Comparison of Volatile Component Formation Arising in Spontaneous and Inoculated Fermentation of Ohio Chardonnay by SPME/GC/MS

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ABSTRACT

Wine making is an ancient art that has yet to reveal all of its secrets. Tactile stimulation, color, taste, and aroma are the factors that influence the value of wine. For this work, aroma components were studied in hopes of increasing the knowledge of the formation of these compounds during the fermentation process. Many studies have addressed the problem of volatile component formation in wine making, but they have only addressed certain compounds or classes of compounds at one time. This study addresses this same problem but tracks many components from many different classes of compounds simultaneously using a SPME/GC/MS method. Some discoveries made were the link between the formation and degradation of ethyl decanoate and decanoic acid, the higher concentration of trace volatiles in the spontaneous fermentation, and the rapid cell growth and death of the yeast in the spontaneous fermentation when compared to the inoculated one. The method developed for this study should be useful in further enquiry into both methods development and the biosynthesis of volatile components in wine or other beverages.

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LIST OF ABBREVIATIONS

Symbol	Definition
CO_2	Carbon Dioxide
C	Celsius
CFU	Colony Forming Units
°/min	Degree per minute
ECD	Electron Capture Detector
FID	Flame Ionization Detector
GC	Gas Chromatography
g/gal	Grams per gallon
g/L	Grams per liter
HPLC	High Pressure Liquid Chromatography
kPa	Kilopascal
MS	Mass Spectrometry
m/z	Mass to charge ratio
µm	Micrometer
µL	Microliter
µg/L	Micrograms per liter
mg/L	Milligrams per liter
mL	Milliliter
min	Minute
ppb	Parts per billion
ppm	Parts per million

LIST OF ABBREVIATIONS (cont.)

Symbol	Definition
ppt	Parts per thousand
pptr	Parts per trillion
PID	Photoionization Detector
SPME	Solid Phase Micro-Extraction
TCD	Thermoconductivity Detector

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CHAPTER I

Introduction/Literature Review

A. Volatile Compounds in Wine

Taste and tactile sensations in wine are primarily determined by nonvolatile components that are present in relatively high concentrations. However, the aroma is predominately determined by the volatile compounds that exist in relatively low concentrations between 10^{-1} and 10^{-10} g/L (1). The fundamental aroma is due to two alcohols (isobutyl and isoamyl alcohols), four esters (ethyl acetate, isoamyl acetate, ethyl hexanoate, and ethyl octanoate), and acetyldehyde (2). Secondary differences in aroma are caused by hundreds of other volatile compounds that act as modifiers to these fundamental compounds (3).

The volatile compounds that form the aroma of the wine have differing origins. Some come directly from the grape, such as terpenes. Others form during the fermentation, such as higher alcohols (over 4 carbons), aliphatic carboxylic acids, and esters. Finally, during the aging process the wine may acquire such volatiles as phenols and acetals.

1. Terpines

Monoterpines such as nerol, linalool, and geraniol tend to exhibit a pronounced flowery, fruity aroma even at low concentrations. When these compounds are present they contribute most of the varietal character, but when they are bound to other molecules

such as in glycosides they are much less volatile and do not contribute to either the aroma or taste.

The grape naturally forms some of the terpenes that are found in wine. The ripeness and quality of the grape may actually be determined by the levels of terpines present (4). During the fermentation the terpene levels may raise due to the liberation of glycosidically bound terpenes that are freed by the yeast or by beta-glucosidase enzyme found in the grape (5). In addition to the liberation of bound terpenes, some species of yeast may actively synthesize new terpenes. *Saccharmyces fermenti* may produce terpenes under fermentation conditions. This has been shown to occur after alcoholic fermentation.

The glycosylation of the terpenic alcohols follow the general pattern of glycosylation found in all alcohols in musts (6). Primary alcohols (ex. geraniol, nerol, or benzyl alcohol) are highly glycosylated and the more sterically hindered secondary and tertiary alcohols (ex. linalool) are much less glycosylated. Hydroxyls that are close to carbon-carbon double bonds (ex. pyran rings or linalool oxides) are almost completely glycosylated unless they are sterically hindered.

Methods for increasing the terpine content, both bound and unbound, that are contributed from the grape are; skin contact, pressing method, heating of the juice, acid hydrolysis, and the use of enzymes. Skin contact can increase the terpine levels due to the fact that most grape produced terpines are found in the skins. This method works best when combined with heat treatment (7,8,9). Higher pressure during pressing may also release terpines. However, it also releases other compounds found within the skins, which may not be desirable (10). Acid Hydrolysis actually involves raising the pH of the wine

from about 3 or 4 to about 5 to allow the indigenous grape enzymes to break down the terpine glycosides (11,12). This de-acidification may have undesirable effects and is probably not a practical approach. Commercial enzyme preparations are actually a mixture of many enzymes with a corresponding mixture of activities. Therefore, a comprehensive understanding of this procedure is currently unavailable since many of variables involved are not yet known (13,14,15). It has also been conjectured that the use of these enzymes may increase the value of a wine immediately after fermentation but then increase the rate of spoiling by an unknown enzyme activity that is still present after bottling.

The biogenesis of terpines by yeast under fermentation conditions has been studied and the first terpene intermediate was found to be geranyl pyrophosphate. This compound arises from isopentenyl pyrophosphate. Geranyl pyrophosphate may be converted directly into nerol or into alpha-terpinol via linalool. Geranyl pyrophosphate may also be used to form sterols by passing through farnesyl pyrophosphate to pre-squalene to squalene and then combining with oxygen to form sterols (5).

Chardonnay wine is known to have low levels of monoterpines, even when the fermentation is inoculated with a strain of yeast known to be capable of producing terpenes when grown on musts from other grapes (6).

2. Alcohols and Aldehydes

The major alcoholic constituent of wine is the psychoactive compound ethanol. This compound is responsible for the intoxication associated with the consumption of wine. Also, ethanol along with the other members of this class of compounds has been

found to be the major taste contributors to wine. Ethanol is also responsible for the final inhibition of the fermentation by the yeast and its bacteriostatic properties may help to prevent the spoilage of the wine.

Alcohols and aldehydes with a six carbon chain length are known to depart a "leafy grassy" quality to the wine (16). These compounds are known to arise enzymatically during aerobic oxidation from linoleic and linolenic acids (17). This class of compounds are known to include; hexanal, (E)-2-hexenal, (E)-2-hexen-1-ol, (Z)-3-hexen-1-ol, (Z)-2-hexen-1-ol, (E)-3-hexen-1-ol, and 1-hexanol (18,19,20).

Generally, the concentrations of these compounds in the wine must are dependent on many factors. The time that the must spends in contact with the skins and leaves of the grape can influence these concentrations as well as the temperature at which the must is stored. The variety and ripeness of the grape can also vary the amounts of these compounds found in the must (21).

The change in concentration of these six carbon alcohols and aldehydes during the fermentation process has been studied (22). The fermentation studied used the *Saccharomyces cerevisiae* strain of yeast. It was found that 1-hexanol and (E)-2-hexan-1-ol were present at higher concentrations than that of (Z)-3-hexan-1-ol. Over the course of the fermentation the 1-hexanol concentrations peaked early, at about 2 days, and then slowly declined to end at a concentration higher than the concentration found in the must. The concentration of (E)-2-hexen-1-ol found in the must was approximately equal to the concentration of 1-hexanol found in the must. However, the (E)-2-hexen-1-ol decreased in concentration over approximately the first 2 days to end up at a level just above zero. This concentration remained constant throughout the rest of the fermentation. The (Z)-3-

hexen-1-ol concentration found in the must was lower than the 1-hexanol and (E)-2-hexen-1-ol must concentrations. However, unlike the two previous compounds, the concentrations of (Z)-3-hexen-1-ol were not significantly changed over the course of the fermentation (22).

The above procedure was carried out in musts that were sterilized/treated with SO_2 and in musts that were not treated. The results from both groups were without significant differences.

Further experiments carried out on this system suggested that the (E)-2-hexen-1-ol was being reduced to 1-hexanol. The evidence for this conclusion was the rapid reduction of (E)-2-hexen-1-ol concentration in the first two days of fermentation coupled with the concurrent formation of 1-hexanol. Fermentations that were spiked with these compounds yielded results consistent with this conclusion. Furthermore, the leafy grassy odor of the wine may be attenuated during fermentation by an increase in 1-hexanol that is formed via this reductive process.

Phenylethanol and benzyl alcohol have been found in grape musts (23). These 2 compounds are normally found bound to glucose. The glycosides of these compounds that have been previously studied are beta-rutinosides and 6-O-alpha-L-arabinofuranosyl-beta-D-glucopyranosides. Benzyl beta-D-glucopyranoside is known to impart a bitter taste. These glycosides may also be substrates for the glycosidases that are known to be present in fermentation vats. Therefore, the amount of free phenylethanol and benzyl alcohol may rise during the course of the fermentation as was discussed earlier in the terpines section.

Other alcohols that have been found in wines and musts include 1-pentanol, 2pentanol, 3-pentanol, 3-methyl-1-pentanol, 4-methyl-1-pentanol, 1-hexanol, 2-ethyl-1-hexanol, 1-heptanol, 2-heptanol, 1-octanol, 2-octanol, trans-2-octenol, 1-octene-3-ol, 1-nonanol, 2-nonanol, 1-decanol, benzylalcohol, 2-phenylethanol, 4-ethylphenol (24), methanol, n-propanol, i-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol (25), 3-oxo-alpha-ionol, vomifoliol, dehydrovomifoliol (26), p-menth-8-ene-1,2-diol (27), p-menth-8-ene-8,9-diol (28), cis-resveratrol, and trans-resveratrol(29).

3. Acids and Esters

Volatile free fatty acids have an acrid, putrid, or rancid smell and typically have a low concentration in the final wine product. A high concentration of free fatty acids may be associated with spoilage of conversion to vinegar. "Acid has a lot to do with the structure of the wine (30)."

However, volatile fatty acid esters and acetate esters impart floral and fruity olfactory properties to wine (31). Fatty acid esters such as, ethyl butanoate, ethyl hexanoate, ethyl octanoate, etc. form from the ethanolysis of acylCoA from fatty acid degradation or synthesis. The acetate esters such as isoamyl acetate, propyl acetate, hexyl acetate, and phenylethyl acetate arise from the reaction of acetylCoA with the higher alcohols that are formed from the degradation of carbohydrates and amino acids.

Many factors affect the formation and degradation of these esters during yeast fermentation. These factors include; the yeast strain (32,33,34,35) and/or fermentation conditions such as temperature, nutrient availability, pH, unsaturated fatty acid levels, sterol levels, and oxygen levels (31,36,37,38,39,40). Esterases are present during

fermentation and participate in enzymatic hydrolysis reactions of the esters. However, during storage and aging where esterases are not present, hydrolysis continues chemically, at a much slower rate (38,41,42).

Studies concerning the formation of volatile esters during the fermentation process have been conducted (43,44,45). The most recent of these studies (45) used an automated SPME/GC/MS headspace analysis to sample the fermentation hourly. It was found that ethyl acetate and isoamyl acetate had similar patterns of production during fermentation. The concentrations of these compounds rose slightly during the first 100 hours after inoculation. Then, at about the midpoint of the exponential growth phase of the fermenting yeast, their concentrations steeply raise and peak at about 180 hours. After the peak, the ester levels remained constant until the end of the fermentation when a second increase in concentration occurred. Finally, after the fermentation was done, these levels dropped rapidly to the previously reported range for finished wines (46).

Hexyl acetate was also studied by Vianna and Ebler (45). It exhibited a slightly different pattern of formation than the above two compounds. The concentration of hexyl acetate in the must was 0.046mg/l. It rose quickly at the beginning of the exponential growth phase of the fermentation. It then peaked at a maximum concentration of 0.1 mg/L on about the eighth day after inoculation. The hexyl acetate peak occurred before the peaks of ethyl acetate and isoamyl acetate. After this highest point, hexyl acetate concentrations dropped to a level of about 0.07 mg/l at about 210 hours after inoculation. Next, a slight increase was noted at about the same time as the ethyl acetate and isoamyl acetate reached their second peak. However, the second peak for the hexyl acetate was not as pronounced as the second peaks for the ethyl acetate and isoamyl acetate. Finally

the concentration of hexyl acetate dropped by day 17 after inoculation to a final level of 0.05 mg/L.

The explanation that Vianna and Ebleler (45) propose for this pattern was that the production of these esters is linked with the production of fusel alcohol, which partly explains the lag noted in their production. However, a better understanding of the production of these alcohols would be necessary to confirm this hypothesis. Also, the activity of alcohol acetyltransferase, the enzyme that catalyzes the formation of both ethyl acetate and isoamyl acetate, is at its highest during the exponential growth phase of the fermentation (42). This explains the rapid increase in the concentrations of these compounds at this point. The decrease toward the end of the fermentation is explained by an increase in the esterase activity (42) during this phase.

Vianna and Ebleler (45) also quantified fatty acid esters that form during fermentaion. The esters studied were ethyl hexanoate, ethyl octanoate, and ethyl decanoate. These compounds have very similar patterns of production and degradation. The major difference was the concentrations of each of these components. The production of the fatty acid esters occurred at the same time as the beginning of the exponential growth phase of the fermentation. Maximum production occurred at about six days, at the midpoint of the exponential phase in the fermentation curve. Concentrations then dropped followed by a slight raise that peaked and then fell. However, ethyl decanoate did not show this second peak. The concentrations of these components fell in the end to levels that were reported previously (46). Ethyl decanoate was found at lower levels than the other fatty acid esters. This is thought to occur because of the difficulty in transporting the compound across the yeast cell membrane.

Vianna and Ebleler (45) also saw Phenethyl acetate and n-propyl acetate but at concentrations below their limits of quantitation. Ethyl dodecanoate, 3-methyl butyl octanoate, 3-methyl butyl decanoate, and ethyl heptanoate were also tentatively identified. These compounds were present throughout the fermentation and their levels dropped at the end of the fermentation. This occurs because the higher weight esters hydrolyze faster than smaller ones, and the transport of the higher weight esters out of the yeast cell is slower due to their size (41).

4. Phenols

Phenol, cresols, and chlorinated phenols were found to be responsible for off-flavors in red wine but some other volatile phenols are valuable flavor compounds (47,48,49). Guaiacol is one of these off-flavor phenols, but some guiacyl compounds have the aroma of clove, smoke, or vanilla. 4-vinylguaiacol is reported to have a clove-like odor and has been found in red wine (50). 4-ethylguaiacol has a higher olfactory threshold than 4-vinylguaiacol (50) but is also found in higher quantities in wine (49). These compounds may come from the oak that is used to contain the wine during aging. These compounds are not found in the musts and are the result of yeast and lactic bacteria action (51). The sources of these compounds are p-coumaric and ferulic acids. These compounds may be decarboxylated by members of the *Saccharomyces* yeast genus to yield the vinylic derivative (52).

Tyrosol is another phenol that is found in white wines. It is derived from the decarboxylation and reduction of the alpha-keto acid corresponding to tyrosine. Found in red wines at a mean concentration of 29 mg/L, white wines at a mean of 22 mg/L, and in

sherry wines at levels higher than 4.9 mg/L (53). When pure it has a weak honey or beeswax-like odor and a bitter taste (54).

Acetovanillone is a phenol with a vanilla-like odor. It comes from the grape and seems to have a weak influence on flavor (55).

B. Method Development

The traditional analytical procedures when applied to monitoring volatile component formation are far to slow, tedious, and laborious. Procedures such as, liquid-liquid continuous extraction, and static or dynamic headspace analysis consumes large amounts of time to complete and also may loose the volatile components that are of interest and therefore have poor sensitivity. This type of procedure typically uses large amounts of ultra high purity solvents such as pentane, Freon 11, or Freon 113 (32,43,56,57,58,59,60). These solvents are both costly to purchase as well as to dispose of.

Stashenko (43) developed another wine analysis method that combined the purging and extraction of volatiles from wine into a single apparatus. This technique still required a timely solvent extraction process. This method was employed to look at the formation of volatile esters during the fermentation process but due to the slow extraction process the data was sparse. However, this is one of the first studies to investigate the formation of volatile compounds in the wine making process.

A recent innovation in sample processing, Solid Phase Micro Extraction (SPME) has overcome all of the before mentioned problems. It uses no solvent at all, is fairly quick, has low amount of preparative work before sampling, and most importantly can be

automated. SPME has been used in the analysis of a wide variety of compounds to include volatiles in foods and beverages. It has been used in the last few years to analyze the volatile components in wine (44,61,62,63,64,65,66,67,68,69,).

The SPME process involves inserting the SPME fiber, which is specially coated, into the sample and allowing enough time to pass so that the fiber can adsorb and/or absorb the compounds of interest. The fiber is made of fused silica that is coated with a polymeric coating. This coating may be selected to match the polarity of the compounds of interest so that the fiber can selectively concentrate them. SPME may be used to analyze both the liquid sample itself or it may be used to sample the headspace above the sample. Vianna and Ebleler (45) used the SPME technique in the headspace mode in their study. The fiber was placed directly above the must inside the fermentation vessel itself. In this way they were able to see what volatiles were being given off during the fermentation but they were not able to see what was in the must itself. Since these analytes are volatile the headspace should be close to equilibrium with the must but the variable amount of CO_2 that is being given off may tend to dilute the headspace to different levels at different times during the fermentation.

CHAPTER II

Statement of the Problem

The art of wine making is ancient but until the advent of modern scientific methods the process that yields a good wine has been shrouded in mystery. The methods used to make wine were handed down from generation to generation as a tradition with no real understanding of the intricate nature of the fermentation happening within their vats. Any advances in the technology of wine making were made by trial and error and slowly incorporated into the tradition.

With the appearance of modern analytical chemical instrumentation the shroud can finally be lifted. Extraction techniques such as continuous (Soxhlet) extraction, solid phase extraction, and solid phase micro extraction can be used to prepare a sample of wine by providing a rudimentary separation and by concentrating the analytes of interest. Then chromatographic techniques such as HPLC and GC can separate this complex mixture of compounds. Also, various detection techniques such as FID, ECD, TCD, and PID can detect these chemicals after separation. Finally, Spectrophotometry and mass spectrometry produce data that can be used to positively identify these chemicals while detecting their elution. By employing different combinations of these techniques great strides have been made in the understanding of what wine is, as well as how it is formed.

Many chemicals have been found in finished wine; some at the macroscopic level (ppt), and many more at the microscopic level (ppm, ppb, and pptr). As an understanding of what chemicals are present in wine and what their sensory threshold for human detection unfolds, the winemaker finally finds him or herself facing definable goals.

What is desirable is still a matter of taste but what makes up a certain flavor is now well on the way to being understood. Since the human sensory experience is the defining goal for these experiments, most of these experiments have only focused on the compounds that exist in wine at or above the threshold of detection for human taste or smell.

An issue that has recently received a large amount of attention from scientists interested in wine making is how the yeast bio-transform grape musts into wine. Many studies have been conducted to trace biochemical pathways and to determine the origin of compounds in wine, whether directly from the grape or compounds that were bio-transformed by the yeast.

When looking at the final product only compounds that were above the human sensory threshold were of interest. However, in a biological system, such as yeast in fermentation, many seemingly unrelated factors may influence each other. Due to this fact, all compounds that exist within the fermentation vat are of interest to the winemaker in trying to control the flavor of the final product. A tasteless compound may give rise to a desirable flavor component and therefore it should be bred for in the grapes or yeasts. A bad tasting undetectable chemical may be the breakdown product of another flavor component and therefore the yeast should be bred to remove the gene that forms the protein that catalyses the breakdown reaction. It may also be possible to control fermentation conditions to persuade the yeast to produce or not to produce certain chemicals.

Whether special breeding (of the grapes or yeasts) or close monitoring and control of vat conditions is chosen as the answer, or simple spiking is used to augment the final flavor, an understanding of yeast biochemistry will benefit the wine industry. Although, it

should be noted that simply spiking the product would not remove any undesirable component, this must be accomplished by more complex methods such as those mentioned earlier.

With this in mind, this project followed the formation of the volatile flavor components of an Ohio Chardonnay fermented with *Saccharomyces bayanus* and an Ohio Chardonnay was allowed to ferment spontaneously. Any other compounds that are detectable and identifiable were also followed. The method of choice for this project was SPME/GC/MS. Samples were taken from the fermenter on a daily basis and frozen until analysis. An internal standard was added to account for variation in the adsorption of the SPME fiber. The ratio of the analyte's response in the MS to the MS response of the internal standard was plotted against the time in days that the fermentation had been working. Even though many other studies have used this strategy in the past, they have always focused on only one class of compound such as esters, fatty acids, alcohols etc. This study was the first to combine members from many different classes to elucidate the overall timeline of the formation of volatiles during the fermentation. Since chemical precursors are expected to occur before their products, this study will allow for the design of further experiments to further the understanding of the biochemistry of wine fermentation. Also, the differences between spontaneous verses inoculated fermentation was be explored.

Therefore, this project was meant to be the first step into understanding the volatile component biochemistry of wine fermentation as a whole. This project was also meant to be a pioneering study, which hopefully will help to guide future inquiry into this subject.

CHAPTER III

Materials and Methods

A. Materials for Analysis

85 μm polyacrylate SPME fibers and a 30 m x 0.25 mm x 0.25 μm film thickness SUPELCOWAX^{TM}-10 Capillary Column was purchased from Supelco Inc. (Bellefonte, PA). Helium was used as the carrier gas and was of ultra high purity grade. It was purchased from Praxair (Cleveland, OH). 12mm x 32mm (2mL) clear screw top vials were purchased from Alltech (Deerfield, IL).

All chemicals used in this work were of the highest purity available and were used as received. Sodium chloride was purchased from Sigma (St. Louis, MO). The 3-decanol was purchased from Lancaster (Pelham, NH). Ethyl alcohol, 200 proof, was purchased from (Pharmco Brookfield, CT). The water used was obtained from a Modulab laboratory reagent grade Type I HPLC water purification unit from Continental Water Systems. All water used measured at least 18 megaohms/centimeter to ensure purity. The chemicals used as standards for identification of peaks in the chromatograms were purchased from Fluka (Milwaukee, WI), Aldrich (Milwaukee, WI), Sigma (St. Louis, MO), and AccuStandard Inc. (New Haven, CT). All these compounds with their associated sources are listed in the Table 2 found in the "Standard Preparation" section.

B. Fermentation

The fermentations were carried out by Dr. John D. Usis, Ph.D. From the Dept. of Biological Sciences, Youngstown State University, Ohio.

Six fermentations were carried out, three spontaneous and three inoculated in 9L fermenter Bottles. The must was obtained from Markko Vineyards (Conneaut, OH). The spontaneous fermentations were carried out without any additives at about 20 C. The inoculated fermentation was treated with 25ppm SO_2 and allowed to settle overnight at 20 C. Then it was inoculated with 2g/gal of *Saccharomyces bayanus* var. Premier Cuvee, Red Star brand, manufactured by Universal Foods Corporation (Milwaukee, WI). One spontaneous fermentation and one inoculated fermentation were used in this study.

C. Methods

1. Chromatographic Methods

A Varian 3800 Gas Chromatograph equipped with an 8200 Autosampler and a 2000 Mass Spectrometer was used to collect the chromatographic data. The GC contained a 1076 split/splitless injection port that was operated in the splitless mode for SPME.

SPME/GC/MS parameters were adapted from and slightly modified from De la Calle Garcia et al. (35,36). The most significant modification was the addition of a heated sample tray, a Varian Autotherm. This allowed the samples to be kept at 60 C during the adsorption/absorption phase of the SPME process.

Table 1. Analytical Method Parameters (SPME/GC/MS)

SPME Parameters	
Fiber	85 μm polyacrylate
Adsorption/Absorption Time	30 min with vibration
Desorption Time	5 min
Desorption Temperature	300 C
Sample Temperature	60 C
GC Parameters	
Injection Port Temperature	300 C
Column	30 m x 0.25 mm x 0.25 μm film thickness SUPELCOWAXTM-10 Capillary Column
Temperature Ramp	30 C (5 min) → 200 C (1 °/min) → 210 C (20 °/min)
Carrier Gas	Helium Ultra High Purity
Carrier Gas Pressure	80 kPa
MS Parameters	
	(C)
Trap	150
Manifold	80
Transfer-line	170

2. Quantitation

Total ion data were collected for all standards and samples. Peak areas were integrated using single ion data. This allowed for overlapping peaks to be accurately quantified. The peak areas of each analyte were divided by the peak area of the internal standard (3-decanol) and used to form the concentration verses time graphs.

3. Identification of Unknown Compounds

The compounds listed in Table 2 in the "Standard Preparation" section were identified by comparison of their retention times with that of the known compounds. The identity of the compound in the chromatogram of the standard solution was confirmed by comparison of its' mass spectrum with that of the mass spectrum found In the 1992 NIST library.

Further compounds were identified by comparison of their retention times with the retention times found in the literature and by comparison of their mass spectrum with those found in the 1992 NIST library.

4. Standard Preparation

The standards used in identification of peaks in the chromatogram were prepared by first making a 200 proof ethyl alcohol stock solution in the ppt range. The stock solution was diluted by taking 1 μL of the ethanolic stock solution and adding 99 μL ethanol and 0.9 mL Modulab water to yield a solution on the ppm level in a matrix of 90% water and 10% ethanol. Table 2 includes all the chemicals that were used to test the sensitivity of the method. Table 2 displays the source of the chemicals and the exact concentration analyzed. The entries in this table that are surrounded by quotation marks are the names given to the stock solutions that were used. Under each category name is the list compounds that were run simultaneously except the terpines, which were all, run one at a time.

Table 2. Chemical Standards with Source and Concentration

Compounds	Source	[Standard] (ppm)
"N.C. Mix"		
R-Limonene	AccuStand.	1.25
Linalool	AccuStand.	1.85
Linalyl Acetate	AccuStand.	2.27
Cinnamyl Alcohol	AccuStand.	2.61
Acetophenone	AccuStand.	2.07
Napthalene	AccuStand.	1.67
Phenylethyl Acetate	Fluka	2.14
"Esters"		
Ethyl Acetate	AccuStand.	3.68
Ethyl Propionate	AccuStand.	3.68
Ethyl Butyrate	AccuStand.	3.68
Ethyl Valerate	AccuStand.	3.68
Ethyl Capronate	AccuStand.	3.68
Hexyl Acetate	Aldrich	3.69
Diethyl Succinate	Aldrich	3.69
i-Amyl Acetate	Aldrich	3.92
"Alcohols"		
1-Propanol	AccuStand.	1.56
2-Methyl-1-Propanol	AccuStand.	1.56
1-Butanol	AccuStand.	1.57
4-Methyl-2-Pentanol	AccuStand.	4.603
1-Pentanol	AccuStand.	4.589
2-Ethyl-1-Butanol	AccuStand.	3.200
3-Methyl-1-Butanol	Aldrich	1.96
Glycerol	Sigma	0.680
cis-3-Hexen-1-ol	Sigma	1.97
"Ketones"		
Diacetyl	Sigma	1.00
gamma-Butyrolactone	Aldrich	1.00
"Acids"		
Propanoic Acid	AccuStand.	3.87
Butyric Acid	AccuStand.	3.87
Valeric Acid	AccuStand.	3.87
Caproic Acid	AccuStand.	3.68
Heptanoic Acid	AccuStand.	3.87
i-Butyric Acid	Aldrich	3.96

Table 2. Chemical Standards with Source and Concentration (cont.)

"R1"		
2,5-Dimethyl-4-Hydroxy-3-(2H)-Furanone	Fluka	2.00
trans-2-Hexen-1-ol	Aldich	1.92
2-Furaldehyde	Aldich	2.00
"R2"		
(2R,3R)-(-)-2,3-Butanediol	Aldich	1.94
2(5H)-Furanone	Aldich	1.96
trans-3-Hexen-1-ol	Aldich	1.96
"Terpines"		
Linalool	Aldich	9
beta-Citronellol	Aldich	11
alpha-terpineol	Aldich	56
Nerol	Aldich	8
Geraniol	Aldich	36
Linalool Oxide	Fluka	18

5. Sample Preparation

Approximatly 300 mg sodium chloride was added to a 2 mL clear vial followed by 1 mL of wine and 10 μL of a 100 μg/L of 3-decanol. Solid salt remained undissolved in the sample vials; therefore these solutions were known to be saturated. A screw top cap with Teflon lined septum was used to seal the vial.

6. Bake out

To minimize interferences from the instrument itself, such as the accumulation of previous samples and the build up of nitrogen from the air onto the SPME fiber, the following bake out procedures were implemented.

a. Mass Spectrometer

The MS portion of the instrument was baked out at the beginning of each week. After each MS bake out the instrument was run for approximately five days. During the bake out procedure the GC portion of the instrument is maintained at the initial analytical conditions. The MS trap was heated to 250 C, the manifold was heated to 120 C, and the transfer-line was heated to 170 C for 16 hours.

b. SPME fiber and Gas Chromatograph

The SPME/GC portion of the instrument was baked out before running any sample after a pause in the processing of the samples. This was not only done to ensure that the fiber was purged of any compounds that may have adsorbed onto it from the air, but to also ensure that the column was cleared of any interferences that may have built up during the period of inactivity. Furthermore, before any samples were analyzed 3 blanks were run to condition the fiber and to stabilize its' extraction efficiency. The blanks consisted of 1mL of water and approximately 300 mg of sodium chloride. The injection port was heated to 300 C and the column was heated to 220 C for the bake out which lasted for 10 min. with the fiber inserted into the injection port. Finally, the blanks were run using the same method as that used for a standard sample run.

CHAPTER IV

Results and Discussion

A. Method Capabilities

The SPME/GC/MS method was able to extract, separate, and detect over 300 peaks in some of the sample chromatograms. Comparing these chromatograms to one that was obtained from a blank (water and sodium chloride) and subtracting the number of peaks in the from the blank from the number in the sample it was found that over 200 volatile compounds from the fermentation were detected with this method. The heating of the sample seems to greatly enhance the ability of the fiber to extract compounds from the sample over the same method, unheated. This enhancement was noted in both the intensity of peaks and in the number of peaks that were detectable.

In a situation where a peak of interest was co-eluting with another compound an accurate quantification was still possible by using a quantitation ion from the mass spectrometer that was unique to the compound of interest. This technique further enhanced the separation and identification power of this method.

In order to deal with the peaks that were present in the blank chromatogram, the largest ones found were added to the compound table of the method so that they would not be confused with a legitimate compound from the sample when the computer analyzed the sample data. These peaks from the blank probably originate from either the fiber, septum, or column bleed.

To identify the peaks present in the samples, the retention times of the compounds in the literature (62) were compared to peaks in the sample chromatogram. Then the mass

spectrum of the compound comprising the peak was compared to the 1992 NIST library to confirm the assignment. One compound, butanedioic acid, was tentatively identified by matching only with the 1992 NIST library.

Peaks tentatively identified by comparison with the 1992 NIST library are listed in Table 3 with their retention time and the quantitation ion used.

Table 3. Compounds tentatively identified by the 1992 NIST library only

Compounds	RT (min.)	Quantitation. Ion (m/z)
butanedioic acid	74.370	39

Peaks identified by comparison of retention times with literature values and then confirmed by comparison with the 1992 NIST library are listed in Table 4 with their retention times and quantitation ion used.

Table 4. Compounds tentatively identified by the 1992 NIST library and retention times from literature

Compounds	RT (min.)	Quantitation Ion (m/z)
3-Methyl-1-Pentanol	36.557	85
Ethyl Octanoate	45.821	88
2-(2-ethoxyethoxy)-Ethanol	67.922	45
Ethyl Decanoate	70.418	88
Eicosene	106.466	69
Decanoic Acid	153.936	129
Ethyl octandecanoate	150.202	312

Then to increase the list of identified compounds, the authentic compounds were diluted to the low ppm level in a matrix consisting of 90% water and 10% ethanol,

saturated with sodium chloride, and run using the same SPME/GC/MS parameters as those used to run the samples. Then the peaks in these chromatograms were identified using the 1992 NIST library. This was necessary since more than one compound was run at a time for the identity standards. Some compounds were not detectable at low ppm levels. The compounds that were detectable and identifiable are listed in Table 5 with their respective retention times and quantitation ions. The entries in this table that are listed without retention time and quantitation ion entries were not detectable at the concentration listed in this table. These compounds were most likely not detectable do to the SPME fiber type used. The use of a different fiber may increase the extraction efficiency to detectable limits.

Table 5. Chemical Standards with Retention Time and Quanitation Ion

Compounds	[Standard] (ppm)	RT (min.)	Quanitation Ion (m/z)
"N.C. Mix"			
R-Limonene	1.25	80.243	208
Linalool	1.85	60.920	93
Linalyl Acetate	2.27	76.613	121
Cinnamyl Alcohol	2.61	135.051	134
Acetophenone	2.07	69.609	105
Napthalene	1.67	78.481	128
Phenylethyl Acetate	2.14	89.130	104
"Esters"			
Ethyl Acetate	3.68		
Ethyl Propionate	3.68		
Ethyl Butyrate	3.68		
Ethyl Valerate	3.68		
Ethyl Caproate	3.68		
Hexyl Acetate	3.69		
Diethyl Succinate	3.69	75.092	39
i-Amyl Acetate	3.92		

Table 5. Chemical Standards with Retention Time and Quantitation Ion (Cont.)

"Alcohols"			
1-Propanol	1.56		
2-Methyl-1-Propanol	1.56		
1-Butanol	1.57		
4-Methyl-2-Pentanol	4.603		
1-Pentanol	4.589		
2-Ethyl-1-Butanol	3.200		
3-Methyl-1-Butanol	1.96		
Glycerol	0.680		
cis-3-Hexen-1-ol	1.97		
"Ketones"			
Diacetyl	1.00		
gamma-Butyrolactone	1.00		
"Acids"			
Propanoic Acid	3.87		
Butyric Acid	3.87		
Valeric Acid	3.87	83.662	208
Caproic Acid	3.68	94.815	60
Heptanoic Acid	3.87	105.697	122
i-Butyric Acid	3.96	64.340	103
"R1"			
2,5-Dimethyl-4-Hydroxy-3-(2H)-Furanone	2.00		
trans-2-Hexen-1-ol	1.92	40.563	45
2-Furaldehyde	2.00		
"R2"			
(2R,3R)-(-)-2,3-Butanediol	1.94		
2(5H)-Furanone	1.96		
trans-3-Hexen-1-ol	1.96		
"Terpines"			
Linalool	9	60.920	93
beta-Citronellol	11	86.024	341
alpha-terpineol	56	77.457	136
Nerol	8	89.074	81
Geraniol	36	94.353	81
Linalool Oxide	18	trans-46.046	
		cis-49.565	40

The fiber used for the SPME portion of the method had a polyacrylate coating. This polyester phase has an intermediate polarity. This fiber proved useful at extracting non-polar compounds from a matrix consisting of 90% water and 10% ethanol saturated with sodium chloride, which is very polar. This can be explained by the fact that the non-polar compounds prefer to adhere to the moderately polar surface of the fiber as opposed to the ionic matrix, whereas the more polar compounds would prefer the ionic matrix.

The terpines had to all be run separately as standards due to their similar structure and same molecular weight. All the terpines used had 10 carbons and most had a single hydroxyl functionality. All of the terpines were extracted at levels sufficient enough to detect and identify when present in the matrix at the levels listed in Table 5 in the methods section. This large amount of non-polar structure as compared to the single hydroxyl allowed for efficient extraction. The linalool oxide chromatogram shows two peaks due to the fact that it is a diasteriomeric mixture. The cis peak was chosen for quantitation. This peak was choosen for quantitation because of other compounds coeluting with the other peak.

The carboxylic acids were fairly well extracted by the polyacrylate fiber. All were identified except the 3 smallest, acetic, propionic, and butyric acids. These 3 having the least amount of non-polar hydrocarbon structure. In addition to the acids identified by comparison with external standards (see list in Table 5), decanoic acid was identified by comparison of the literature retention time value with the compounds retention time and then confirming its' identity by comparison of its' mass spectrum with the 1992 NIST library.

Esters were adsorbed/absorbed to a much lesser extent on the polyacrlyate SPME fiber and only diethyl succinate could be identified. The esters that could not be identified all contained 8 carbons or less. Esters that contained more than 8 carbons were identified in the sample chromatograms by comparison of their retention times with literature values and the 1992 NIST library. Ethyl octanoate, ethyl decanoate, and ethyl octadecanoate were identified in this manner. Their long hydrocarbon tails allowed them to be extracted by the polyacrylate fiber even when the smaller esters were not absorbed.

Alcohols were also adsorbed/absorbed poorly on the polyacrylate SPME fiber, only 4 of the standard compounds could be identified. Cinnamyl alcohol, trans-2-hexen-1-ol, trans-3-hexen-1-ol, and cis-3-hexen-1-ol were the only alcohols with enough non-polar hydrocarbon structure to be extracted at high enough levels to be identified. In addition to the 4 alcohols confirmed by injecting authentic standards, 3-methyl-1-pentanol was identified by its' mass spectrum and retention time in the total ion chromatogram.

The only ether that was found albeit an ether alcohol was 2-(2-ethoxyethoxy)-ethanol. This compound is basically a polymer of diethyl ether, which is relatively non-polar. This compound was found by matching its' elution time to that previously reported in the literature and by comparison of its' mass spectrum with the 1992 NIST library. No simple ethers were run as external standards.

The only ketone that was identified from the external standards was acetophenone. The only other ketone that was run as a standard was diacetyl. The acetophenone has a phenyl ring attached that is non-polar enough to be extracted out of the matrix by the SPME fiber. Diacetyl has 2 carbonyl groups and only 4 carbon atoms.

In this compound, the polar carbonyls overwhelm the small non-polar methyl groups, making for a very polar molecule which is not selectively extracted.

The compounds from AccuStandard in the N.C. mix all adsorbed/absorbed well and could all be detected and identified. Most of these compounds were simple hydrocarbons. Note that not all compounds in the standard mixes from Accustandard (such as Naphthalene) were expected to be present in the wine samples. Phenethyl Acetate was added to the N.C. Mix; it was also detected and identified. All the compounds in this group were fairly non-polar with large portions of hydrocarbon structure.

3-Decanol and 3-octanol were both run simultaneously as an internal standard. The 3-octanol could only be recovered in high enough levels in a few of the sample runs. Both of these compounds were spiked into the samples at the 100 ppb level. Also, when 3-ocanol was detectable, it always had a lower response than that of the 3-decanol. This is probably due to the fact that the 3-decanol has a two-carbon longer hydrocarbon chain than 3-octanol, making easier to extract with the fiber. Therefore, in the later runs used in this study, the 3-octanol was abandoned as an internal standard and only 3-decanol was added.

All the concentrations of the compounds are reported as a ratio of their chromatographic peak area relative to the chromatographic peak area of the 3-decanol internal standard. This use of an internal standard will account for any variation in the extraction efficiency or recovery from the fiber.

B. Volatile Compound Formation

1. Yeast Growth

The yeast growth study was carried out by Lynnette L. Thomas, Graduate Student in the Dept. of Biological Sciences at Youngstown State University.

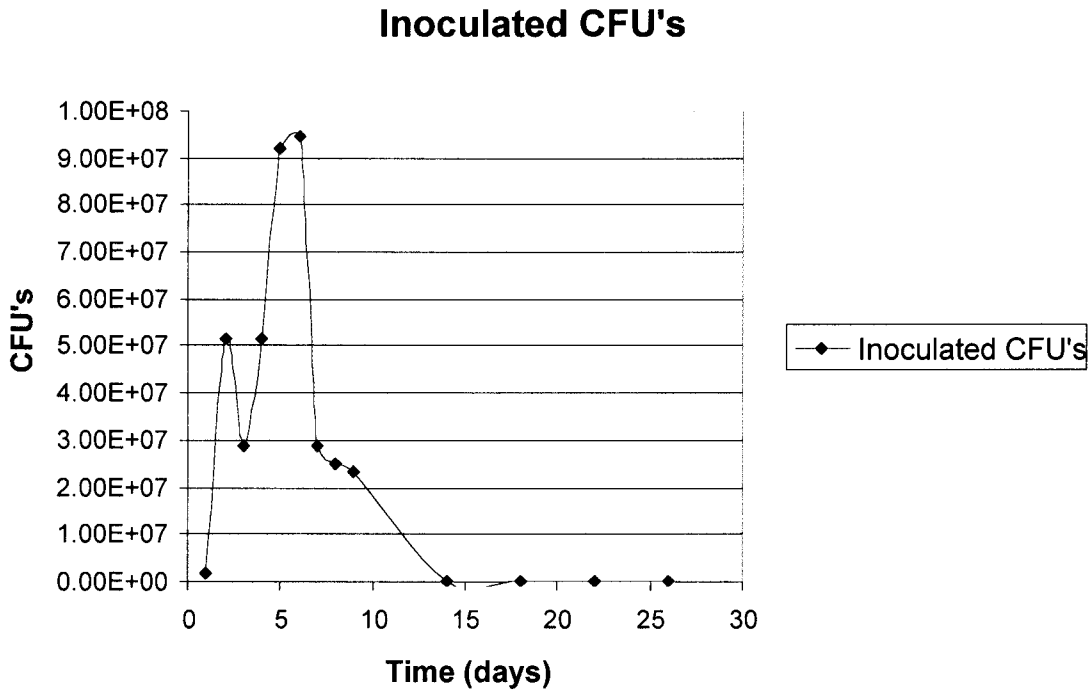

Figure 1. CFU's vs. Time for the Inoculated (*Saccharomyces bayanus*) Fermentation

The colony forming units (CFU) is a rough measure of yeast growth. Figure 1 demonstrates that the inoculated yeast fermentation's experienced a rapid growth spurt (expositional growth) up to the peak at day about 6. Between days 5 and 6 the fermentation reaches the stationary growth phase which is followed by a rapid cell death phase. By day 14, the culture reaches the cell death phase (at about 0 CFU's on the graph) and stays there throughout the rest of the fermentation. This information is summarized in Table 6.

Table 6. Inoculated Yeast Culture Growth Phases

	Day
Initial Level	0
Exponential Phase	0-5
Stationary Phase	5-6
Cell Death Phase	6-14
Zero CFU	14-26

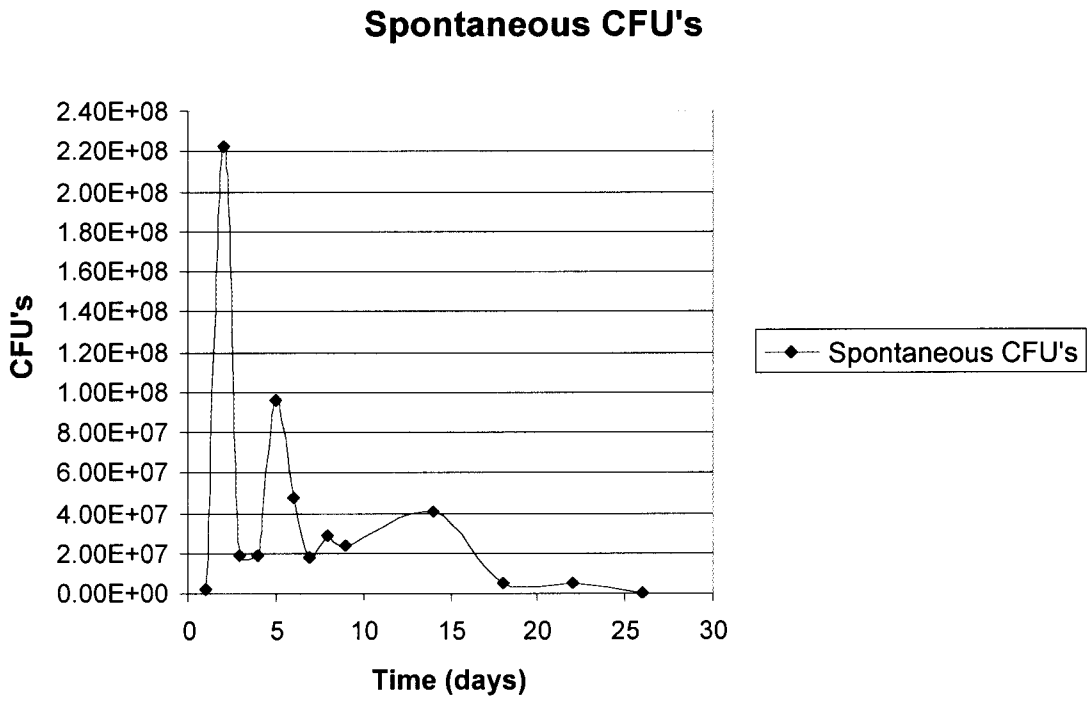

Figure 2. CFU's vs. Time for the Spontaneous Fermentation

The spontaneous yeast fermentation experienced a much faster onset of the exponential growth phase as seen in Figure 2. However, the growth only lasted for about one day. The peak of the spontaneous fermentation was slightly higher than the peak of the inoculated fermentation. The stationary phase of this fermentation was very short, lasting less than a day. Then, the cell death phase was rapid and was about down to zero CFU's on day 18.

Table 7. Spontaneous Yeast Culture Growth Phases

	Day
Initial Level	0
Exponential Phase	0-2
Stationary Phase	2
Cell Death Phase	2-18
Zero CFU	18-26

Table 7 refers to the overall CFU values. This is not a complete picture for the spontaneous fermentation, due to the presence of at least 3 different types of yeast. A further breakdown of the yeast population is possible with some preliminary data obtained from identifying yeast cultures taken from the CFU plates. Based on phenotypical differences, 5 samples of each phenotype was taken and grown up. Then each sample was identified by carbon and nitrogen assimilation tests. This data was plotted in Figure 3 to provide an approximate insight into the yeast distribution. The 3 yeast species identified in the first six days were *Pichia membranifacieus*, *Hanseniaspora Uvarum*, and *Saccharomyces bayanus*.

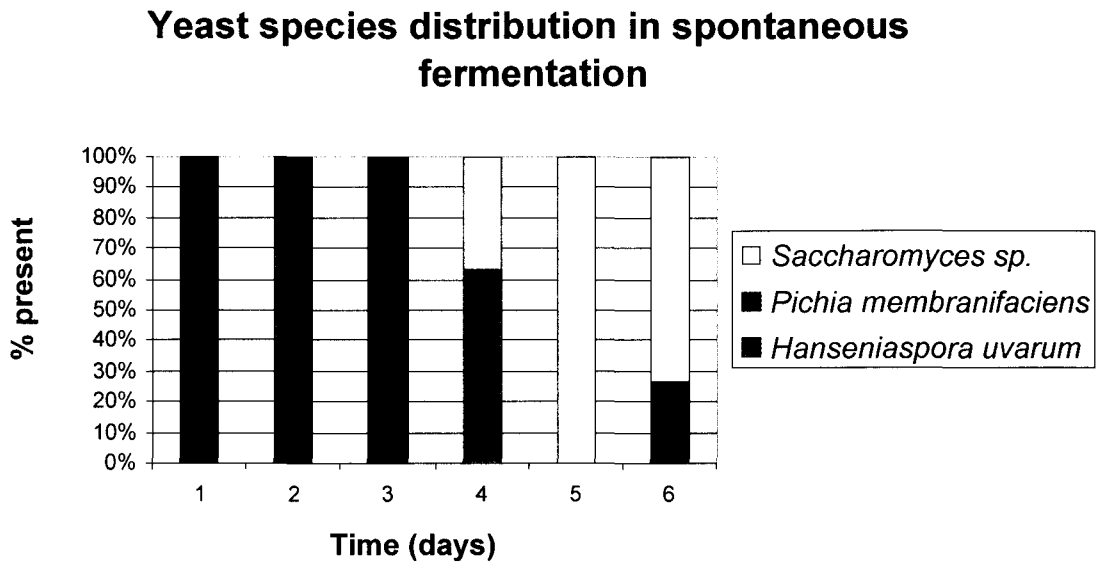

Figure 3. Yeast Species Distribution in Spontaneous Fermentation

The rest of this section will deal with the relationship of the yeast growth to the chemicals formed using the overall growth data; not using the separate growth data. If more complete and accurate data were available a more in depth study using the data for each of the separate yeast species would be possible.

2. Terpines

No Terpines were found in the inoculated or the spontaneous samples at the levels listed in the methods section. The terpines that were run as a standards include; linalool, beta-citronellol, alpha-terpinol, nerol, geraniol, and linalool oxide. The concentrations of these in the standard solution range from 9 to 56 ppm. The exact concentrations of these standards are listed in Table 5. This result agrees with what has been reported in the

literature, as Chardonnay wines are not expected to contain any appreciable amounts of terpines (6).

3. Acids

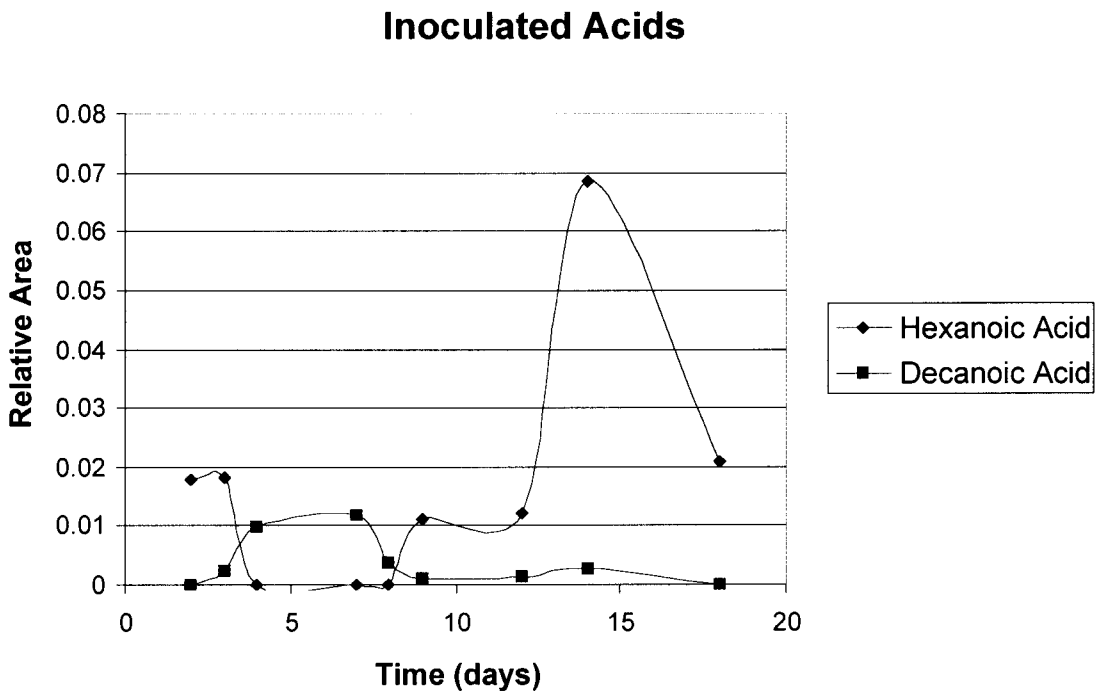

Figure 4. Relative Area vs. Time for Inoculated Acids

In the inoculated fermentation, only hexanoic acid and decanoic acid were detected. The hexanoic acid dropped from its' initial concentration at day 3 to about 0 relative peak area at day 4. This occurred during the exponential growth stage of the yeast fermentation. The hexanoic concentration remained at this level until day 8 when it started to increase. Then by day 12 the concentration of hexanoic acid abruptly rose to its' peak and then began to drop. The increase in hexanoic acid roughly corresponds to the cell death phase of the fermentation and the drop in its' concentration occurred at the

same day as the CFU's dropped to 0. The decanoic acid concentration rose from initial concentrations between day 3 to 4 and remained there until about the day 7. Then it dropped back to about the initial concentrations by day 9. This was roughly the inverse of the hexanoic acid concentration during this time period. Then, at day 14 the decanoic acid concentration rose slightly and then returned to the initial concentration. This slight rise occurred concurrent with the concentration increase of the hexanoic acid.

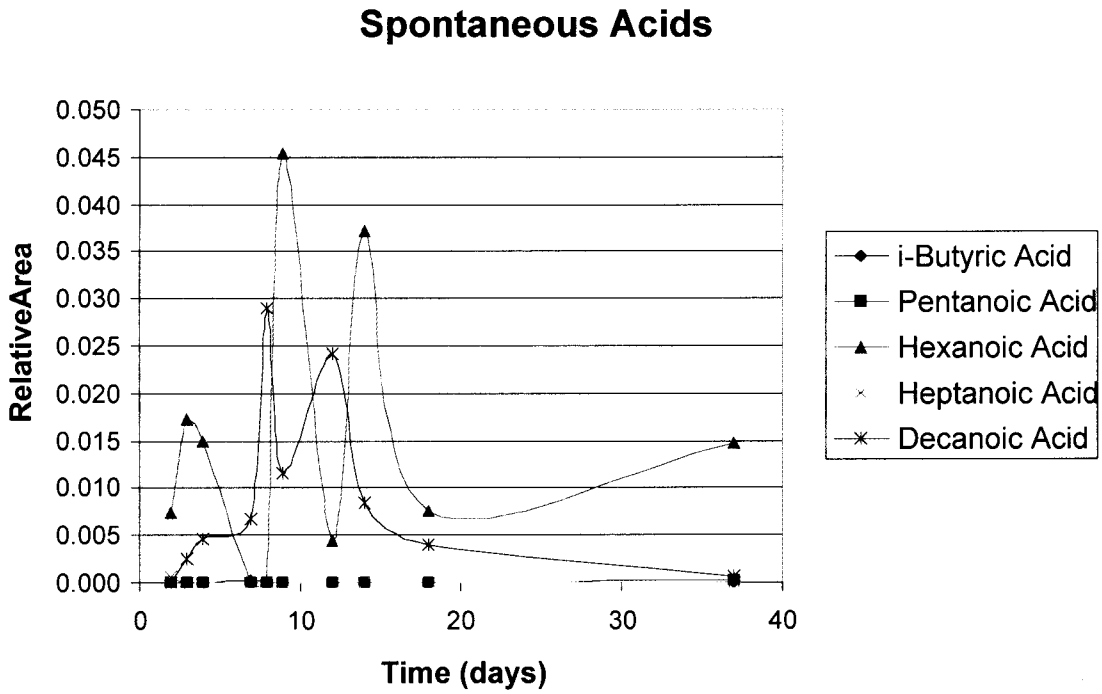

Figure 5. Relative Area vs. Time for Spontaneous Acids

In the spontaneous fermentation many more acidic compounds were found. Hexanoic and decanoic acids were still the major compounds present. The other compounds found were iso-butyric acid, pentanoic acid, and heptanoic acid; these compounds were only detected sporadically at very low concentrations. The major

components started and ended at roughly the same concentrations as in the inoculated fermentation. However, during this spontaneous fermentation the pattern of their occurrence was totally different. The concentrations of both hexanoic and decanoic acid rise initially during the first 3 days of the fermentation, then they began rising and falling erratically until about day 18. The initial rise was during the exponential growth phase and the rest of this time was in the cell death phase. During this period their concentrations peaked 2 or 3 times but not at the same time for the two acids. The hexanoic acid peaked at day 3, 9, and 14. The decanoic acid peaked at 8 and 12. After day 18 the concentration of hexanoic acid slowly rose while the concentration of decanoic acid slowly dropped to about 0 relative peak area.

4. Esters

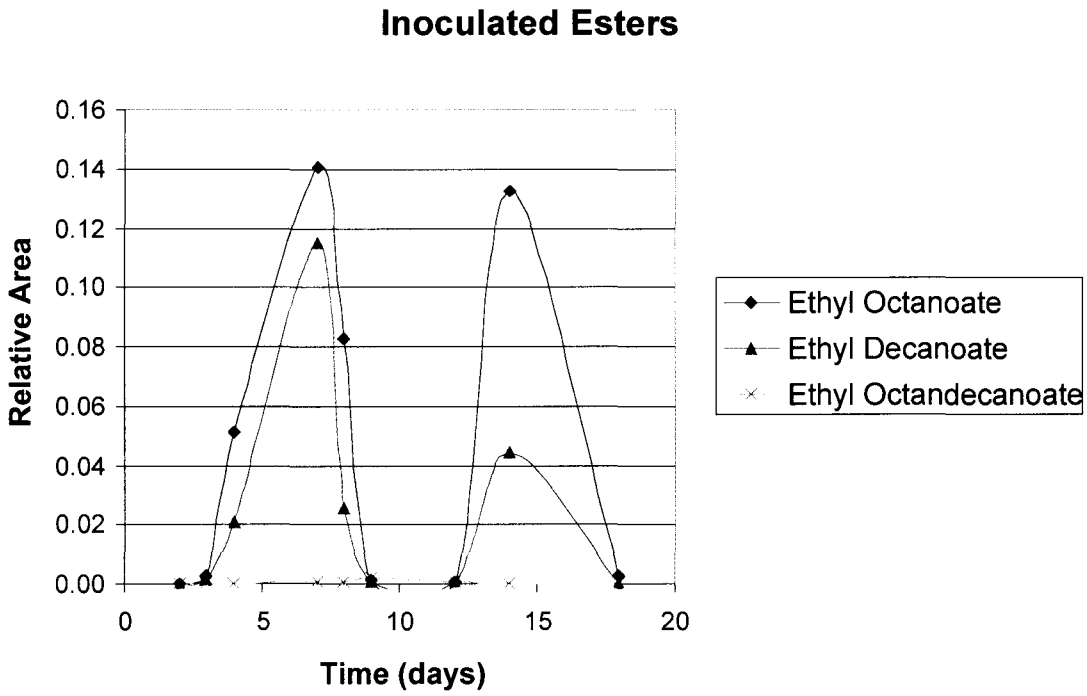

Figure 6. Relative Area vs. Time for Inoculated Esters

Three esters were detected in the inoculated fermentation. The ethyl octadecanoate was only detected in about half of the samples and had the weakest response of all the ethyl esters that were present. The 2 major esters detected were ethyl octanoate and ethyl decanoate. These 2 compounds follow about the same pattern of formation and degradation within the fermentation. They both start out at about 0 relative area concentration and then rise over the first 7 days to their first maximum. This corresponds to the exponential and stationary phases of cell growth. Then both ester concentrations drop to about 0 relative area by day 9. At day 12, which is near the end of the cell death phase, their concentrations both rise again. This time the ethyl octanoate raises to about the same concentration as the first ethyl octanoate maximum. However the concentration of ethyl decanoate only raises to about half of its' initial maximum at day14. By day 18 both the ethyl octanoate and ethyl decanoate concentrations drop to about 0 peak area counts. The increase observed in the decanoic acid concentration roughly coincides with the first increase in the ethyl decanoate concentration. This two peak pattern was also noted in the study by Vianna and Elbeler (45).

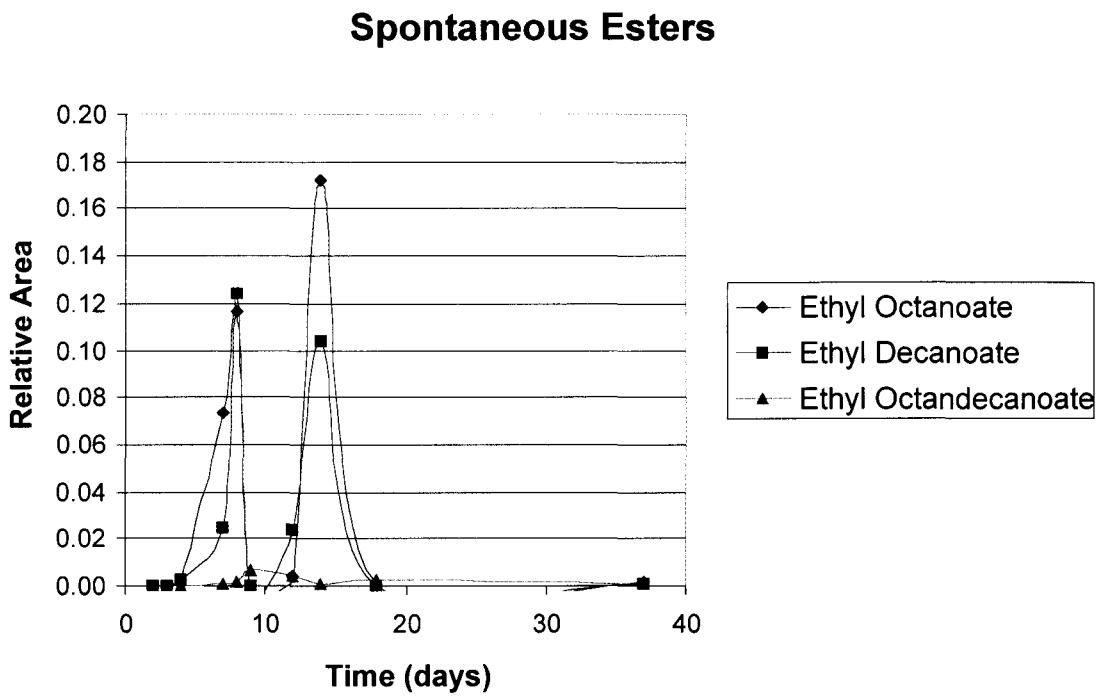

Figure 7. Relative Area vs. Time for Spontaneous Esters

The same three esters were detected in the spontaneous fermentation and a very similar pattern was observed for all three of them. The ethyl octadecanoate was only a minor component although it was detected at higher levels than in the inoculated fermentation. The same two peak pattern was seen for both ethyl octanoate and ethyl decanoate. Although, in this fermentation the second peak concentration for both of these esters was higher. Other differences were the timing of the peaks with reference to the cell growth phase of the fermentation. In this fermentation both of the peaks occurred within the cell death phase. The peak times in the decanoic acid concentrations in this fermentation roughly match the peaks in the ethyl octanoate concentration.

5. Alcohols

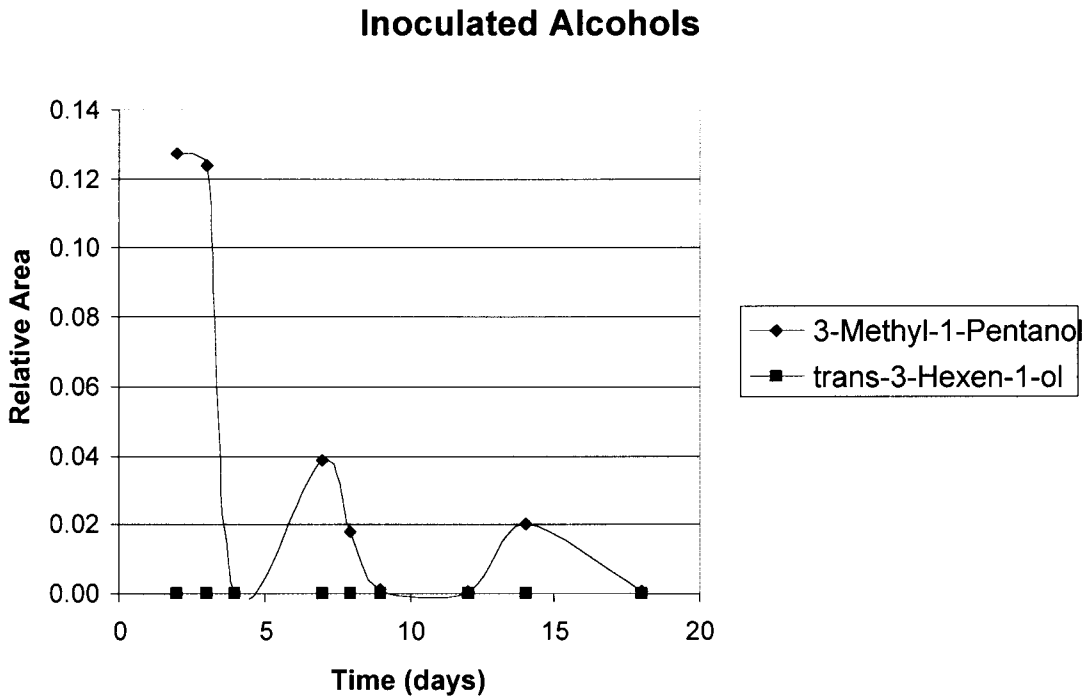

Figure 8. Relative Area vs. Time for Inoculated Alcohols

Only two alcohols were detected in the inoculated fermentation. Trans-3-hexen-1-ol was only detected in one sample and in very low concentrations. 3-methyl-1-pentanol, however, was detected in a large enough amount to detect a pattern in its' formation and degradation. The 3-methyl-1-pentanol level initially begins at the highest level seen during the fermentation. Then it drops to about 0 relative area by day 4 and then oscillates between a peak value and zero twice and finishes at 0 relative peak area. The first drop in concentration of 3-methyl-1-pentanol occurs during the exponential phase of cell growth. The next peak forms during the stationary cell growth phase, and peaks at the start of the cell death phase. The second peak forms during the cell death phase and peaks on the first day when there are 0 CFU's present.

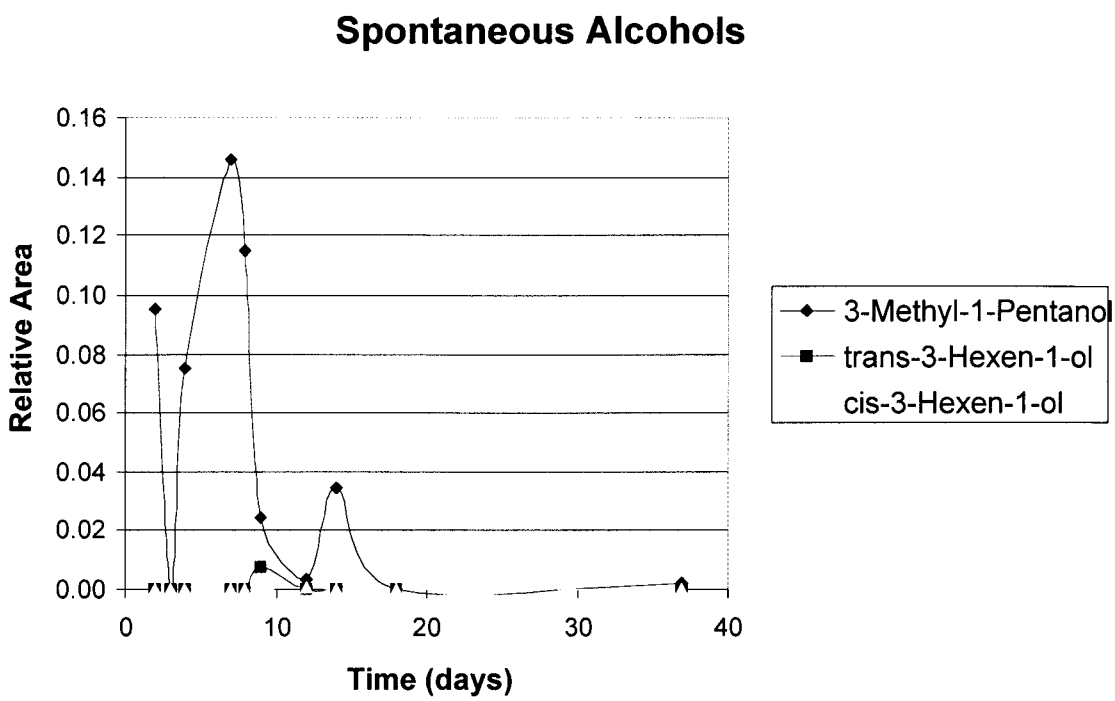

Figure 9. Relative Area vs. Time for Spontaneous Alcohols

Three alcohols were detected in the spontaneous fermentation. Cis-3-hexen-1-ol and trans-3-hexen-1-ol were found in very low levels or not detected throughout the entire fermentation. As noted before, the spontaneous fermentation demonstrated higher levels of the minor compounds. In this fermentation the 3-methyl-1-pentanol pattern fairly closely follows the pattern observed for this same compound in the inoculated fermentation. The exception being that the first peak was a much higher amount than in the spontaneous fermentation and there was a slight mismatch in the timing both by day and by the phase of cell growth. In this fermentation both of the peak concentrations occur during the cell death phase.

6. Ethers

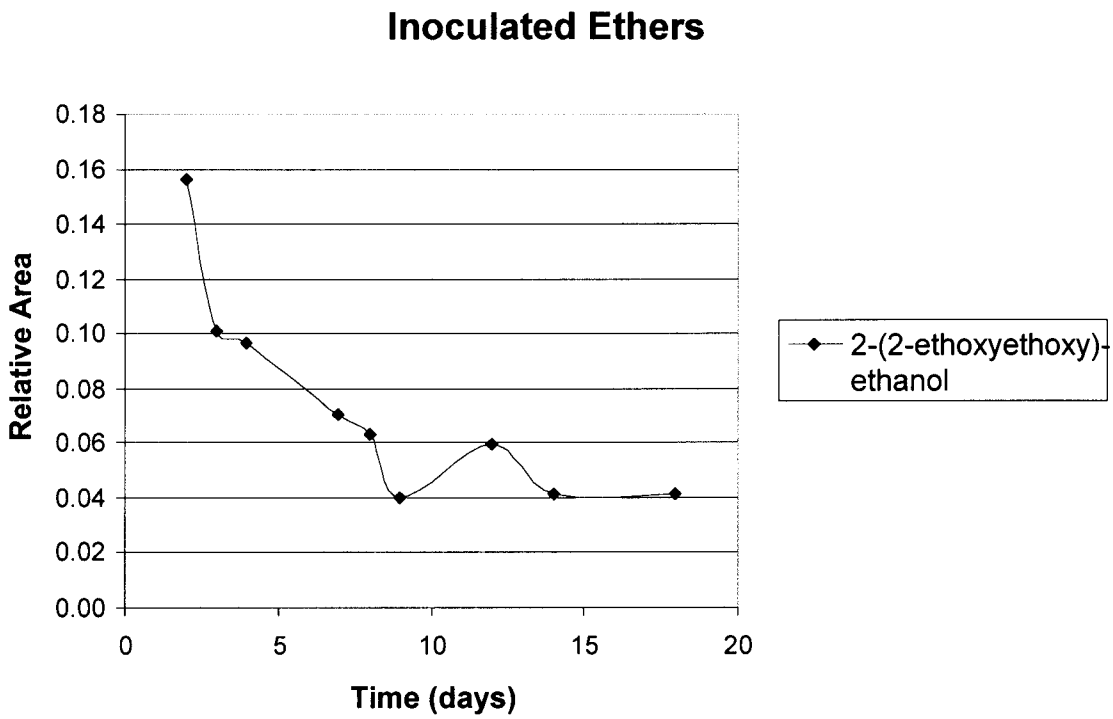

Figure 10. Relative Area vs. Time for Inoculated Ethers

The only ether (or ether-alcohol) found in the inoculated fermentation was 2-(2-ethoxyethoxy)-ethanol. The concentrations of this ether was found to be highest initially in the must and then it slowly dropped during the course of the fermentation to a lower level, to about an eighth of its initial amount but not to zero relative area.

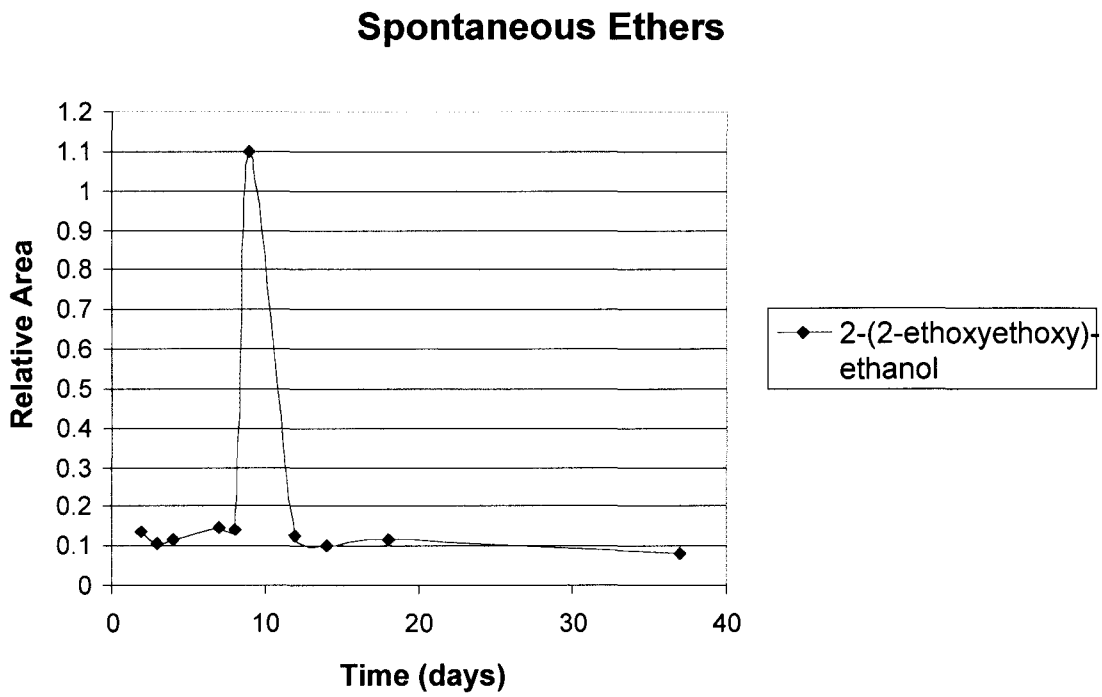

Figure 11. Relative Area vs. Time for Spontaneous Ethers

As in the inoculated fermentation, the only ether found in the spontaneous fermentation was 2-(2-ethoxyethoxy)-ethanol. Its concentration did not follow the same pattern of formation and degradation as in that fermentation. The levels in the must are about the same in both fermentations. In the spontaneous fermentation the levels of 2-(2-ethoxyethoxy)-ethanol remain constant except for one spike on day 10 which occurs during the cell death phase and then quickly returns to the initial concentration. There is no reasonable explanation for this spike in concentration.

7. Ketones

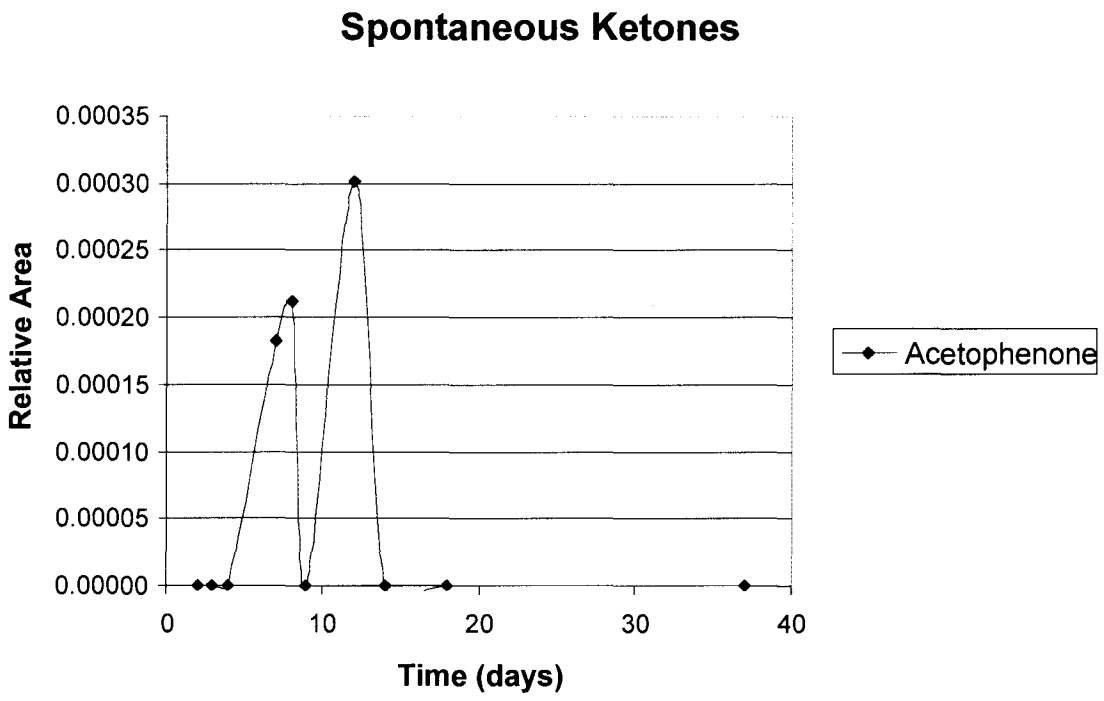

Figure 12. Relative Area vs. Time for Spontaneous Ketones

The only ketone found in either fermentation was acetophenone. It was also found only in the spontaneous fermentation. The pattern of formation and degradation of acetophenone follows closely the pattern seen for the esters, ethyl octanoate and ethyl decanoate in the spontaneous fermentation. It follows fairly closely the peaks and troughs of the esters and also follows closely the timing of these events.

8. Hydrocarbons

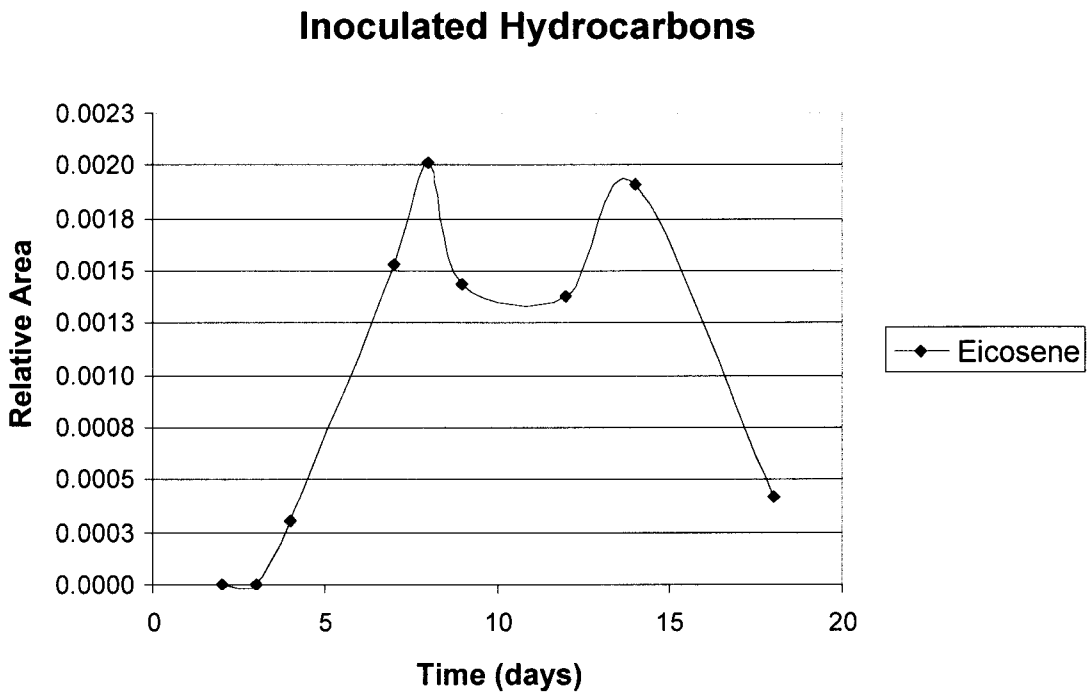

Figure 13. Relative Area vs. Time for Inoculated Hydrocarbons

Eicosene was the only hydrocarbon found in the inoculated fermentation. It starts at about 0 relative area and climbs from day 3 to a peak on day 8. This time period covers both the exponential and stationary phase of the fermentation. During the cell death phase the level of eicosene drops slightly and then climbs again to a second peak on day 14 to about the same level as the first maximum. Day 14 coincides with the beginning of the zero CFU phase and from here the eicosene concentration drops but does not reach 0 relative area.

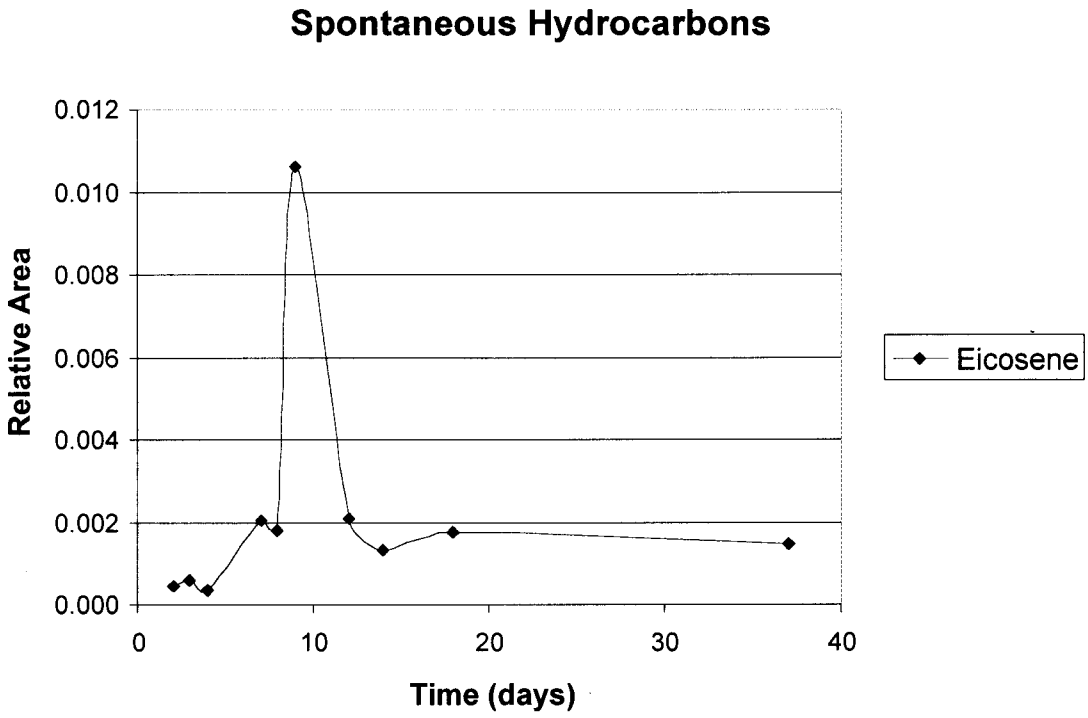

Figure 14. Relative Area vs. Time for Spontaneous Hydrocarbons

As in the inoculated fermentation, eicosene was the only hydrocarbon detected in the spontaneous fermentation showed in Figure 14. However, in this fermentation its' pattern of formation and degradation is very different. It climbs slowly for the first 7 days and then suddenly shoots up in a spike that was seen with 2-(2-ethoxyethoxy)-ethanol in Figure 10. Also, as seen in the 2-(2-ethoxyethoxy)-ethanol pattern, the eicosene quickly falls to the same level as it was on day 8 and remains roughly constant until the end of the fermentation. The spike forms during the middle of the cell death phase. There is no good explanation for this spike in concentration at day 10. There was no major maintenance event on that day such as changing the SPME fiber or equipment malfunctions and these spikes will require further investigation and/or conformation.

CHAPTER V

Conclusions

This research has demonstrated the successful use of SPME/GC/MS in the relative quantitation of volatile compounds found in Chardonnay wine. Absolute concentrations were not evaluated; instead the relative increase and decrease of the compound's concentration with respect to an internal standard were monitored to indicate their patterns of formation and degradation. The patterns of formation and degradation of each compound analyzed was compared with the others that were found. The formation of the compounds was then correlated to the fermentation activity using the CFU values. This illustrates the overall dynamics of Chardonnay wine maturation in both inoculated and spontaneous fermentations.

The observed patterns showed some correlation between a few related compounds, such as, a similarity in the decanoic acid and ethyl decanoate patterns. This correlation is expected due to the acid-ester relationship between the two compounds, the acid needing to be present before the ester can form. Also, other possible relationships that are not as obvious or expected were uncovered; these include the simultaneous spike in concentrations at day 10 of 2-(2-ethoxyethoxy)-ethanol and eicosene in the spontaneous wine fermentation.

In the comparison of the spontaneous and the inoculated wine fermentations, the spontaneous fermentation contained more compounds at higher concentrations (stronger signals) for all of the compounds studied. The spontaneous fermentation went through the cell growth phases faster than the inoculated fermentation and there was no correlation

between the compound formation and time in days or in cell growth phases. This lack of correlation may be due to the fact that the spontaneous fermentation contained at least three different yeast species and the inoculated wine fermentation contained only a single yeast species.

CHAPTER VI

Future Work

Time and funding limitations prevented the absolute concentration values from being determined. Developing the method to quantitate these compounds along with using other methods under different conditions (e. g. different SPME fibers) to detect and quantitate other compounds is the next logical step for this project.

Exploring the use of different SPME fibers to optimize the extraction of various classes of compounds would be a valuable next step in this project. The purchase and/or synthesis of more standard compounds to confirm the identity of unidentified peaks, as well as to verify the tentatively identified peaks, is also of value. Finally, the method should be refined so that better quantitation is possible. Also, finding a way to shorten the GC-MS analysis time from four hours would be a welcome improvement to this project.

Future work in the biochemical study of fermentation would include a more in depth study of the exact sequence of the rise and fall of the different yeast species in the spontaneous fermentation and also their relationship with the formation and degradation of the various volatile components.

Afterwards, other experiments could be devised to trace the biosynthesis of the compounds of interest. These experiments could include fermenting synthetic musts that could have various components added or left out, or spiking the must with isotopically labeled compounds and tracing the pathways by following the label.

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