was satisfactorily done by plotting concentration against (peak area/internal standard area) to the power 0.6, an exponent that was found to give a closer approximation to linearity with these compounds and this system than the theoretically more firmly based square root.

To help in the identification of unknown volatile sulfur compounds in beers and wines, a synthetic medium was prepared containing glucose (10 g/L) and methionine (20 mg/L) and buffered to pH 4.7 with potassium dihydrogen phosphate (3 g/L). This medium was incubated in semi-aerobic conditions with a *Saccharomyces cerevisiae* strain of commercial baker's yeast for 65 h at 30 °C with magnetic stirring. The head-space volatiles from the metabolized medium were swept out in a stream of nitrogen and adsorbed on a Chromosorb 101 trap (500 \times 6 mm glass tube). They were desorbed from the porous polymer at 170 °C into a slow stream of nitrogen and condensed in a glass capillary immersed in liquid nitrogen. The condensate was analyzed by combined GC-MS.

The identification of volatile sulfur compounds in beer and wine samples was supported by comparing retention times with those of authentic compounds on nonpolar DC-200 (4 m, 12% on Chromosorb W), polar Triton X-305 (6 m, 10% on Chromosorb W), the porous polymer Chromosorb 101 (1 m), and a 23-m SCOT column coated with SP 2100.

RESULTS AND DISCUSSION

There are only a limited number of volatile sulfur compounds that are stable in alcoholic beverages, and, to support the chromatographic evidence, chemical characterization can eliminate many components. For example, thiols are bound by heavy metal salts, esters can be hydrolyzed, compounds containing carbonyl functions are bound by dinitrophenylhydrazine, thiazoles cause strongly tailing peaks, etc.

Many of the volatile sulfur compounds in fermentation products ultimately derive from the *S*-containing amino acids in the proteins of the raw materials. Preliminary experiments showed that from a medium consisting of glucose, methionine, and water only, baker's yeast could produce a component identical with an unidentified sulfur compound present in the head-space of beers and wines. The dominant volatile sulfur compounds from the metabolized methionine were hydrogen sulfide, methanethiol, and methyl thiolacetate, with methyl sulfide and methyl disulfide present at lower concentrations (Figure 2).

Table I. Volatile Sulfur Compounds ($\mu\text{g/L}$) in Finnish Beers

brewery	n^a	Me_2S	Me_2S_2	MeSAc	EtSAc
A					
medium ^c	4	93	0.8	22	2.4
strong ^d	2	92	0.8	33	2.6
B					
medium	5	56	0.3	16	3.3
strong	3	63	0.4	24	4.1
C					
medium	3	57	0.2	5	<i>b</i>
strong	4	63	0.3	9	0.7
D					
medium	3	48	0.4	13	1.2
strong	3	54	0.5	27	2.4
E					
medium	1	95	0.3	14	1.8
strong	2	84	0.5	27	2.2
F					
medium	4	63	0.3	13	1.2
strong	2	63	0.3	18	1.3
G					
medium	3	54	0.8	20	1.9
strong	3	50	0.8	28	2.8
H					
medium	5	65	0.5	27	2.1
strong	5	65	0.5	31	3.3
I					
medium	4	71	0.3	23	2.7
strong	1	58	0.4	20	1.6
J					
medium	4	114	0.4	18	2.0
strong	2	67	0.4	26	2.8
K					
medium	3	65	0.2	17	1.2
strong	3	66	0.5	27	3.2
average					
medium		71	0.4	17	1.8
strong		66	0.5	25	2.5

^a Number of samples analyzed. ^b Trace. ^c 10.5% original extract. ^d 12% original extract.

Methyl thiolacetate was identified in the head-space condensate by its mass spectrum: m/e (rel intensity) 92 (3), 91 (3), 90 (28), 75 (2), 48 (5), 47 (11), 46 (5), 45 (15), 43 (100), 42 (9), and 15 (15). The identity of EtSAc was strongly supported when it was noted that both it and MeSAc disappeared from the head-space of alkaline samples at closely similar rates, presumably because of hydrolysis. The internal standard and the other sulfides did not fall off in this way.

Methyl thiolacetate has been identified before as a metabolite of microorganisms, in spoiled poultry (Freeman et al., 1976), but not previously in beverages. Thiolacetates are quite stable esters, the rate of acid-catalyzed hydrolysis of ethyl thiolacetate in water-acetone being only about one-thirtieth that of ethyl acetate (Schaeffgen, 1948). Nevertheless, because thiolacetates can be hydrolyzed to free thiols and acetic acid at low pH, these esters are potential precursors of off-flavors in alcoholic beverages despite their high sensory thresholds.

Wainwright (1972) has shown that, in addition to hydrogen sulfide, a large amount of thiol is formed by *Saccharomyces cerevisiae* fermenting a synthetic medium containing methionine. The thiols were detected by sweeping the head-space from the acidified medium at 70 °C for 2 h into a mercuric acetate trap. In these conditions part of the thiol detected could clearly derive from hydrolysis of thiolacetate.

It seems reasonable to postulate that thiol esters are formed in a way analogous to the formation of ordinary oxygen esters but with thiol replacing alcohol. However, it is clear that additional work is necessary to investigate

Table II. Volatile Sulfur Compounds ($\mu\text{g/L}$) in White and Rose Wines

wines	Me_2S	Me_2S_2	MeSAc	EtSAc	CS_2
white					
Austrian					
brand 1	44	0.3	6	1	0.8
brand 2	26	+ ^a	6	1	1.2
Bulgarian	5	+	9	2	0.8
French	6	0.1	7	1	2.6
Finnish ^c	7	+	13	1	0.7
German					
brand 1	9	+	4	+	1.4
brand 2	21	+	6	1	10
Hungarian					
brand 1	15	1.1	5	- ^b	1.2
brand 2	36	0.5	2	-	0.5
Italian	34	+	9	+	6.5
Jugoslav	6	+	7	2	+
Turkish	5	+	6	+	1.1
rose					
French	20	+	10	+	1.4
Finnish ^c	7	+	15	+	1.1
Hungarian	5	0.5	11	+	0.7

^a Trace. ^b Not detected. ^c Finnish white and rose wines are made from cultivated berries.

Table III. Volatile Sulfur Compounds ($\mu\text{g/L}$) in Red Wines

red wines	Me_2S	Me_2S_2	MeSAc	EtSAc	CS_2
Algerian	10	0.3	14	+ ^a	+
Bulgarian	28	0.5	14	4	0.9
French					
Bordeaux	28	0.5	11	3	1.1
Burgogne	24	0.4	9	2	2.0
Rhône	21	0.6	13	4	1.2
Midi	18	0.4	9	1	2.1
Finnish ^b	13	0.3	16	2	1.0
Hungarian	10	1.6	8	+	1.3
Italian	12	0.3	10	+	0.8
Jugoslav	16	0.5	9	3	1.0
Spanish	32	0.5	7	+	1.1

^a Trace. ^b Finnish red wine is made from cultivated berries.

the origin and behavior of thiolacetates in alcoholic beverages and to decide whether they are, in fact, of importance in the generation of off-flavors, either as esters or after transformation to the much more readily noticeable thiols.

Beer Samples. Medium strength beer (10.5% original extract) and strong beer (12% original extract) taken from 11 Finnish breweries 2–3 weeks after bottling were compared by analyzing the volatile sulfur compounds in an average of three samples of each class from different months. The 39 samples of Finnish medium beer contained on average 71 $\mu\text{g/L}$ of Me_2S , 0.4 $\mu\text{g/L}$ of Me_2S_2 , 17 $\mu\text{g/L}$ of MeSAc , and 1.8 $\mu\text{g/L}$ of EtSAc . The corresponding values for the 30 samples of Finnish strong beer were 66, 0.5, 25, and 2.5 $\mu\text{g/L}$ (Table I and Figure 3). Strong beer contained significantly more (matched pair *T* test, $p < 0.05$) of both thiolacetates than medium beer from the same brewery. The concentration of ethyl thiolacetate was roughly 10% that of methyl thiolacetate. There was no statistically significant difference in the content of methyl sulfide between the two classes of beer, neither when matched by brewery nor when pooled.

Wine Samples. The wines analyzed were imported to Finland in bulks of 20 000–200 000 L. The samples were taken soon after bottling from a single bulk so that they truly represent the average of many thousands of liters.

The average contents of Me_2S in the white and red wines examined were, respectively, 18 and 19 $\mu\text{g/L}$. The corre-

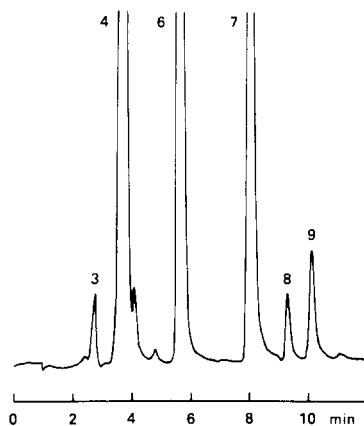


Figure 3. Chromatogram of volatile sulfur compounds from Finnish beer; DC-200 column: (6) methyl ethyl sulfide (internal standard), (9) ethyl thiolacetate, other peaks as in Figure 1.

sponding values for Me_2S_2 were less than 0.1 and 0.4 $\mu\text{g}/\text{L}$ (Tables II and III). The Hungarian wines differed from the others in that their Me_2S_2 content was clearly higher. The average contents of methyl thiolacetate were, respectively, 6.7 and 11 $\mu\text{g}/\text{L}$ in white and red wines. The content of carbon disulfide varied between a trace and 10 $\mu\text{g}/\text{L}$. Carbon disulfide has previously been tentatively identified in raw grain spirit (Ronkainen et al., 1973). The separation of Me_2S and CS_2 was difficult and accomplished readily only on nonpolar columns.

It is clear that methyl thiolacetate is a normal metabolite formed by the action of yeast on methionine. Methyl and ethyl thiolacetates are commonly present in beers and wines and, together with other volatile sulfur compounds, can be accurately determined routinely by concentrating

the volatiles on a porous polymer trap before GC analysis. The content of thiols in beers and wines is normally very low, but the apparently ubiquitous thiolacetates form a potential source of thiols in these beverages.

LITERATURE CITED

- Anderson, R. J., Howard, G. J., *J. Inst. Brew., London* **80**, 357 (1974).
- Bertuccioli, M., Montedoro, G., *J. Sci. Food Agric.* **25**, 675 (1974).
- Clark, R. G., Cronin, D. A., *J. Sci. Food Agric.* **26**, 1616 (1975).
- Freeman, L. R., Silverman, G. J., Angelini, P., Merritt, C., Jr., Esselen, W. B., *Appl. Environ. Microbiol.* **32**, 222 (1976).
- Jansen, H. E., Strating, J., Westra, W. M., *J. Inst. Brew., London* **77**, 154 (1971).
- Jennings, W. G., Wohleb, R., Lewis, M. J., *J. Food Sci.* **37**, 69 (1972).
- Nakajimi, S., Narziss, L., *Brauwissenschaft* **31**, 254 (1978).
- Pickett, J. A., Coates, J., Peppard, T. L., Sharpe, F. R., *J. Inst. Brew., London* **82**, 233 (1976).
- Ronkainen, P., Denslow, J., Leppänen, O., *J. Chromatogr. Sci.* **11**, 384 (1973).
- Schaeffgen, J. R., *J. Am. Chem. Soc.* **70**, 1308 (1948).
- Schreier, P., Drawert, F., Junker, A., *Brauwissenschaft* **27**, 205 (1974a).
- Schreier, P., Drawert, F., Junker, A., *Z. Lebensm.-Unters.-Forsch.* **154**, 279 (1974b).
- Schreier, P., Drawert, F., Junker, A., *Chem. Microbiol. Technol. Lebensm.* **4**, 189 (1976).
- Takahashi, T., Nakajima, S., Konishi, I., Miedaner, H., Narziss, L., *Brauwissenschaft* **31**, 1 (1978).
- Wainwright, T., *Eur. Brew. Conv., Proc. Cong.* **13**, 437 (1972).
- Wenzel, F. W., Jr., Reid, E. E., *J. Am. Chem. Soc.* **59**, 1089 (1937).
- Williams, A. A., May, H. V., Tucknott, O. G., *J. Inst. Brew., London* **84**, 97 (1978).

Received for review March 29, 1979. Accepted October 5, 1979.

Isotopic Composition of Carbon in Apple Juice

Landis W. Doner,* Harold W. Krueger, and Richard H. Reesman

The variability of the $^{13}\text{C}/^{12}\text{C}$ ratio in pure apple juices has been measured to determine base line values for a method to detect the undeclared addition of cane or corn sugars to apple juice. Forty apple juice samples representing 18 varieties of apples were analyzed by isotope ratio mass spectrometry. The mean $\delta^{13}\text{C}$ (ppt) value for all samples is -25.4 (ppt), and the coefficient of variation is 4.88%. There are no significant variations in $\delta^{13}\text{C}$ values with regard to apple variety or geographical origin of the apples, and the uniformity of the data suggests that it can be used to detect the adulteration of apple juice.

Plants use either the Calvin (C_3) or Hatch-Slack (C_4) pathway for photosynthetic carbon dioxide fixation, as revealed by differences in both leaf anatomy and $^{13}\text{C}/^{12}\text{C}$ ratios in their organic carbon. Plants using the C_3 cycle produce 3-phosphoglycerate as the first product of pho-

tosynthesis, while in plants using the C_4 cycle, oxalacetate, malate, and aspartate are the initial products. All plants are slightly lighter in ^{13}C than is the carbon dioxide of the atmosphere and C_3 plants discriminate against ^{13}C to a greater extent than do C_4 plants, as established by Bender (1971) and Smith and Epstein (1971).

Recently, practical uses have resulted from the finding that the $^{13}\text{C}/^{12}\text{C}$ ratio of a plant derived material reflects the photosynthetic pathway operating in the plant from which it was derived. Cane (C_4 plant) sugar sucrose can be distinguished from beet (C_3 plant) sugar sucrose by $^{13}\text{C}/^{12}\text{C}$ analysis (Smith and Epstein, 1971), a distinction not possible chemically. Also, the undeclared addition of cane sugar to maple (*Acer saccharum*, C_3 plant) sirup

Krueger Enterprises, Inc., Cambridge, Massachusetts 02139 (H.W.K.), Geochron Laboratories Division, Krueger Enterprises, Inc., Cambridge, Massachusetts 02139 (R.H.R.), and the Eastern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118 (L.W.D.).