

Monitoring Volatile Chemicals Formed from Must during Yeast Fermentation

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Volatile flavor chemicals formed from grapes during yeast fermentation were collected at different periods of time using a purging/extraction apparatus. The volatiles recovered at each time period were analyzed by gas chromatography. The major compounds formed from grapes during fermentation were alcohols, acetates, and ethyl esters of C₄-C₈ fatty acids. 1-Pentanol (gas chromatographic peak area % = 33.4) and its acetate (33.2%) comprised almost two-thirds of total volatiles formed on the fourth day of fermentation. With the exception of alcohol formation, which maintained an equal level throughout the fermentation period, volatile formation was greatest on the third day of fermentation and then decreased gradually until the end of fermentation (after 11 days).

INTRODUCTION

Many volatile chemicals, some of which possess characteristic flavor, have been reported in wine (Schreier, 1979). Although a number of these flavor chemicals are also found in the original grapes (Schreier et al., 1976), most flavor chemicals found in wine are formed during yeast fermentation (Margalith and Schwartz, 1970). Because flavor components play an important role in the quality of wines, it is important to understand the formation of flavor chemicals during fermentation. Nakajima and Yamagata (1983) found a positive correlation between yeast population and production of volatile chemicals, including various alcohols and ethyl esters, in fermenting must.

Monitoring volatiles formed in a food or a beverage during certain processes such as heat treatment, light irradiation, and fermentation is a difficult experimental procedure. Quantitative analysis of volatiles formed at specified time intervals is particularly difficult. A trapping agent such as porous polymer is most commonly used to trap headspace volatiles of foods and beverages including wine. Hoff et al. (1978) obtained gas chromatographic headspace volatile profiling of beer using the porous polymer Porapak Q. Noble (1978) used Tenax to recover headspace volatiles from wine for a study on the correlation between sensory and instrumental evaluation of wine aroma. Recently we used a newly developed purging/extraction apparatus to monitor volatile chemicals formed by bananas during the ripening process (Macku and Shibamoto, 1991). In the present study, this newly developed apparatus was used to monitor volatile chemicals formed by must during yeast fermentation.

MATERIALS AND METHODS

Materials. Grapes (Thompson Seedless) were purchased from a local market. Sulfur dioxide (6% solution), tridecane (99%), and 1-octanol (99%) were bought from Aldrich Chemical Co. (Milwaukee, WI). Yeast (*Saccharomyces cerevisiae*, Montrachet strain) was purchased from Red Star (Universal Foods Corp., Milwaukee, WI).

Sample Preparation. The berries (10 kg) were separated from the stems and homogenized with 2 L of deionized water. The skins and tissues were filtered off with a cheesecloth. The

juice was mixed with a prediluted solution of sulfur dioxide to a final concentration (in the must) of 50 ppm. A suspension of yeast was added to the must to a final concentration of 0.25 g/L.

The must was placed on a 10-L three-neck round-bottom flask and stirred with a magnetic stirrer during fermentation. The temperature of the must was kept at 25 °C with a water bath. The whole setup was wrapped with aluminum foil to avoid the effect of light. The headspace volatiles were recovered using a simultaneous purging and solvent extractor devised by Umano and Shibamoto (1987). Purified air (flow rate, 60 mL/min) was used to purge the headspace for the first day; purified nitrogen was used for 10 additional days at the same flow rate. Dichloromethane was used for extraction.

The dichloromethane extract (50 mL) was removed from the simultaneous purging and solvent extractor every 24 h for a period of 11 days. Using a Vigreux column for distillation, the extract was concentrated to a final volume of 2 mL. Before analysis, tridecane (50 ppm) was added to the samples as a gas chromatographic internal standard.

Instrumental Analysis of Headspace Volatiles. The gas chromatographic (GC) retention index (Kovats, 1965) and mass spectral data of each GC component were compared with those of the authentic compounds for qualitative analysis. A flame photometric detector (FPD) was used to confirm the presence of sulfur-containing compounds.

A Hewlett-Packard (HP) 5890 gas chromatograph equipped with a 60 m × 0.25 mm i.d. DB-Wax bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA), a flame ionization detector (FID), and an FPD was used for sample analysis. The GC peak areas were integrated with a Spectra Physics 4290 integrator (San Jose, CA). The injector temperature was 250 °C, and the detector temperatures were 250 °C for the FID and 230 °C for the FPD. The oven temperature was held at 35 °C for 5 min and then programmed to 180 °C at 3 °C/min. The linear helium carrier gas flow rate was 30 cm/s.

An HP 5890 gas chromatograph interfaced to a VG Trio II mass spectrometer with a VG 11-250 computer data system was used for MS identification of the GC components. The column and oven conditions for GC/MS were described above.

Determination of Ethanol Content in Fermented Must. A small portion of the fermented must (5 mL) was removed from the flask after 11 days. The must was centrifuged at 3000 rpm to separate the solid materials from the solution. After the supernatant was diluted 1000-fold from deionized water, the solution (1 µL) was injected into a 30 m × 0.25 mm i.d. DB-5 bonded-phase fused-silica capillary column installed in an HP 5790 GC equipped with an FID. The GC peak areas were integrated with an HP 5880A series terminal. The injector temperature was 300 °C, and the detector temperature was 300 °C. The oven temperature was isothermally held at 150 °C. A standard curve, established between 50 and 500 µg/mL, was used to measure the ethanol content.

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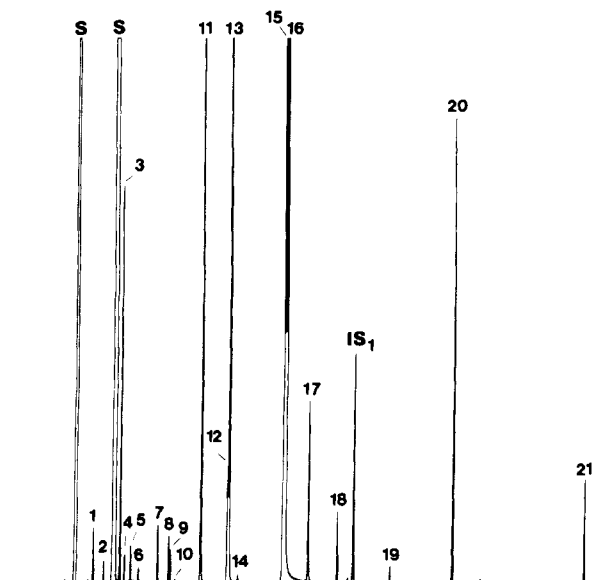


Figure 1. Typical gas chromatogram of headspace volatiles collected from must on the fourth day of fermentation. S, solvent (dichloromethane); IS₁, internal standard (tridecane). See Table I for peak identification.

Table I. Volatile Compounds Formed from Must on the Fourth Day of Fermentation

peak no. in Figure 1	compound	<i>I</i> ^a	GC peak area %
1	unknown	857	0.24
2	methyl propyl ether	877	<i>b</i>
3	ethanol	938	1.31
4	unknown	950	0.27
5	ethyl propionate	964	0.50
6	propyl acetate	983	0.22
7	isobutyl acetate	1019	1.10
8	unknown	1038	0.14
9	ethyl butanoate	1040	0.61
10	methyl thioacetate	1047	<i>b</i>
11	unknown	1089	2.93
12	isopentyl acetate	1127	2.29
13	pentyl acetate	1129	33.2
14	unknown (alcohol)	1142	<i>b</i>
15	isopentanol	1203	10.1
16	pentanol	1206	33.4
17	ethyl hexanoate	1237	3.19
18	unknown (alcohol)	1276	1.70
19	hexanol	1350	0.24
20	ethyl octanoate	1440	7.12
21	ethyl decanoate	1700	1.32

^a Kovats index on DB-Wax. ^b GC peak area % <0.1.

RESULTS AND DISCUSSION

The must fermented for 11 days contained 11.01% alcohol, which is consistent with commercial wine (10.5–12%). The pH of the must at the end of the fermentation period was 4.0.

Figure 1 shows a typical gas chromatogram of headspace volatiles collected from the must on the fourth day of fermentation. Table I shows volatile compounds identified in the headspace of the must on the fourth day of fermentation. Methyl thioacetate (peak 10), which has been reported in the flavor of cheese (Adda et al., 1978; Dumont et al., 1981), was identified in wine for the first time. It was also found in beer and postulated to be a product of the interaction between brewers' yeast and methyl mercaptan or methionine during the fermentation of wort (Matsui and Amaha, 1981). Methyl thioacetate was later proved to be formed from methyl mercaptan by the action of a yeast, *S. cerevisiae* (Matsui et al., 1981), which was used for the present study.

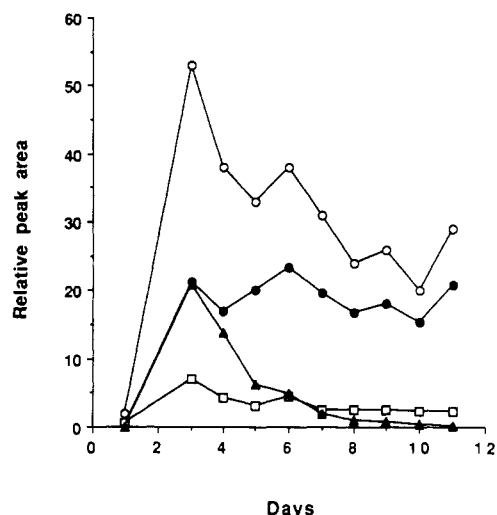


Figure 2. Formation of total volatiles (O), alcohols (●), acetates (▲), and ethyl esters (□) on different days. Values are means of two duplicate experiments.

The major compounds formed in the headspace of the must during fermentation were alcohols, acetates, and ethyl esters. The headspace volatiles collected on the fourth day of fermentation were composed of 47% alcohols, 37% acetates, and 13% ethyl esters. 1-Pentanol (33.4%) and its acetate (33.2%), which comprised the largest quantity among the acetates, accounted for almost two-thirds of total headspace volatiles. It is interesting that ethanol was not recovered in large quantity due to its high solubility in water. On the fourth day of fermentation, ethyl esters of long-chain fatty acids—such as hexanoic (3.19%), octanoic (7.12%), and decanoic (1.32%)—were recovered in greater amounts than those of short-chain fatty acids such as ethyl acetate (0%) and ethyl propionate (0.5%).

Figure 2 shows the relative peak area (GC peak area account number of a compound/GC peak area account number of the internal standard) of total volatiles and the title of each chemical group on different days. The volatiles formed in greatest quantity on the third day of fermentation. The formation of alcohols increased until the third day and then plateaued. Acetate formation increased during the first three days and reached a maximum and then steadily decreased to almost zero at the end of fermentation. Among the acetates produced in wine during fermentation, isopentyl acetate reportedly formed in the greatest amount and reached a maximum on the sixth day (Miller et al., 1987), whereas in the present study pentyl acetate was found to form the greatest amount and reached a maximum on the fourth day.

A series of acetates C₂–C₅ were also reportedly formed from Thompson Seedless by *S. cerevisiae* (Daudt and Ough, 1973). Formation of ethyl esters generally remained constant during the fermentation except on the third day, when they formed at greatest rate. The constant formation of ethyl esters of the C₄–C₈ fatty acids during fermentation in wine was also previously reported (Miller et al., 1987).

Acetates, ethyl esters of fatty acids, and alcohols are three major components which influence wine aroma strongly (Schreier, 1979). The present study tracks quantitatively the formation of these components as fermentation progresses. A newly developed headspace collection method for volatiles gave satisfactory results and may be useful for monitoring flavor development of foods and beverages during treatments which include fermentation.

ACKNOWLEDGMENT

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Registry No. Methyl propyl ether, 557-17-5; ethanol, 64-17-5; ethyl propionate, 105-37-3; propyl acetate, 109-60-4; isobutyl acetate, 110-19-0; ethyl butanoate, 105-54-4; methyl thioacetate, 1534-08-3; isopentyl acetate, 123-92-2; pentyl acetate, 628-63-7; isopentanol, 123-51-3; 1-pentanol, 71-41-0; ethyl hexanoate, 123-66-0; 1-hexanol, 111-27-3; ethyl octanoate, 106-32-1; ethyl decanoate, 110-38-3.