CHAPTER 1: LITERATURE REVIEW

AROMA AND FLAVOR

The aroma of wine is caused by hundreds of different compounds (Rapp and Mandery 1986), and is defined as the fragrant perception that is derived from aromatic grape constituents (Margalit 1997).   This perception is a result of the odor-active volatiles traveling into the nose and binding with odor binding proteins that are found in the mucous membrane of the olfactory epithelium (Snyder et al. 1988).   Once the aroma compound is bound, it activates adenylate cyclase, which in turn opens ion channels causing a depolarization and the firing of that receptor (Beauchamp and Bartoshuk 1997). This impulse is then carried from the basal cells of the receptor to the olfactory bulb and higher areas of the brain for processing.

Most aroma compounds activate multiple receptors (Malnic et al. 1999), all of which have varying levels of sensitivity to each group of aromatic compounds.   Volatile compounds also have many different chemical natures, covering a wide range of polarity, solubility, volatility, and pH (Ortega-Heras et al. 2002). Generally, volatile compounds can be detected at very low levels, in the order of $10^{-4} - 10^{-12}$ g/L (Rapp and Mandery 1986). An important number of aroma-active volatiles, many of which are unstable, are found at low concentrations (μg/mL). They may be easily oxidized and degraded by heat or pH, creating new compounds as well as artifacts (Ortega-Heras et al 2002).

Flavor, as an attribute of foods and beverages, has been defined (Amerine et al. 1965) as the sum of perceptions resulting from stimulation of receptors that are grouped together at the entrance of the alimentary and respiratory tracts. For purposes of practical sensory analysis, flavor is the interaction of chemical constituents with the sense of taste as well as smell. Flavor is, therefore, composed of volatile compounds, responsible for the aroma, as well as nonvolatile compounds, responsible for taste sensations (Meilgaard et al. 1999, Rapp and Mandery 1986).

Aroma and flavor constituents of wines have been studied extensively over the last 20 years, and reviews of work on wine aroma and flavor list hundreds of volatile substances detected in different wines. The specific importance of fermentation-derived volatiles responsible for aroma, and their effects on wine quality, has also been extensively investigated (Simpson and Miller 1984a, Amerine and Roessler 1976, Webb and Ingraham 1963, Rapp and Mandery 1986). The presence or absence of these volatile substances is crucial to the identity and complexity of wine. Based upon this, evaluation of winemaking techniques that would function to improve the profile of desirable volatile compounds would be beneficial in the creation of high quality wines.

AROMA ACTIVE-VOLATILES

Aroma and flavor compounds are found and released in three distinct categories: 1) nascent in the grape (released only by crushing), and compounds produced by enzymatic interactions at crush, 2) fermentation derived, and 3) the result of aging (Rapp and Mandery 1986). Complex arrays of volatiles as well as their precursors are nascent in the

grapes prior to vinification (Schreier 1979, Muller et al. 1993). These aroma compounds are released by crushing and are further modified by enzymatic interactions. These nascent volatiles are, however, outnumbered by the volatiles that are formed by metabolites during alcoholic fermentation (Bertrand 1983, Dumont and Dulau 1996). Aroma compounds liberated during fermentation are the main contributors to wine aroma (Rapp and Mandery 1986). The last category of contributors to wine aroma are the volatiles that are formed as a result of the transformation of aroma products due to aging. This volatile constituency is commonly referred to as the bouquet of the wine. Bouquet is also defined as the fragrant sensation in wine derived from aromatics produced during fermentation, maturation or aging (Rapp and Mandery 1986).

The aroma compounds are then classified based upon the constituents' comparative importance. Aroma compounds can be defined as impact compounds, contributing compounds or insignificant compounds (Ferreira et al. 1995). The impact compounds have a marked and distinctive effect on wine fragrance and generally give the wines varietal uniqueness. Contributing compounds on the other hand do not have anything to do with varietal uniqueness; rather they are responsible for the overall complexity of the wine. For example, the acetate esters of ethanol and the higher alcohols are often the volatiles providing the major aroma impact of freshly fermented white wines (Houtman et al. 1980, Houtman & Du Plessis 1981).

Chardonnay has aspects of its aroma profile that are distinct to the cultivar, but it does not possess clearly defined aroma impact compounds (Sefton et al. 1993). The compounds

contributing the most to Chardonnay juice aroma are linalool, damascenone, β-ionone and α-terpeniol (Simpson and Miller 1984b).   The varietally distinctive odors of Chardonnay may, therefore, arise from quantitative rather than qualitative aromatic differences (Le Fur et al. 1996, Simpson and Miller 1984b, Ferreira et al. 1995).

CHEMICAL CHARACTERISTICS OF WINE AROMA

Chemical classes of compounds found in wine include esters, alcohols, acids, lactones, carbonyl compounds, acetals, phenols, sulfur containing volatiles, nitrogen-containing volatiles as well as other miscellaneous substances (Schreier 1979, Etievant 1991, Rapp and Mandery 1986). The basic aroma of white wine, however, can be attributed to four esters: (ethyl acetate, isoamyl acetate, ethyl caproate and caprylate), two alcohols (isobutyl, isoamyl) and acetaldehyde (Schreir 1979). The vast majority of the other compounds typically function to modify the basic odor (Avakyants et al. 1981). The important fermentation-derived aroma compounds found in the highest quantities include 2-methyl-propanol, 3-methyl-butanol, 2-methyl-butanol and 2-phenol ethanol, which collectively account for 50% of the total volatiles (Rapp and Hastrich 1978).

Simpson (1979a) reported that the most important white wine aroma volatiles produced during fermentation are drawn from three chemical classes. Ethyl esters of medium chain fatty acids (*ethyl butyrate, hexanoate, octanoate, decanoate and dodecanoate*) which are fruity and wine-like; acetate esters that are responsible for tropical fruit and banana-like aromas and a third group of higher alcohols such as isobutanol, isoamyl alcohol and

hexanol which are harsh and unpleasant when alone. These compounds are an integral and desirable part of wine aroma if collectively below 300 mg/L.

ETHYL ESTERS AND ACETATE ESTERS

The largest qualitative constituents of wines are esters, a condensation product between the carboxyl group of an organic acid and the hydroxyl group of an alcohol or phenol. The majority of esters are ethyl esters of organic acids, acetates, and ethyl esters of fatty acids. More than 160 esters have been specifically identified with many being found in trace amounts with little contribution to wine aroma. The more common esters however, are generally found at levels higher than sensory threshold (Simpson and Miller 1984a, Ribereau-Gayon 1978). They occur in wines as major volatile constituents, and are typically responsible for 'fruity' wine odors. Esters are the main contributors to the intense and characteristic aroma of non-vinifera varieties such as *V. labrusca* and *V. rotundifolia.* Ethyl esters are found in the largest quantities while acetates, though in less quantity, contribute to some of the intensity and quality of wine aroma (Van der Merwe & van Wyk 1981). These esters identified in the wine are found in the grape, but their main origin is from the secondary metabolism of yeasts during fermentation (Schreir 1979).

Esters are either cyclic (phenolic) or straight chain (aliphatic) in structure. Phenolic esters have a low volatility and have no significant sensory impact. Aliphatic esters are made up of three groups (monocarboxylic, di- or tri- carboxylic and hydroxyl or oxo-acid esters) with only the monocarboxylic acid esters having any contribution to

Chardonnay aroma (Sefton et al. 1993, Vernin et al. 1986). As the length of the carbon chain increases fruity odors tend to become soft, then soap-like and finally stearic (Rapp and Mandery 1986).

Esters based on ethanol and saturated fatty acids such as hexanoic, octanoic and decanoic acids generally contribute to the 'fruity' or 'wine-like' aroma of wine, their presence even at sub-threshold levels having a possible additive effect (Simpson 1979a, Amerine and Roessler 1976). While this theory has not been validated it was determined that ethyl acetate, the most investigated ester, generally has a suppressive effect on the perception of these other fruity esters. Maintaining an ethyl acetate level below 50-100 mg/L is important, especially in white wines such as Chardonnay, in which this fatty acid esters profile is important (Rapp and Mandery 1986).

Simpson and Miller (1984b) evaluated Chardonnay wine and found that the ethyl esters of fatty acids, based upon their concentration in the wine and subsequent flavor thresholds, are important contributors to aroma. They are fairly volatile with limited solubility in must and wine. Further, ethyl n-hexanoate, and the branched chain heptanoic acid were found in quantities higher than threshold, which would be expected to impact the 'estery' or 'wine-like' aroma (Simpson 1979a).

The distinctive aroma of Chardonnay may also be assisted in its development by aging in oak (Simpson and Miller 1984b, Sefton et al. 1993). Oak lactones (*cis-* and *trans-*β-methyl-γ-octalactone) as well as hexanal, octanal and decanal, formed by oxidation of the

corresponding alcohols, are the result of storage of the Chardonnay wine in oak casks (Simpson and Miller 1984b). Lactones are a subgroup of esters formed by an internal esterifcation between the carbonyl and hydroxyl group, which results in a cyclic compound. Most lactones in wine appear to be produced during fermentation, with those found in the grape generally not involved in the development of varietal odors.

Ester formation during fermentation parallels ethanol formation (Rapp and Mandery 1986) and is affected by any factor that decreases the speed of fermentation, such as a decrease in temperature or an increase in pH (Simpson 1979a, Ribereau-Gayon 1978, Van der Merwe and van Wyk 1981, Bertrand 1983). These two factors, in combination with strict anaerobic conditions and low SO_2 levels were found to dramatically increase the amount of esters formed (Simpson and Miller 1984b, Nykanen 1986). Ammonia levels, yeast used and level of non-soluble solids are also directly related to the formation of esters important in the wine aroma (Bertrand 1983). Any changes in ester concentration post-fermentation are due to alcohol esterification of acids, or hydrolysis of the esters (Etievant 1991).

Ethyl and acetate esters are fairly volatile when compared to other aroma compounds, with limited solubility in must or wines (Simpson and Miller 1984b). Consequently these compounds are partially transferred onto the carbon dioxide produced during fermentation and can easily be entrained or swept away.

ALDEHYDES AND KETONES

Aldehydes and ketones are carbonyls produced in relatively small amounts and do not play an intrinsic role in the creation of varietal wine aromas. Suomalainen and Keranen (1967) showed that aldehydes and keto-acids are essential for amino acid synthesis as well as for the formation of fusel alcohols. Aldehydes are distinguished by the terminal location of the carbonyl group, and are either formed via carbohydrate degradation, originate from lignins or are formed during wine aging (Rapp and Mandery 1986). Ketones are related compounds, with the carbonyl functional group located on an internal carbon.

Six carbon hexanals and hexenals, which differ in their level of saturation, are found in the most significant quantities and are associated with grassy and herbaceous descriptors. The most common aldehyde found in wine is acetaldehyde as it makes up more than 90% of the aldehyde content (Nykanen 1986). Acetaldehyde is produced as an intermediary product of yeast metabolism (from pyruvate through irreversible decarboxylation by the pyruvate decarboxylase multienzyme complex) during alcoholic fermentation and as a result of oxidation of ethanol during storage (Schreir 1979). Normal levels in newly fermented wine are less than 75 mg/L with sensory thresholds between 100 and 125 mg/L. At levels above these values acetaldehyde imparts overripe, bruised fruit, and sherry like aromas (Zoecklein et al. 1999, Ebeler and Spaulding 1998). Most other aldehydes are only detectable in the initial phases of fermentation. This lack of aldehydes may be due to the reduction of aldehyde to alcohol during fermentation (Schreir 1979).

Not many volatile ketones are found in wine, but those that are present in the grape usually survive through fermentation (Etievant 1991). Volatile ketones that are typically seen include β-damascenone, α-ionone, β-ionone and diacetyl. β-damascenone likely plays a role in the aroma profile of many white wines, as well as Chardonnay (Strauss et al. 1987, Simpson and Miller 1984b) because it is found at higher than usual concentrations (Le Fur et al. 1996), and has a desirable aroma profile and low odor threshold (Nykanen 1986).

HIGHER ALCOHOLS, FUSEL ALCOHOLS, FUSEL OILS

Ethanol and glycerol are quantitatively the largest group of alcohols found in wine, followed by diols, higher alcohols (more than two carbon atoms) and esters, which account for 0.2-1.2 g/L in white wines (Rapp and Mandery 1986). While ethanol contributes to the structural and textural aspects of wines, higher alcohols (fusel alcohols) are generally found to be responsible for aroma due to the fact that they are found in quantities above perception threshold. Perception threshold is the minimal concentration in which the component can be detected (Margalit 1997), and varies widely for fusel alcohols with values of 150 mg/L for butanol (Shinohara and Watanabe 1976) to 25-105 mg/L (Amerine and Roessler 1976) for 2-phenylethanol. Perception thresholds, commonly called flavor thresholds, pertain specifically to aroma when dealing with volatile or aromatic compounds. Aroma units can be calculated from flavor thresholds by dividing the concentration of the compound present in the beverage by flavor threshold value.

Other volatile compounds present can affect this threshold as they can act synergistically to exceed threshold levels. At low levels (< 300 mg/L), straight chain higher alcohols generally add complexity to the bouquet of a wine (Amerine and Roessler 1976). Quantities above 400 mg/L are regarded as negatively impacting wine quality (Rapp and Mandery 1986, Ribereau-Gayon 1978, Bidan 1975) and are frequently described as petroleum or tar-like (Zoecklein et al. 1999). In table wines, the fusel alcohol contribution is typically found to range from 140-420 mg/L (Amerine & Ough 1980).

The fusel alcohols found in the largest quantity in wine are 1-propanol, 2-methyl-1-propanol (isobutyl alcohol), 2-methyl-1-butanol, and 3-methyl-1-butanol (isoamyl alcohol). Quantitatively, isoamyl alcohol generally accounts for more than 50% of all fusel alcohol fractions (Muller et al. 1993). Of the benzene-derived higher alcohols, 2-phenylethanol is the most important, and is also the only fusel alcohol described with positive terms such as sweet, perfumed, and dry rose (Simpson 1979a).

Formation of fusel alcohols, like esters, is found to parallel ethanol formation (Rapp and Mandery 1986), and is mainly produced by the fermentation of sugars by yeasts using two different metabolic pathways. Bidan (1975) states that one-fourth of the fusel alcohols are due to the catabolism of sugars, and three-fourths are due to amino acid degradation. They may originate from grape-derived aldehydes by the reductive denitrification of amino acids or via synthesis from sugars (Nykanen 1986). The formation of higher alcohols during fermentation is influenced by winery practices.

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Factors such as yeast strain, low amino acid levels, low temperature, low pH of the juice, and level of anaerobiosis result in significant decreases in formation of fusel alcohols (Etievant 1991, Nykanen 1986). Temperature stressed and nutrient stressed yeast, however, are known to produce increased levels of fusel oils (Muller et al 1993).

PRODUCTION DURING FERMENTATION

The major compounds found in the headspace of fermenting must are typically alcohols, acetates, and ethyl esters. The higher alcohols and esters are formed in the early phase of fermentation, paralleling yeast growth (Kunkee & Goswell 1977). Aroma volatiles were found to peak in evolution on day 3 (Stashenko et al. 1992) and then significantly decrease until the end of fermentation. This is likely due to the increasing alcohol level and the possible higher solubility of volatiles (Williams and Rosser 1981). Significant loss of acetate ester and ethyl esters of fatty acids occurred during fermentation (Miller et al. 1987). Early on in the fermentation, headspace volatiles were found to consist of alcohols (47%), acetates (37%) and ethyl esters (13%). 1-Pentanol and its acetate ester comprised the largest quantities of acetates and were responsible for approximately two-thirds of the total headspace volatiles (Stashenko et al. 1992).

Miller, Amon and Simpson (1987) quantified the concentration of higher alcohols and esters entrained with carbon dioxide throughout fermentation, and found that levels changed with different compounds reaching peaks at different points throughout fermentation. The general pattern of compound evolution was found to be an increase in concentration then a decrease in the later part of fermentation. When compared to the

other compounds quantified, hexylacetate peaks first and ethyl decanoate peaks at the latest point during fermentation. 2-phenylethanol did not increase until later in the fermentation and followed patterns previously described (Williams et al. 1983). Negligable portions of higher alcohols were found bound to carbon dioxide (Milller et al. 1987).

EFFECT OF TEMPERATURE ON FERMENTATION VOLATILES

The aroma profile of a wine is affected by any factor that decreases the speed of fermentation, specifically a decrease in temperature (Simpson 1979a, Ribereau-Gayon 1978, Van der Merwe and van Wyk 1981, Bertrand 1983). Higher temperature fermentations result in larger losses of volatile components than do low temperature fermentations (Muller et al. 1993). There are, however, a number of high boiling point esters that require higher temperatures in order to increase in concentration (Killian and Ough 1979). While these esters are known to contribute to many of the 'fruity' aspects of wine aroma, white wine aroma profiles still seem to benefit from slower and cooler fermentations. It has been shown, however, that there is no sensory advantage to low ($\leq$ 15ºC) temperature fermentation for Chardonnay (Cottrell and McLellan 1986, Killian and Ough 1979). This may be due to the fact that Chardonnay obtains its varietal characteristics based upon the balance of esters and aldehydes, which have a higher temperature of volatilization.

The majority of the literature does, however, indicate that white wine fermentations that proceed at lower temperatures ($\approx 15ºC$) have a resultant minimization in loss of primary aroma components (Miller et al. 1987). While there are a number of factors involved in the extent to which volatiles are lost, temperature adjustment and the modification of fermentation dynamics to decrease the fractions of volatile components lost is a feasible tool that can be utilized. Muller et al (1993) indicated that the possibility of fermenting white wines at higher temperatures ($27ºC$) would be practicable if methodology to trap and return escaping entrained volatiles were developed. While fermentation at low temperature has been shown to reduce loss of volatiles (Reynolds et al. 2001, Cottrell and McLellan 1986, Muller et al. 1993) there has been no temperature specific analysis of the effect of temperature on the subsequent entrainment of these volatiles with carbon dioxide.

AROMA & FLAVOR TRAPPING

Several procedures for trapping volatiles from the escaping CO_2 stream in wine have been reported (Simpson and Miller 1984a, Muller et al. 1993, Miller et al. 1987, Todd et al. 1990). It has been shown that a large number of volatile components can be lost to the atmosphere during vinification due to their inherent volatility and the ease with which they are entrained to the escaping carbon dioxide (Muller et al. 1993). There are a number of factors that affect the extent to which the volatiles are lost. Simpson et al (1984a) state that the extent of loss of aroma compounds depends upon temperature, rate of gas evolution and the type of fermentation vessel utilized. Vapor phase concentration

is also affected by ethanol concentration and carbon dioxide evolution (Boulton et al. 1996).

These factors, along with the effects of temperature, affect the way in which the wine volatiles are released and arranged in the headspace of the fermentation vessel and are carried out. Higher temperature fermentations result in larger losses of volatile components than do low temperature fermentations (Muller et al. 1993), and constituents with the highest volatility are more readily entrained and lost into the atmosphere. Miller et al. (1987) quantified the proportional effect of the loss of volatile components during fermentation. They concluded that it was possible to determine the effective concentration loss of volatile components by measuring the quantity of each compound entrained with carbon dioxide and dividing that number by the volume of the new wine. Up to 25% of the acetate esters and the ethyl esters of the fatty acids were removed by the carbon dioxide. The higher alcohols were reduced by no more than 1%. The alcohols are retained in the wine in contrast to the hydrophobic esters. Loss of major esters, quantified in aroma units (AU) ranged from 0.1 for hexyl acetate and ethyl *n*-butanoate, to 1.6 AU for ethyl *n*-hexanoate.

This early loss of fermentation-derived volatiles can lead to a decrease in the pool of volatiles that provides positive sensory attributes and has been a concern of winemakers. Capture and return methodology has been developed based upon this perceived loss in order to enhance or improve wine quality (Muller et al. 1993, Todd et al. 1990, Zoecklein 2000). Research involving volatile trapping methodology has involved different

techniques many of which have been developed based upon technology that has been implemented in the petroleum and chemical industries. Charcoal filtration systems have been developed (Muller et al. 1993, Todd et al. 1990) which rely on heat exchangers to remove moisture prior to adsorption to the activated charcoal filters. Upon saturation of the charcoal with volatiles, dry countercurrent steam is used for desorption. The volatiles are then captured and concentrated. Membrane filter systems have also been evaluated (Zoecklein et al. 2000). This type of filter system would be beneficial in that it requires less equipment, is economically more viable and also uses no heat which can degrade volatile flavor components.

Simpson and Miller (1984a) quantified the formation and loss of 10 flavor compounds and concluded that the extent of entrainment was considerable. They evaluated the effects carbon dioxide entrainment, removal of yeast lees, bentonite fining and filtration on aroma and flavor of the wine. Isoamyl acetate, ethyl octanoate, ethyl decanoate and ethyl hexanoate were produced and lost in fairly large quantities during fermentation. Removal of yeast lees also had an appreciable effect on levels of these aroma compounds. This may be due to the large size of the esters, such as ethyl decanoate or ethyl dodecanoate impeding their diffusion through and out of the yeast cell. Another consideration would be the lipophilic nature of ethyl esters greater than six carbons (Zoecklein et al. 1997) causing these compounds to be adsorbed to the yeast matter. Aroma compounds were also reduced as a result of bentonite fining and filtration. Based upon these conclusions modifications in fermentation temperature, lees management, and fining or filtration would be ways in which aroma and flavor could be conserved.

In the early nineties the California wine industry was subjected to scrutiny regarding the effect of ethanol emissions on the environment (Todd et al. 1990). The loss of ethanol is proportional to the square of the concentration of sugar utilized (Williams and Boulton 1983). Therefore fermentation of juice at 22º Brix would result in 600-mg/L loss of ethanol into the atmosphere, with large tanks releasing upwards of 3.0×10^6 mg of ethanol. This volatile ethanol is considered by some to be a precursor of photochemical smog. In response to investigations by the California Air Resources Board, California State University Fresno began developing emission control devices centered on charcoal or tenex adsorption traps, with or without solvent extraction (Muller et al. 1993). These traps were used to monitor ethanol emissions, and were then subsequently used to capture and analyze fermentation emission volatiles for their identification as well as their role in quality enhancement (Nasrawi et al. 1990). The total concentration of volatile compounds produced during fermentation is 1% of the ethanol content or 0.8-1.2 g/L (Rapp and Hastrich 1978). This loss could be sensorially significant (Muller et al. 1993).

It follows that methodology to recover the loss would be highly beneficial to the production of high quality wine. This task is complicated, however, by the relatively low concentration of aroma active volatiles compared to carbon dioxide volume as well as complex interactions between the volatiles and variations in fermentation temperature (Miller et al. 1987). Furthermore, not all of the fermentation volatiles that are produced would be beneficial to wine quality. For example, sulfur-containing compounds, which

impart negative sensory features expected to 'blow off' during fermentation, may be retained.

In order to modify these techniques to conserve flavor, Muller et al (1993) developed capture hoods connected to charcoal filters. Removal of adsorbed volatiles was performed using countercurrent steam, which was then condensed and collected. Lost aroma and flavor constituents were then added back to the wine and compared to control wine that did not have reintroduction of the lost volatile compounds. It was determined that there was a moderate difference in intensity of aroma and bouquet with the treatment wine being preferred over the control. They also determined that the later fractions of volatile components actually detracted from wine quality due to the highly fusel nature of the volatiles. Fractions from the early parts of fermentation, however, did improve wine quality and were found to be more desirable. They concluded that it is possible to enhance wine quality when reincorporating lost volatile fractions. The results of this study are particularly significant in that they introduce the possibility of fermenting white wines at red wine temperatures in order to release volatiles that require a higher fermentation temperature and would typically not be released in a conventional white wine fermentation.

Boulton et al. (1996) states that while these techniques are presently not considered to be financially advantageous, options that might make gas collection processing systems to recover the carbon dioxide and escaping fermentation volatiles would be desirable. Based upon this, work has been done to evaluate the use of alternate capture and return or

flavor trapping systems (Zoecklein et al. 2000). In the current study, we investigated the efficacy of a modified capture and return system, and subsequently evaluated whether this system could be used on a commercial scale in order to retain fermentation volatiles that are typically lost to the atmosphere.

The specific aims of this study, *Evaluation of the Effects of Capture and Return on Volatiles of Chardonnay (Vitis vinifera L.) Wine,* were as follows:

1- To quantitatively determine the effects of capture and return methodology in a commercial setting.

2- To quantitatively determine the effects of capture and return methodology in a small scale research setting.

3- To determine if there is a correlation between the presence of the trap and levels of odor active compounds.

4- To identify, and quantitatively determine the volatile organic compounds retained by capture and return methodology (trap effluent) using a modified trap system.

5- To quantitatively determine the effects of fermentation temperature on trap efficacy.

In summary, the overall goal of this study was to evaluate the effectiveness of methodology to trap and return wine fermentation volatiles as a means of improving white wine quality. In the current study we evaluated the effects of the trap in a lab setting and a commercial setting, identified volatile compounds retained by the trap, and evaluated the effects of temperature on trap efficacy in order to elucidate the functionality of the capture and return system as a means of retaining desirable aroma and flavor compounds during fermentation.

CHAPTER 2

Effects of temperature on the capture and return of Chardonnay (*Vitis vinifera* L.) fermentation volatiles.

INTRODUCTION

Volatile compounds can be lost to the atmosphere during fermentation due to their inherent volatility and entrainment with carbon dioxide. The most important white wine aroma volatiles are from three chemical classes (Simpson and Miller 1984b). Ethyl esters of medium chain fatty acids (ethyl butyrate, hexanoate, octanoate, decanoate and dodecanoate) which are fruity and wine-like, acetate esters (isoamylacetate and hexylacetate), responsible for tropical fruit and banana-like aromas, and higher alcohols such as isobutanol, isoamyl alcohol and hexanol which may be individually harsh and unpleasant. Some of these compounds are found nascent in the grape, but the majority are formed by yeast during alcoholic fermentation (Webb and Muller 1972, Schreier 1979) and help to determine identity and complexity of wine (Muller et al. 1993).

A number of volatile components can be lost to the atmosphere during vinification due to their inherent volatility and entrainment with carbon dioxide (Simpson and Miller 1984a). The extent of loss depends on a number of factors including fermentation temperature (Killian and Ough 1979), amino nitrogen content (Vos et al. 1978, Bell et al. 1979), and yeast species and strain (Soles et al. 1982). Williams and Boulton (1983) illustrated that the loss of the quantitatively most important compound, ethanol, was proportional to the square of the concentration of sugar utilized. Fermentation of 22 Brix juice results in a small but not insignificant loss of ethanol of approximately 600 mg/L. Aroma volatiles

are present in approximately 1.0% of the ethanol concentration, or about 0.8-1.2 g/L (Rapp and Hastrich 1978). Therefore, fermentation can decrease the pool of volatiles, which may impact the sensory attributes (Miller et al. 1987).

Fermentation temperature is a stylistic tool in white wine production (Ough and Amerine 1967, Simpson 1979a, Ribereau-Gayon 1978). Compounds typically retained at fermentation temperatures ≤ 20ºC include acetate esters such as isoamyl acetate, isobutyl acetate, and hexylacetate (Killian and Ough 1979). High fermentation temperatures (≥ 20ºC) result in the release of more volatile constituents (Ough and Amerine 1967) primarily by promoting the production of higher-boiling esters such as ethyloctanoate, ethyldecanoate, and phenethylacetate (Killian and Ough 1979). Increased production at these temperatures is associated with larger losses into the atmosphere of those constituents with the highest volatilities (Muller et al. 1993) resulting in a loss of some primary aroma components (Miller et al. 1987). Chardonnay varietal characteristics are based, in part, on a balance of esters and aldehydes (Simpson and Miller 1984b) which may not be volatilized at low temperatures (Killian and Ough 1979).

Several procedures for trapping escaping fermentation volatiles have been reported using carbons, Tenex or other adsorbants, with or without solvent extraction, with limited success (Simpson and Miller 1984a, Miller et al. 1987, Todd et al. 1990, Muller et al. 1993). Miller et al. (1987) quantified the proportional effect of the loss of volatile components during fermentation. They found that 2-24% of the acetate esters, up to 25% of the ethyl esters of fatty acids and no more than 1% of the higher alcohols were lost.

Muller et al. (1993) determined that it was possible to enhance the quality of wine by reincorporating lost volatile fractions.

Zoecklein et al. (2000) evaluated the use of a membrane filter to capture and return fermentation volatiles. This research further evaluated capture and return to quantify and identify its effects and the influence of fermentation temperature on the chemical and sensory properties of Chardonnay wines.

MATERIALS AND METHODS

Trap evaluation consisted of: 1) control wines fermented with a conventional fermentation lock (twin bubble lock), 2) a membrane filter (capture and return; described below) and 3) a modified trap (capture and remove), in which the captured and condensed volatiles were collected via gravity into a separate collection vessel.

The aerosol filters (Pall Trincor, Sealkleen, Ultrafine Filtration Company, 2200 Northern Bouldevard, East Hills, NY 11548-1289) were 0.2-micron absolute membranes composed of modified polyvinylidene fluouride with 0.2-micrometer absolute liquid filtration capacity, 0.1-micrometer absolute gas filtration capacity, 0.5 square foot filtration area and a gas volume capacity of 6.0 cubic feet per meter.

Winemaking. *Commercial Scale Study:* Capture and return treatments were evaluated from 1998 to 2002. Control and treatment vessels were randomly assigned and consisted of three or six replications of each depending on producer and year. Capture and remove treatments were only developed and incorporated in 2002. Four commercial scale capture and return trials were evaluated using Chardonnay (*Vitis vinifera* L.) grown at Pellegrini Vineyards, Long Island, New York (1998 to 2000), and White Hall Vineyards, Virginia (2000 and 2002). Fruit harvested from Pellegrini vineyards came from four vineyard blocks: front block, west block, north block, and C96. Fruit soluble solids at harvest ranged from 20.0-22.0 Brix. All wines were produced using standard winemaking procedures, which included hand harvesting, whole cluster pressing (White

Hall Vineyards) or machine harvesting, destemming and crushing (Peligrini Vineyards), and the use of a thin-layer bladder press (Wilmes, 100L), pressed to approximately 1.0 Bar.

Juices were enzyme-treated at 15 mL/ton (Pec-5L, Scott Laboratories, Petaluma, CA), cold settled at 7ºC for 24 to 48 hours, racked and nitrogen supplemented using a combination of Fermaid-K (Lallemand, Montreal, Canada) and diammonium phosphate (DAP) (Fisher Scientific Fair Lawn, N.J.) to a fermentable nitrogen concentration of 300 mg/L. Juices were warmed to 19°C, inoculated with an actively growing culture of *Saccharomyces cerivesiae* strain VL-1 or CY3079 (3% inoculum by volume, Lallamond, Montreal Canada). Capture and return treatments were affixed directly to the bunghole of the barrel. Wines were fermented to 18° Brix and transferred to 500L puncheons (Pellegrini) or 200 L barrels (Whitehall). All wines were fermented to dryness (0.25 g/L residual sugar), at which point capture and return treatments were removed. Post fermentation, wines were inoculated with malolactic bacteria (*Leuconostoc oenous)* and remained in barrels 4 to 6 months. After completion of malolactic fermentation, wines were racked with an addition of 30 mg/L of SO_2, bottled in 750 mL bottles and treated with dimethyldicarbonate (Velcorin, Scott Laboratories Petaluma, CA.) at a rate of 200 mg/L. The wines were stored in completely full containers at 4°C for four to six months until volatile and sensory analysis.

Small Scale Temperature Evaluation. In order to ascertain the effects of fermentation temperature on capture and return of fermentation volatiles, small-scale laboratory

winemaking techniques were used. Capture and return as well as capture and remove treatments were evaluated at two temperatures using laboratory scale fermentations. Clone 4 Chardonnay grapes were harvested (230 kg) in 2002 at 22 Brix from the Allison J. Smith Extension and Research Center (Winchester, VA.), chilled to 7ºC and whole cluster pressed with a Wilmes (100 L) thin layer bladder press, to approximately 1.0 Bar. Sulfur dioxide was added at a rate of 10 mg/L, and the juice was cold settled at 7ºC for 24 hours, racked, nitrogen sparged, and frozen at -10°C in six, 20-liter plastic carboys until fermentation. The thawed juice was sparged with nitrogen, 30 mg/L of SO_2 was added, and nitrogen supplemented as described above. A juice volume of 3.5 liters was placed into 18 one-gallon glass carboys, warmed to 19°C, and inoculated with an actively growing culture of *Saccharomyces cerivesiae* strain D-254 (3% inoculum by volume) (Lallamond, Montreal Canada). Two treatment temperatures were evaluated (15ºC and 30ºC). Fermentation vessels were randomly assigned and consisted of three replications each of capture and return, capture and remove and control at each temperature.

The fermentation vessels were weighed, and put into a circulating water bath in a random order and brought to either 15ºC, or 30ºC (± 0.5 ºC). Temperatures were recorded every hour (Campbell Data Logger, Logan, Ohio 43138) within fermentation vessels and traps. Vessels were briefly removed once per day and weighed (Ohaus Scale CQ10 , Brooklyn, NY) with an accuracy of 0.002kg. Rate of fermentation was monitored by change in weight as described by El Haliou et al. (1987). At dryness (0.25 g/L residual sugar) wines were racked into full containers, and 45 mg/L of SO_2 and 200 mg/L dimethyl

dicarbonate (Velcorin, Scott Laboratories Petaluma, CA.) were added. Storage temperature was 4ºC, and volatile analysis was performed 4 to 6 months after bottling.

Chemical Analysis. Analyses of alcohol, pH, titratable acidity, SO_2, reducing sugar, and volatile acidity were conducted for all wines using standard procedures as described by Zoecklein et al. (1999). The concentration of tartaric, malic and lactic acids was determined by HPLC (Hewlett Packard model 1100, Palo Alto, CA) using a Synergi 4μ Hydro-RP80A column (Phenomenex Torrence, CA).

Volatile Analysis. A 15 mL wine sample was taken from each pooled replicate, spiked with 6μL of 204 mg/L propylbenzoate (internal standard), and stored in a 16 mL Teflon-capped amber vial at 16°C. A 4-mL aliquot of each sample was pipetted into a 10 mL sample vial, and 1.0 g of NaCl was added to increase partition coefficients (Steffen and Pawliszyn 1996). Using solid phase micro extraction techniques (SPME) (Whiton and Zoecklein 2000), the headspace was sampled for 30 minutes at 22ºC with a 65μ Carbowax-divinyl benzene fiber (Supelco, Bellefonte, PA) with agitation (5sec/2sec).

Gas chromatography/mass spectrometry. The fiber was desorbed into a 5890 Gas Chromatograph (GC) interfaced to a 5972 Mass Spectrometer (MS) (Hewlett Packard, Palo Alto, CA). The GC column was a 30 m x 0.25 mm DB-WAX (J & W Scientific, Folsom, CA) with a 0.25 μm coating, carrier gas of helium at a linear flow velocity of 36 cm/sec, with the injector temperature at 240°C, and the column held at 40°C for 5 minutes, and programmed at 6°C/minute to 230°C, injection mode was splitless for 5

minutes. The MS operated in full scan mode under Autotune conditions with 70 eV electron impact ionization. Chromatographic peak areas were determined using HP Chem Station (Version 5.02).

All samples were analyzed in triplicate for 23 volatile compounds listed in Table 1. Analyte peaks were integrated from extracted ion plots, and the responses for each compound were quantified using a standard calibration curve. Calibration with a 23 compound solvent standard was multi-point with the original sample, a 50:100 dilution a 1:100 dilution and a 1:1000 dilution. The standards (Sigma-Aldrich, Milwaukee, WI) were prepared in 10% ethanol with 1mM tartaric acid.

Condensate Analysis. The condensate in the capture and remove treatment was collected daily and stored in a sealed container at 4ºC prior to analysis. The ethanol concentration of each sample was determined using direct injection (7673 GC/SFC Autoinjector, Hewlett Packard, Palo Alto, CA) of a 1 μL sample into the injection port of a 5890 Series II Plus Gas Chromatograph (GC) interfaced to a flame ionization detector (FID), at 250ºC (Hewlett Packard, Palo Alto, CA), with a 30 m x 0.32 mm RTX-5 column (Restek, Bellefonte, PA), with a 1.0 μm coating. The method was adapted from the 2003/2004 Supelco Method for ethanol determination. The carrier gas was helium at a velocity of 20 cm/sec., injection mode was split 10:1 for five minutes with 5 ng of sample on column. The injector temperature was 225°C, run isothermally at 40°C. Chromatographic peak areas for selected ions were calculated using HP Chem Station version 5.02. Samples were diluted using distilled deionized water, to 13% (v/v) ethanol

concentration and analyzed for volatile compounds (Table 1) using the methodology previously described. Aroma units were determined by dividing the concentration of each compound present by the reported sensory threshold value (Simpson and Miller 1984a) (Table 1).

Sensory Analysis. Sensory analysis was conducted four to six months after bottling, using pooled treatment replications. Discrimination and preference sensory evaluation occurred between 0900 and 1400 in the sensory evaluation laboratory in the Department of Food Science and Technology. Panelists were seated in individual sensory booths. Twenty mL of wine at 15ºC was poured into standard ISO glassware, covered with a watch glass, and immediately evaluated. Panelists consisted of students that had attended three two-hour training sessions which included structural components as well white wine varieties.

Panelists were asked to evaluate wine aroma using the triangle difference test as described by Meilgaard et al. (1999). Panelists performed two difference tests per seating. The preliminary 1998 and 1999 sensory sessions consisted of evaluations by 13 to 30 panelists. In 2000 and 2002, 40 panelists evaluated each treatment. When differences were observed, the treatments were also evaluated for preference (n=40) using the paired preference method at a separate session one week after the discrimination test. Panelists received two samples per session.

Wines that were determined to be significantly different were also evaluated using consensus training methods by a panel, made up of commercial winemakers (n=16). Assessment occurred between 0900 and 1600 in a room modified to follow sensory evaluation standards described by Meilgaard et al. (1999). Twenty mL of wine at 15°C was poured into a randomly coded, standard ISO glass, covered with a watch glass, and immediately evaluated. Panelists were asked to rate the intensity, on a 12 cm scale (0= weak to 12= strong), of a modified list of standard white wine descriptors (honey, butterscotch, butter, ethylacetate, floral/fruity, apple, cherry, pear grape, citrus and cut grass), selected from the wine aroma wheel (Noble 1987). Each response was measured and converted to a numerical value. Evaluation consisted of three half-hour sessions with 6 samples per session and one hour between sessions. Panelists conducted these ratings independently with no discussion.

Statistical Analysis. Average concentration of analytes in the trap evaluations were compared using the General Linear Model and average concentration of analytes in the temperature evaluations were compared using a two by three factorial structure with sub-sampling. SAS statistical software (version 8.1; SAS Institute, Inc. Cary, N.C.) was used with an α-level of 0.05. Analysis of difference and preference testing was conducted using published tables (Meilgaard et al. 1999), at an α-level of 0.05 and a β-level of 0.10. Consensus training methods were analyzed using paired-comparison t-test. Significance was assigned an α-level of 0.05.

RESULTS

The pH, titratable acidity and alcohol (% v/v) of wines produced in these studies are given in Table 2 (commercial study) and Table 3 (temperature controlled study). Treatment wines frequently had elevated alcohol levels compared to the control, and treatment wines fermented at 30ºC had lower alcohol levels than treatment wines fermented at 15ºC. Both capture and return, and capture and remove had higher titratable acidities the exception being 2002. Organic acids of commercially produced wines were comparable ($p>0.05$) and wines successfully completed malolactic fermentation, except for one 2000 vintage (data not shown). Temperature controlled fermentations did not undergo malolactic fermentation.

Commercial scale evaluation of capture and return over four vintages indicated a trend of increased concentration of esters and ethyl esters of fatty acids (Table 4). These compounds were typically above sensory threshold, with the exception of diethylsuccinate. Ethyldodecanoate, ethyloctanoate, and ethylhexanoate were the only ethyl esters found in lower concentrations in the treatment wines. Differences in fusel alcohol acetates were seen in hexylacetate and isoamylacetate, with a trend of decreased concentration in the treatment. Isoamylacetate was consistently in lower concentrations in treatment wines and below sensory threshold. Fatty acids were minimally affected by capture and return (Table 4). Octanoic acid, decanoic acid and acetic acid had lower concentrations in the treatment wines (Table 4). None of the fatty acids were determined to be above sensory threshold except for acetic acid in the 2002 control wine. There

appeared to be no strong correlation between molecular weight, temperature of volatilization and compound retention. Higher alcohols demonstrated few significant differences based upon treatment (Table 4). Two compounds, 2-methylpropanol and n-hexanol were found in increased concentrations in capture and return wines, while 3-methylbutanol and 2-phenethylacetate were consistently lower in treatment wines. The only higher alcohol that was not above sensory threshold was 2-methylpropanol.

In 2002, the commercially produced capture and remove wines had nine compounds that were significantly different from the control (Table 5). Diethylsuccinate was the only compound with higher concentrations than the control. The remaining eight compounds consisted mainly of fatty acids and fatty acid esters and were found in lower levels in capture and remove when compared to the control. Capture and remove wines consistently had lower concentrations of volatile compounds compared to the capture and return with the exception of acetic acid and diethylsuccinate.

Analysis of trap condensate collected in 2002 indicated that fifteen of the 23 compounds were retained, six in concentrations above aroma threshold (Table 6). Hexanoic acid was the only compound collected in the condensate that was not also found to be significantly different in commercially fermented capture and return wines. Compounds with concentrations above sensory threshold were primarily esters and higher alcohols, with lower concentrations than in the wine. Only one fusel alcohol acetate, isoamylacetate, was above sensory threshold and in higher concentrations in the condensate than in the wine.

Evaluation of temperature effects indicated that fatty acids and ethyl esters of fatty acids were impacted (Table 7). The majority of analytes were found in higher concentrations at 30ºC compared to 15ºC in control, capture and return and capture and remove wines. These were generally of higher molecular weight and higher temperatures of volatilization compared to the other compounds quantified. Minimal temperature effects were seen on the fusel alcohol acetates and the higher alcohols. Compounds impacted by temperature were typically found in the highest concentration in capture and remove (Table 7), with the exception of ethyldodecanoate, decanoic acid and phenethylacetate.

SENSORY ANALYSIS

The results of sensory analysis were variable depending upon year, and source (Table 8). In 1998, the Chardonnay West demonstrated significant differences ($p \leq 0.05$), as well as a preference (27/40, $p<0.05$) for the capture and return wine. These wines were evaluated again eight months later and, there was still a preference for the treatment (32/40). Consensus evaluation of the wines indicated a higher perception of butter, grape and pear, and lower levels of cut grass in the treatment wines (Figure 2). Triangle difference testing in 1999 demonstrated a significant difference in one of the three blocks evaluated (Table 8). Other vintages resulted in no sensory difference (Table 8). Panelists were able to determine differences between wines fermented at 15ºC and 30ºC, but unable to determine differences between control, capture and return, and capture and remove wines fermented at the same temperature (Table 9).

DISCUSSION

Capture and return and capture and remove treatments had a trend of elevated titratable acidities, and ethanol concentrations (Table 2,3) throughout the study. Ethanol increase may be due to retention of higher alcohols and retention of volatile constituents in the second highest concentration next to water which is ethanol. Lower ethanol concentrations were observed in capture and return and capture and remove treatments fermented at 30ºC. This is consistent with previous research (Ough 1964, Ough 1966). Capture and remove and capture and return wines fermented at 30ºC also consistently had elevated TA's. Several studies have reported elevated TA with increased fermentation temperature, which has been accounted for by increased production of acetic and succinic acids (Ough et al. 1967).

Ethyl esters of fatty acids were consistently found in higher concentrations in capture and return wines, possibly due to the relatively hydrophobic nature of these compounds. With the exception of ethyldodecanoate, most compounds were in concentrations above aroma threshold (Table 4). Many of these compounds result in fruity and estery characteristics, and have been positively correlated to wine quality (Duplessis 1975).

Fusel alcohols and their acetates generally contribute more to wine aroma intensity than quality (Etievant 1991). In general, fusel alcohol acetate concentrations appeared to be negatively associated with capture and return. In both capture and return and capture and remove treatments, minimal temperature effects were seen on the fusel alcohol acetates,

which is in concordance with work done by Rankine (1968), Shinohara and Watanabe (1976) and Killian and Ough (1979). Phenethylacetate, however did exhibit temperature effects, but was below sensory threshold.

Fatty acids and higher alcohols had the least differences in concentrations between control and capture and return and capture and remove treatments. Miller et al. (1987) also indicated a negligible proportion of higher alcohols were entrained to carbon dioxide. This may be due to low level of hydrophobicity and the fact that they are formed in the early growth phases of the yeast (Kunkee and Goswell 1977), and readily solubilize as the ethanol content of the wine increases (Williams and Rosser 1981).

Analysis of trap condensate in 2002 indicated that fifteen of the 23 compounds were retained, four in concentrations above aroma threshold (Table 6). The condensate was primarily made up of esters and ethyl esters of fatty acids. This is in concordance with our capture and return wine analysis, which indicated a trend of increased retention of these compounds. Four of the fifteen compounds retained were fatty acids, each of which was found in concentrations below threshold. Higher alcohols were also identified in the condensate and were generally above threshold. This was surprising due to the lack of differences in concentrations of higher alcohols in the capture and return wines. The differences observed may have been due to hydrolysis. These compounds were collected in the form of condensate from the fermenting vessel, therefore the aroma unit calculation, is effectively the loss of aroma due to fermentation. Loss of ethyl esters of

fatty acids, in this study, was higher than those observed by Miller et al. (1987) for unknown reasons.

Ethyloctanoate and ethylhexanoate were found to be significantly lower in the 30ºC fermentations in the control, capture and return and capture and remove treatments (Table 7). Killian and Ough (1979) indicated that fermentation at 30ºC can result in slowed formation of esters and more rapid losses. Ethyldecanoate and ethyldodecanoate, however, were found in higher concentrations in the 30ºC wines. This may be due to the fact that they are higher boiling point esters, and are thus less likely to be lost during fermentation.

Higher alcohols, fatty acids and fusel alcohol acetates were produced in higher concentrations in the 30ºC fermentation and were also in higher concentrations in capture and return wines compared to the control (Table 8). The 30ºC fermentation appeared to have increased formation of these compounds, and therefore, capture and return treatments subsequently had increased concentrations compared to the control at both temperatures. Elevated fermentation temperature showed increased concentration and retention of fusel alcohols, fatty acids and higher alcohols. At 15ºC, the effects of capture and return were primarily seen in ethyl esters of fatty acids. It is interesting to note that compounds that were in increased concentration as a result of higher fermentation temperature were mainly sensory contributing compounds (AU<1.0), while those that had lower levels as a result of increased fermentation temperature were mainly sensory impact compounds (AU>1.0).

While the higher alcohols were generally found in lower concentrations in the 30ºC wines in both capture and return and capture and remove, the fatty acids were consistently in higher concentrations. While the fatty acids were not often significantly different in capture and return wines compared to a control fermented at the same temperature, there were significant differences in capture and return wines fermented at 30ºC compared to a control fermented at the same temperature (Table 8). Fatty acids (decanoic acid, acetic acid, and octanoic acid) were in higher levels in the capture and return and capture and remove wines, with a trend of increased compound concentrations in the 30ºC wines. However, none of the fatty acids were above sensory threshold.

The results of sensory evaluation follow volatile analysis, in which most compounds found to be significantly different between treatments were below sensory threshold. Therefore, a consumer panel was not able to determine differences between control and capture and return wines. Significant differences between control and capture and return wines were observed in vintages in which larger proportions of compounds were found above sensory threshold (AU>1).

The purpose of this study was to evaluate capture and return for the partial retention of fermentation volatiles as a means of improving white wine quality. Compound concentration trends in capture and return wines were variable from vintage to vintage with a trend of increased concentrations of ethanol, esters and ethyl esters of fatty acids and decreased concentrations of fusel alcohol acetates, fatty acids and higher alcohols in

treatment wines. Fermentation temperature at 30ºC increased concentration and retention of fusel alcohols, fatty acids and higher alcohols compared to 15ºC. Sensory analysis using triangle difference testing indicated inconsistent differences in aroma among treatments.