Monitoring Ester Formation in Grape Juice Fermentations Using Solid Phase Microextraction Coupled with Gas Chromatography−Mass Spectrometry

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Volatile esters contribute important floral and fruity sensory properties to wine. Numerous factors influence the biosynthesis and hydrolysis of esters throughout yeast fermentation; however, methods to monitor the dynamic changes in ester production that occur during winemaking processes are limited. In this study, we showed that solid phase microextraction (SPME), a rapid, solventless sampling procedure, combined with GC/MS analysis is a useful method for the nearly continuous analysis of volatile compounds such as esters that are produced during fermentation. Accuracy, precision, and limits of quantification were comparable to those of other sample preparation methods such as liquid−liquid extraction. Using GC/MS−SPME to monitor fatty acid ethyl esters and acetate esters, we obtained detailed information on the production patterns of ester formation during fermentation. This method now enables the monitoring of volatiles during fermentation and can provide greater insight into yeast metabolism and flavor formation.

Keywords: *Esters; fatty acid esters; acetate esters; grape juice; wine; yeast; fermentation; formation; solid-phase microextraction; analysis; GC-MS*

INTRODUCTION

One of the most important groups of aroma compounds in wine are the fatty acid and acetate esters that are formed enzymatically during fermentation and that contribute floral and fruity sensory properties to the wine (*1*). Fatty acid ethyl esters (e.g., ethylbutanoate, ethylhexanoate, ethyloctanoate, etc.) are obtained from ethanolysis of acylCoA that is formed during fatty acid synthesis or degradation. Acetate esters (e.g., isoamyl acetate, propyl acetate, hexyl acetate, phenethyl acetate) are the result of the reaction of acetylCoA with higher alcohols that are formed from the degradation of amino acids or carbohydrates. Ester concentration and relative distribution is governed by yeast strain (*2*−*5*) and fermentation conditions (e.g., temperature, nutrient availability, pH, unsaturated fatty acid/sterol levels, and oxygen levels) (*1*, *6*−*10*). Enzymatic hydrolysis of esters also occurs during fermentation (via esterases) while chemical hydrolysis occurs during storage and aging (*8*, *11*, *12*). Therefore, several factors contribute to both the synthesis and the hydrolysis of esters, and these factors differ in the time at which they may become significant during winemaking.

The interconversion of esters and their corresponding acids and alcohols in aqueous solutions, and the dynamic system that results from this phenomenon mandates the understanding of conditions that may affect this equilibrium. However, monitoring volatiles during fermentation is a challenging procedure. Traditionally, liquid−liquid extraction (using pentane, Freon 11, or Freon 113 as the extracting solvent), simultaneous distillation/extraction, and dynamic and static headspace sampling methods have been used for fermentation ester analysis (*2*, *6*, *13*−*16*). However, these methods can require the use of large amounts of purified solvents that are costly to use and to dispose of and that can suffer from poor sensitivity and loss of analytes during extraction and concentration. In general, these methods are also fairly laborious, require relatively expensive glassware, and are not easily automated.

Stashenko et al. (*14*) developed a combined purging/extraction apparatus for analysis of volatiles produced during fermentation. This is one of the few studies that has monitored the evolution of volatiles during fermentation; however, the solvent extraction was still relatively lengthy and only allowed for sparse sample collection, and the additional concentration step was time-consuming. As a result, each sample was collected over a 24 h period, and collection did not begin until the third day of fermentation.

More recently, solid-phase micro-extraction (SPME) has been used for analysis of a wide range of flavor compounds, including esters, in wine and other alcoholic beverages (*17*−*26*). SPME involves the concentration of analytes by adsorption (or absorption) onto a polymeric material that is coated onto the end of a fused silica fiber. Extraction is based on partitioning of the analyte among the three phases present in the sampling vial: the liquid, the headspace of the vial, and the SPME fiber (*27*).

Vas et al. (*25*) used SPME to compare production of volatiles, including ethyl esters (ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and diethyl succinate), acetate esters (linalyl acetate, hexyl acetate, isoamyl acetate, and butyl acetate) and terpene alcohols, by different yeast strains

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during the fermentation of Muscat Ottonel grape juice. Total acetate ester and ethyl ester amounts were evaluated by measuring total peak areas, not actual concentrations. Vas et al. (*25*) observed that acetate esters reached their maximum concentration later in fermentation (approximately 300 h after inoculation) than the ethyl esters (200−250 h after inoculation). However, data collection in this study was sporadic; samples were taken only every 25 h, and there was a gap in sampling between the 50th and 150th hour.

The lack of solvent use, the relatively short extraction times, and the potential for automation make SPME a promising method for continuous sampling of volatiles during fermentation. The goal of the present study is to optimize SPME coupled with GC/MS as a quantitative method for "continuous" monitoring of ester production during the fermentation of grape juice. Using this method, the patterns of fatty acid ethyl ester and acetate ester production will be evaluated.

MATERIALS AND METHODS

Fermentation. Duplicate 1-L fermentations of Chardonnay juice (pH 2.9, 22.1 Brix) from the UC Davis Tyree vineyards (Davis, CA) were performed and are referred to as fermentation A and fermentation B. Fermentation A was inoculated 1 h prior to fermentation B with Premier Cuvee − *Saccharomyces bayanus* var. *Prise de Mousse* (Red Star, Milwaukee, WI) at 0.38 g/L of juice. Yeast was first hydrated in 40 mL of juice which was warmed to 38 °C and then added to complete 1 L of juice. Diammonium phosphate (DAP) was added to each fermentation at 0.4 g/L to ensure sufficient nitrogen content. Fermentations were carried out in 1-L glass fermentors (Applikon, Holland) that were kept in a water bath maintained at 18 °C and were stirred constantly at 100 rpm. Fermentations were monitored by weight loss (CO_2 evolution) starting at inoculation and continuing every 12 h until the weight stabilized. Clinitest tablets (Miles Inc., Elkhart, IN) were used to confirm dryness (*28*). After all sampling was finished, the juice was racked, pH was measured by pH meter, and ethanol levels were determined by ebulliometry (*28*).

Sampling. SPME fibers were purchased from Supelco (Bellafonte, PA). Both PA-85 (polyacrylate; 85 μm) and PDMS [poly(dimethylsiloxane); 100 μm] fibers were evaluated. Preliminary trials showed that PA-85 coated fibers did not give as sensitive of a response for the ester analysis as did the PDMS coated fibers; therefore, PDMS fibers were used for all subsequent analyses. Headspace sampling involved manually exposing the SPME fiber through a rubber septum in the lid of the fermentor for an absorption time of 1 h followed by immediate injection into the GC/MS. During sampling, the tip of the SPME fiber was approximately 3 cm above the liquid in the fermentor, and the needle was offset from the center of the fermentor by about 3 cm to avoid hitting the stirrer. Two SPME fibers were used (denoted A and B), and they were consistently used for the same fermentor (A or B) throughout the experiment. Quantitation for each fiber was based on a standard curve obtained with that fiber. SPME samples of headspace from fermentors were taken starting 3 h after inoculation and were subsequently taken every other hour until the juice fermented to dryness. Samples were taken for several days after dryness with additional data collected up to 2 weeks after fermentation was completed.

Standard Curves. The following standards were purchased from Aldrich (Milwaukee, WI): ethyl acetate, *n*-propyl acetate, ethyl butyrate, isoamyl acetate, hexyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and phenethyl acetate. All ester standards were of greater than 99% purity.

Model wine solutions were used for the standard curves. These were prepared with distilled water and contained 0.75 g/100 mL tartaric acid and 12% ethanol. The pH was adjusted to 3.5 with 6 N NaOH. Stock solutions of each ester were prepared first in methanol then diluted to appropriate levels in the model wine solutions. One liter of solution was prepared for each standard, and the standards were sampled by SPME in the same fermentors used for the juice/wine samples to duplicate the headspace and volume conditions of the actual fermentations. Table 1 summarizes the concentrations of esters found in each standard. Standards were also prepared in a similar manner for determining precision and accuracy of the sampling. Ester concentrations for these standards were 25 mg/L for ethyl acetate, 2.5 mg/L for isoamyl acetate, and 0.25 mg/L for all other esters.

Table 1. Ester Concentrations Used for Preparation of Standard Curves[a]

	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6
ethyl acetate	5.0	25.0	50.0	75.0	100.0	150.0
n-propyl acetate		0.25	0.50	0.75	1.0	
isoamyl acetate	0.5	2.5	5.0	7.5	10.0	15.0
hexyl acetate	0.05	0.25	0.50	0.75	1.0	1.5
phenethyl acetate		0.25	0.50	0.75	1.0	
ethyl butyrate		0.25	0.50	0.75	1.0	
ethyl hexanoate	0.05	0.25	0.50	0.75	1.0	1.5
ethyl octanoate	0.05	0.25	0.50	0.75	1.0	1.5
ethyl decanoate	0.05	0.25	0.50	0.75	1.0	1.5

[a] All concentrations are given in mg/L.

SPME adsorption time was 1 h for each standard and desorption time in the GC/MS was 5 min, which was identical to the sampling protocol used during fermentation.

GC-MS Analysis. The GC/MS system used was a Hewlett-Packard HP 6890 series gas chromatograph equipped with a 5972 Mass Selective detector and Chemstation software (Agilent, Avondale, PA). GC/MS parameters were adapted and slightly modified from Jelen et al. (*22*). The oven starting temperature was held at 40 °C for 4 min. It was raised at 5 °C/min to 100 °C, followed by an increase of 10 °C/min to 220 °C. The temperature then remained at 220 °C for 9 min. The total run time was 40 min. The injection port temperature was 250 °C, and the detector transfer line was 260 °C. The column used was a DB-5MS (Cross-linked 5% phenyl methyl Siloxane, J&W Scientific, Folsom, CA). Column dimensions were as follows: 30 m × 0.25 mm id; 0.25 μm film thickness. The carrier gas used was helium at a flow rate of 36 cm/s. Splitless injections were made with use of a split/splitless 0.7-mm glass liner. The electron multiplier voltage was set at 200 eV over the autotune value, and a solvent delay of 1.8 min was used. All ester peaks were well separated with the following retention times: ethyl acetate, 2.4 min; *n*-propyl acetate, 3.8 min; ethyl butyrate, 6.2 min; isoamyl acetate, 8.9 min; ethyl hexanoate, 13.4 min; hexyl acetate, 13.9 min; ethyl octanoate, 19.9 min; phenethyl acetate, 21.3 min; and ethyl decanoate, 23.9 min.

Quantitation. Total ion data were collected for all standards and samples. Peak areas for each compound were plotted against the actual concentration to obtain standard curves for each ester. The ester concentration of the samples was then calculated by using the linear regression equation. Equations for the standard curves are presented in the results section.

Identification of Unknown Compounds. Identification of unknown compounds was based on matches to the Chemstation Wiley Spectral Library, calculated retention time indices available from the literature (*29*) and injection of authentic standards whenever possible.

RESULTS AND DISCUSSION

Fermentation. Fermentation curves were virtually identical for the replicate fermentations A and B with both showing a lag time of approximately 48 h. The exponential phase also was approximately of the same duration for both samples (approximately 12 days), and both samples reached dryness simultaneously after 12.2 days of fermentation, typical of fermentations conducted at 18 °C. Final pH for wine A was 3.10 and the final

Table 2. Range and Linearity of Standard Curves Used for Quantitation of Individual Esters

ester	range (mg/L)	R^2	regression equation
ethyl acetate A[a]	5−150	0.9751	$y = 78742x - 495619$
ethyl acetate B		0.9684	$y = 72761x + 94672$
n-propyl acetate A	0.25−1.9	0.9816	$y = 63400x - 106213$
n-propyl acetate B		0.9503	$y = 666603x - 129781$
isoamyl acetate A	0.5−15	0.9713	$y = 3.00E+06x - 1E+06$
isoamyl acetate B		0.9573	$y = 3.00E+06x - 169735$
hexyl acetate A	0.05−1.5	0.9464	$y = 1.00E+07x - 460863$
hexyl acetate B		0.9662	$y = 1.00E+07x - 610051$
phenethyl acetate A	0.25−1.0	0.9802	$y = 3.00E+06x - 497309$
phenethyl acetate B		0.9697	$y = 3.00E+06x - 399649$
ethyl butyrate A	0.25−1.0	0.9189	$y = 2.00E+06x - 331196$
ethyl butyrate B		0.9495	$y = 3.00E+06x - 282825$
ethyl hexanoate A	0.05−1.5	0.9310	$y = 1.00E+07x - 116332$
ethyl hexanoate B		0.9314	$y = 1.00E+07x - 144393$
ethyl octanoate A	0.05−1.5	0.9446	$y = 8.00E+07x + 4E+06$
ethyl octanoate B		0.9850	$y = 9.00E+07x - 922693$
ethyl decanoate A	0.05−1.5	0.9519	$y = 4.00E+08x - 7E+07$
ethyl decanoate B		0.9813	$y = 3.00E+08x - 3E+07$

[a] A and B denote the two different SPME fibers used with the corresponding fermentors throughout the experiment. Four replicate analyses were obtained at each concentration to construct the standard curves.

Table 3. Precision and Accuracy for Ester Standards Spiked in Model Wines

compound	spiked conc (mg/L)	mean measured conc (mg/L)	SD	%RSD
ethyl acetate A[a]	25	29.5	2.4	8.2
ethyl acetate B	25	27.6	1.9	6.8
n-propyl acetate A	0.25	0.27	0.03	12.0
n-propyl acetate B	0.25	0.28	0.01	5.1
isoamyl acetate A	2.5	3.2	0.06	1.9
isoamyl acetate B	2.5	2.9	0.08	2.6
hexyl acetate A	0.25	0.29	0.02	5.1
hexyl acetate B	0.25	0.36	0.01	3.6
phenethyl acetateA	0.25	0.33	0.06	16.6
phenethyl acetateB	0.25	0.19	0.01	4.7
ethyl butyrate A	0.25	0.30	0.01	2.6
ethyl butyrate B	0.25	0.24	0.04	13.6
ethyl hexanoate A	0.25	0.27	0.01	2.5
ethyl hexanoate B	0.25	0.29	0.01	3.4
ethyl octanoate A	0.25	0.33	0.01	2.1
ethyl octanoate B	0.25	0.29	0.01	4.3
ethyl decanoate A	0.25	0.31	0.02	7.6
ethyl decanoate B	0.25	0.32	0.03	8.8

[a] A and B denote the two different SPME fibers used with the corresponding fermentors throughout the experiment. Six replicate analyses were obtained at each concentration for each fiber.

pH for wine B was 3.13. Ethanol levels for the wines were 12.6 and 12.9%.

Method Validation and Quantitation. Originally, we attempted to quantitate each ester with the use of an internal standard added to the fermentors. However, this method was unreliable since the internal standard concentration changed as a result of the repeated sampling from the same fermentor during the fermentation. We then attempted to expose the fiber to an internal standard solution for 10 min prior to each sampling interval. However, poor reproducibility of the internal standard peak areas was oberved. Therefore, external standard quantitation was used for all analyses. Using this procedure, good linearity was obtained for all analytes with r^2 values ranging from 0.919 to 0.996 (Table 2). A standard curve was also obtained at the end of the experiment to check for the viability of the SPME fibers used. This standard curve showed similar total peak areas and linearity as the initial standard curves, demonstrating that the quality of the fibers had not been compromised after extensive use (approximately 200 analyses/SPME fiber).

Signal-to-noise (S/N) ratios for the lowest standards of ethyl acetate, isoamyl acetate, hexyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were 20, 18, 9, 9, 60, and 200, respectively. Therefore, lower limits of quantitation may be possible for some of these esters; however, ester concentrations in wine are typically well above the lowest standards used for these analytes. Limits of quantitation for ethyl butyrate, *n*-propyl acetate, and phenethyl acetate should be improved for future studies since levels as low as 0.02 mg/L have been reported in wine (*28*). S/N ratios calculated at 0.25 mg/L for these esters were 6, 10, and 10, respectively. It is important to note that for this study the MS detector was run in the total ion mode which is not as sensitive as selected-ion monitoring. Selected-ion monitoring would significantly lower the limits of quantitation for these esters.

The method had good precision with relative standard deviations (RSDs) for repeated sampling being generally less than 10% and ranging from 1.9 to 16.6% (Table 3). The method was also accurate with relatively small deviations from the mean expected concentrations for spiked samples (Table 3).

All standards used for quantitation were prepared in a 12% ethanol model solution. However, during fermentation ethanol concentration changes, and it is not clear how this may influence the measurement of absolute ester concentrations. De la Calle Garcia et al. (*17*) used SPME to sample terpene alcohol concentrations in wines and model systems. They observed that as ethanol concentration increased from 2 to 12% the measured concentrations of geraniol, nerol, citronellol, linalool, and alpha-terpineol were reduced. In contrast, Vas et al. (*25*) suggest that esters, unlike alcohols, are not as likely to be affected by the ethanol concentration of a solution. These authors did not observe significant effects of ethanol concentration on headspace concentrations of esters using a SPME procedure. Similarly, Conner et al. (*30*) showed that for solutions of less than 17% (v/v) ethanol, there were no significant differences in headspace concentrations of esters (i.e., ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and ethyl dodecanoate) as measured by a static headspace procedure. However, a decrease in headspace concentration was observed at ethanol levels above 17% (v/v) ethanol. At this ethanol concentration, the ethanol molecules tended to form clusters and the esters partitioned into the clusters thus increasing their solubility and decreasing the headspace concentrations. Future studies evaluating the effects of ethanol levels on the SPME measurement of volatiles in the headspace are needed.

Production of carbon dioxide during fermentation may also influence quantitation of analytes. For example, vigorous CO_2 production during fermentation may decrease the efficiency of SPME extraction. However, Scarlata and Ebeler (*31*) observed that carbonation in beer did not affect the quantitation of dimethyl sulfide using a SPME headspace sampling procedure. In addition, CO_2 production may influence the rate of volatilization (and subsequent headspace concentration) of individual compounds. The kinetics of this volatilization during fermentation as well as effects on quantitation by SPME headspace sampling are not fully known and require further study.

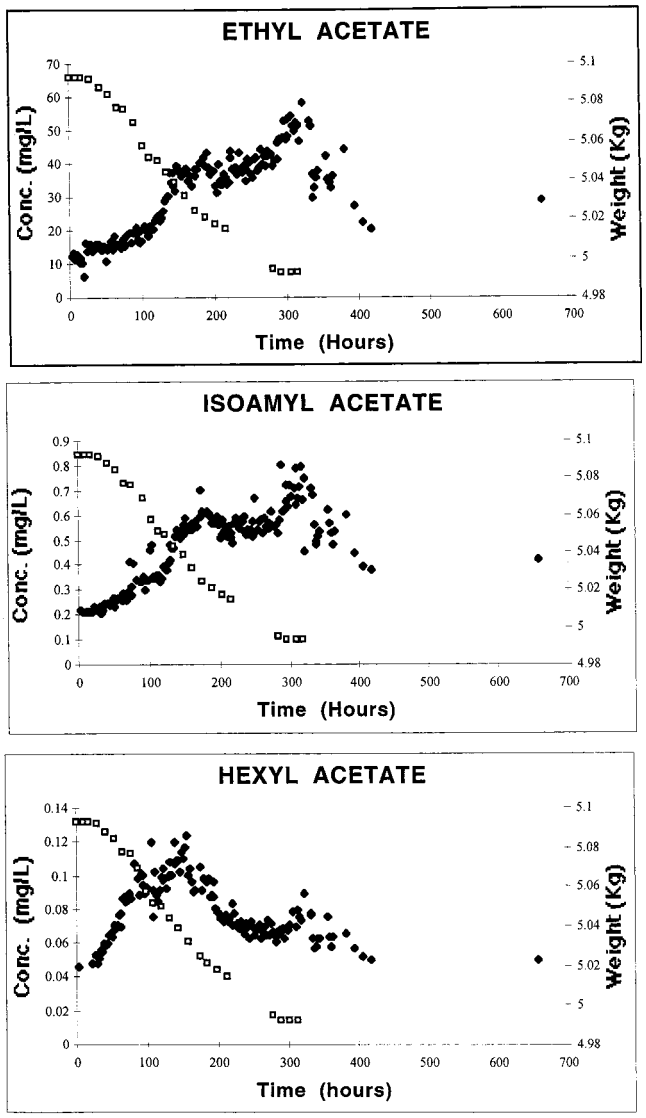

Figure 1. Production of acetate esters during fermentation. Open squares indicate progression of the fermentation as monitored by weight loss. Data points represent the average of fermentations A and B.

Formation of Esters during Fermentation. The two replicate fermentations showed identical patterns of ester production for all esters assayed. Therefore, data from both fermentors were combined and average values are presented.

Acetate Esters. Ethyl acetate and isoamyl acetate followed very similar patterns of production throughout the fermentation (Figure 1). The levels of these esters increased only slightly during the first 100 h after inoculation. Approximately midpoint in the exponential phase of the fermentation, a steep increase in concentration was observed resulting in an initial peak in concentration approximately 180 h (7−8 days) after inoculation. The ester levels remained constant until near the end of the fermentation when a second increase in production occurred with both esters reaching a maximum value shortly after dryness. The ester levels then began to decrease rapidly, reaching final concentrations of 20.4 and 0.38 mg/L for ethyl acetate and isoamyl acetate, respectively, on the 17th day after inoculation. A subsequent measurement taken 10 days later (27 days after inoculation) showed no significant change in concentrations of these two esters. These final concentrations fall within the previously reported ranges observed in finished wines (*28*).

Hexyl acetate showed a slightly different pattern of production as compared to ethyl acetate and isoamyl acetate (Figure 1). Hexyl acetate levels increased rapidly at the beginning of the exponential phase of the fermentation. A maximum concentration of 0.1 mg/L was reached on the eighth day after inoculation, well before dryness and before ethyl acetate and isoamyl acetate reached their peak levels. The hexyl acetate levels then declined, reaching a plateau of ∼0.07 mg/L about 210 h into the fermentation. A second slight increase was observed shortly after dryness, but this increase was much less pronounced than that of the other acetate esters. Hexyl acetate levels then declined again to a final concentration of 0.05 mg/L measured 17 and 27 days after inoculation. This final concentration was nearly identical to the original concentration measured in the juice prior to fermentation (0.046 mg/L).

The observed profiles of acetate ester production reflect some of the known aspects of ester biosynthesis by yeast. For example, production of these esters is dependent on the production of the corresponding fusel alcohol which may partially explain the lag in their production. Future studies monitoring alcohol production are necessary to better understand this relationship. In addition, Mauricio et al. (*12*) used two different strains of *S. cerevisiae* (*S. cerevisiae* var. *cerevisiae* and *S. cerevisiae* var. *capensis*) to show that maximum activity of the enzyme, alcohol acetyltransferase (AAT), which is involved in the synthesis of both ethyl acetate and isoamyl acetate, is highest at the midpoint of the exponential phase of fermentation. This is also the point at which a sharp increase in concentration of both these esters occurred in our study. Mauricio et al. also observed increased hydrolysis-related esterase activity at the end of the fermentation, consistent with the decrease in ethyl acetate and isoamyl acetate concentrations observed in our study after 12−13 days (∼300 h). The number of sampling times used by Mauricio et al. was limited, however, and consisted of sampling after 1, 2, 3, 10, 31, and 134 days of fermentation, and so more detailed patterns of ester production could not be obtained.

Reasons for differences in production of hexyl acetate as compared to ethyl acetate and isoamyl acetate are unknown. However, our data are in agreement with Moreno et al. (*32*) who showed that hexyl acetate was synthesized during the first days of fermentation and reached a maximum concentration within the first 5 days. Moreno et al. used a fermentation temperature of 25 °C; the fermentation was carried out by *S. cerevisiae*, and samples were collected only on the first, fifth, 20th and 30th days of fermentation.

Phenethyl acetate and *n*-propyl acetate were found in very small amounts throughout fermentation. However, the amounts present were below the limits of quantitation of this method. Future studies are needed to improve method sensitivity and quantitation of these esters.

Fatty Acid Esters. Ethyl hexanoate, ethyl octanoate, and ethyl decanoate showed nearly identical patterns of production, with the main difference being the total amount of each ester produced (Figure 2). Production of the fatty acid esters was coincident with the beginning of the exponential phase of the fermentation; the

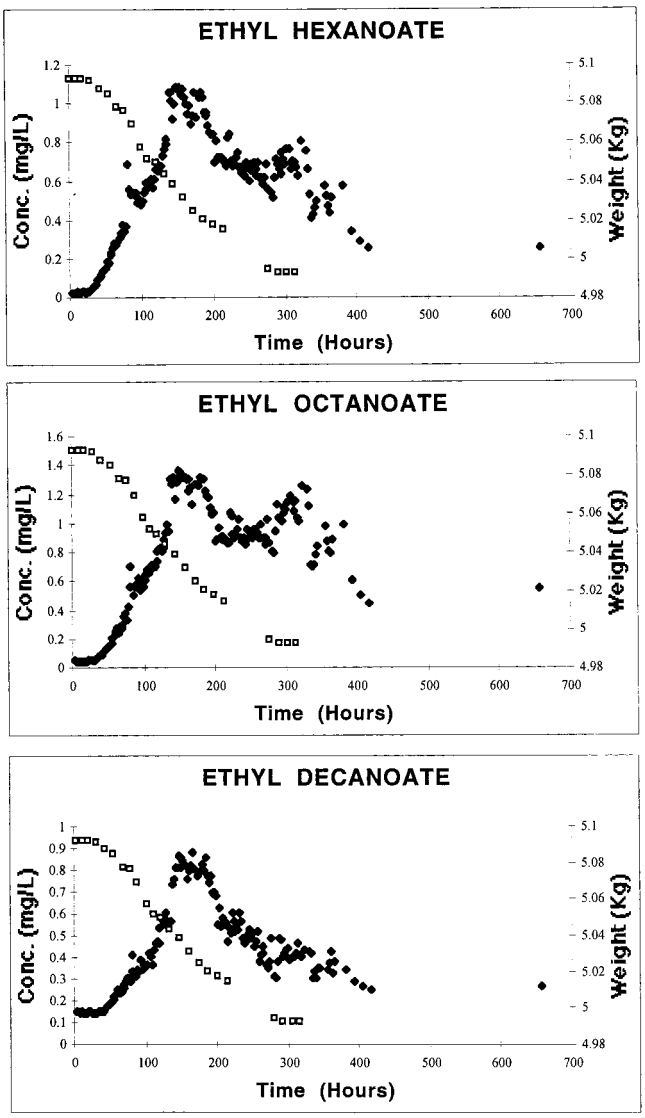

Figure 2. Production of fatty acid ethyl esters during fermentation. Open squares indicate progression of the fermentation as monitored by weight loss. Data points represent the average of fermentations A and B.

initial lag phase of the fermentation curve was nearly identical to the initial lag in ester production. Maximum fatty acid ester production occurred at approximately the midpoint of the log phase in the fermentation curve (~150 h or 6 days of fermentation). Concentrations subsequently dropped quickly until the end of the fermentation, when ethyl hexanoate and ethyl octanoate levels increased slightly before again declining. This second peak in production was most pronounced for ethyl octanoate and was not observed for ethyl decanoate.

Final concentrations for ethyl hexanoate, ethyl octanoate, and ethyl decanoate were 0.26, 0.56, and 0.27 mg/L, respectively, consistent with previously published studies (*28*). Ethyl decanoate is typically present at lower levels as compared to ethyl octanoate or hexanoate (*9*, *33*). This may be explained by the fact that ethyl decanoate is the ester that is most difficult to transport from the yeast cell into the medium, while both ethyl hexanoate and ethyl octanoate are more readily transported out of the yeast cell (*34*).

Several studies using different yeast strains and fermentation temperatures have consistently shown that fatty acid ethyl esters reach their maximum levels early in the fermentation (day 3−7 depending on fermentation conditions) (*9*, *14*, *25*). The enzyme ethyl hexanoate synthase is known to be inhibited by high levels of ethanol (35). If a similar enzyme is responsible for synthesis of ethyl octanoate and ethyl decanoate, this may explain why concentrations increase early in fermentation and then decrease rapidly as fermentation progresses. In contrast, AAT, the enzyme involved in synthesis of ethyl acetate and isoamyl acetate, has been shown to maintain its activity at higher ethanol levels than ethyl hexanoate synthase (*35*) and may explain why ethyl acetate synthesis peaks later in the fermentation, as previously discussed.

The multipeak pattern of ester production observed in this study for all esters except ethyl decanoate has not been previously reported. Previous data indicated that ester production follows a pattern of consistent increase to a maximum level followed by a consistent decrease in concentration (*9*, *12*, *14*, *25*, *32*). The second increase in concentration observed in the current study occurred immediately following the end of fermentation. Charpentier et al. (*36*) showed that there is a loss of both amino acids and glucans in the cell wall of yeasts during autolysis which results in a structural loosening of these cell walls. Therefore, the observed second increase in ester concentration may result from their release from the yeast cells at the end of fermentation. Future studies should include a measurement of viable yeast cells and the extent of autolysis at the end of fermentation to provide better insight into this phenomenon.

Other Volatiles. Several other compounds were tentatively identified in the samples, all of which have been previously reported in wine. Three additional peaks could not be identified. In general, concentration of these compounds were low at the beginning of fermentation, increased slightly as fermentation progressed and then decreased in the later stages of fermentation.

One of the compounds present at highest concentrations (approximately 0.2−0.5 mg/L) was tentatively identified as ethyl dodecanoate. Lesser amounts of 3-methyl butyl octanoate, 3-methyl butyl decanoate, and ethyl heptanoate were present throughout fermentation. As expected, the levels of all of these esters were very low at the end of fermentation since larger molecular weight fatty acid esters hydrolyze more rapidly than the smaller molecular weight esters and they are not readily released by the yeast cell (*11*).

The PDMS (polydimethyl siloxane) SPME fiber used for this study is particularly suitable for the analysis of esters (*18*) and may explain why all of the identified compounds in this study were esters. Other fibers, such as PA-85 (polyacrylate) may be more suitable for the analysis of other classes of flavor compounds and could be used for future studies.

SUMMARY

The SPME procedure used in this study was readily adaptable for the "nearly continuous" monitoring of ester production throughout fermentation. The PDMS fiber was ideal for quantifying esters, while other fiber types may be applicable for other flavor compounds.

Using this procedure, differences in production of acetate and fatty acid ethyl esters were observed and related to progression of the fermentation. In addition,

a multipeak pattern of ester production was observed which has not previously been reported. Future studies using SPME that correlate synthesis and activity of the appropriate enzymes, different yeast strains, and fermentation conditions to the production of a range of volatile compounds, including esters, will prove valuable to the further understanding of yeast metabolism and flavor formation during fermentation.

ABBREVIATIONS USED

SPME, solid-phase microextraction; GC/MS, gas chromatography/mass spectrometry; DAP, diammonium phosphate; PA, polyacrylate; PDMS, poly(dimethylsiloxane); RSDs, relative standard deviations; AAT, alcohol acetyltransferase

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