

METHODS AND OBSERVATIONS FOR THE INFLUENCE OF  
TEMPERATURE ON VOLATILE LOSS FROM  
WINE FERMENTATION

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by  
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## ABSTRACT

### Methods and Observations for the Influence of Temperature on Volatile Loss from Wine Fermentation

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**Background and Aims:** Volatile loss of carbon dioxide, ethanol, esters and other compounds occurs during wine fermentation. When collected nondestructively, valuable ethanol and aroma compounds can be preserved for various uses while mitigating production restrictions and regulations regarding volatile organic compound (VOC) loss from wine production. Knowledge of the volume of volatiles lost during wine fermentation contributes to a better understanding of the magnitude of possibilities for resource recovery/aroma recovery, the implications of volatile loss on wine composition as well as a more clear understanding of the possible effect of alcoholic fermentation on air quality. The aim of this study was to contribute to a better understanding of how the loss of volatiles from wine fermentation varies with temperature.

**Methods and Results:** Temperature controlled microscale fermenters were developed and infrared detection technology was adapted to study the effect of temperature on volatile loss. Results are presented for the rates and volumes of volatile loss from the fermentation of California Syrah at constant temperatures (17, 23, 27, 33°C) in 1.9L containers. Observed volatile losses are compared to theoretical losses based on kinetic and stoichiometric principals. Each ferment started with 1200g of fruit and was adjusted to 23.5°B. Following Brix adjustment, final volumes ranged from 1129.16mL to 1160.10mL.

**Conclusion:** The loss of VOC from fermentation increases exponentially with temperature. Total VOC and CO<sub>2</sub> loss appears to be slightly less than theory predicts.

**Significance of the Study:** A significant loss of compounds occurs during wine fermentation. Commercial and environmental benefits may be achieved if efforts are made to recover and make use of these otherwise wasted compounds. Funding provided by the Agricultural Research Initiative, California State University.



Keywords: Wine, ethanol, fermentation, esters, aroma, volatile, ethanol recovery, aroma recovery, temperature, VOC, dissolved carbon dioxide.

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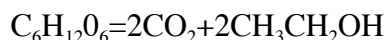
## SECTION 1

### LITERATURE REVIEW

#### Volatile Loss from Wine Fermentation: Challenges and Opportunities

##### Introduction

When yeast ferment juice into wine, one mole of glucose/fructose is converted to two moles of carbon dioxide (CO<sub>2</sub>), and two moles of ethanol (EtOH):



The majority of total CO<sub>2</sub> produced and a fraction of total EtOH produced are lost to the atmosphere. Recognition of these losses is attributed first to Gay-Lussac in 1821 (Williams and Boulton 1983). Subsequent interest in these losses came about in 1978, when ethanol emissions from wine production were identified as a source of smog precursors in the San Joaquin Valley of California (Fresno County Air Pollution Control District 1978). Most recently, interest in these losses has been expressed for the potential to collect and reuse the associated aroma compounds (Bach 2001).

The following literature review explores the relationship between wine fermentation, volatile loss, air quality regulations, wine composition and aroma recovery. The story begins in the San Joaquin Valley (SJV) of California (CA), where the relationship between wine fermentation and air quality was first confronted. We then briefly consider the extent of this relationship in other parts of CA and the world. We discuss the affect that air quality regulations have had on wineries, we consider the

impact that a stricter national air quality standard for ozone would have on many wine producing regions in CA and we present the options available for wineries to mitigate production regulations. We then consider the composition of fermentation volatiles, the implications that the loss of ethanol and aroma has on wine composition and the potential to collect and utilize these otherwise wasted compounds. Research regarding the volume of volatile organic loss and the mechanisms responsible for these losses are presented and discussed while the need for new research is highlighted.

A better understanding of volatile loss from wine fermentation is of interest for both resource recovery and air quality implications. Today, the majority of attention devoted to this topic is due to the environmental implications. Thus, we shall first review the history of wine production and air quality.

### Wine Fermentation and Air Quality

Volatile organic compounds (VOC) react with nitrous oxides in the presence of sunlight to form ground-level ozone, more commonly referred to as smog (Carter 1994). While the majority of VOC are produced by the combustion of fossil fuels, wine production is a significant source in many areas. For example, wine production accounts for about 30% of the stationary sources of VOC in the SJV (Cox 2009). This is a significant, especially when we consider the fact that the SJV has the second worst smog pollution in the US (Carter 1994). Given the magnitude of wine production as a source of smog precursors, wine production in the SJV has a history

of being evaluated for and subjected to regulatory efforts intended to decrease the loss of VOC from wine fermentation for the purposes of improving air quality (Table 1).

Table 1. One third of a century of attempts to control ethanol loss from wine fermentation.

YEAR	KEY EVENTS
<b>1978</b>	Ethanol emissions were identified as a source of oxidant precursors in the San Joaquin Valley, leading to interest in the control of ethanol emissions from wine fermentation by the California Air Resource Board (CARB).
<b>1980</b>	Fresno County Air Pollution Control District (FCAPCD) was identified as the lead agency for developing Suggested Control Measures (SCM).
<b>1982</b>	FCAPCD produced a draft SCM which required a 90% reduction in ethanol emissions for all fermentation tanks with a capacity greater than 100,000 gallons. Condensation was recommended as the most cost effective method. In response, the Wine Institute, representing over 80% of wineries in CA, prepared an alternative proposal based on temperature control of wine tanks (30% efficiency).
<b>1985</b>	FCAPCD revised the SCM, indicating temperature control (30% efficiency) as the most cost effective method of control. CARB evaluated the cost analysis of various control methods and showed that temperature control was not the most cost effective. CARB took over as lead agency for further development of the SCM due to lack of resources at FCAPCD.
<b>1986</b>	ARBs revised SCM were considered. The ARB was invited to tour wineries to see plant layout and operations unique to each facility, wine industry submitted comments/ held meetings. Deferred action pending outcome of demonstration.
<b>1987</b>	1st phase of demonstration program. The Wine Institute and Wine Growers of California jointly funded a pilot project at CSU Fresno. Found water scrubbing, catalytic incineration and carbon adsorption to be 90% efficient or greater but not feasible due to disposal of ethanol laden water (water scrubbing), high initial capital cost (catalytic incineration), operational problems (carbon adsorption). Condensation was not evaluated.
<b>1988</b>	2nd phase of demonstration program. Tests utilized carbon adsorption exclusively given that it was the most promising method tested in '87. Better operation of the system was achieved.
<b>1990</b>	Demonstration project at E&J Gallo Fresno. Tested adsorption on 207,000 gallon tank. 90% control efficiency. Mandatory fermentation control still under decision. Cost to achieve control as primary concern (Heredia 1993).
<b>2005</b>	Rule 4694 adopted by the San Joaquin Valley Air Pollution Control District (SJVAPCD). Requires 35% reduction in Volatile Organic Compounds (VOC) emissions from wine fermentation and storage, or equivalent reductions from alternative emission sources (ARB 2005).
<b>2013</b>	The SJV SIP draft was amended (by the SJVAPCD due to EPA concerns) and accepted by the EPA to only pertain to wine storage. EPA requested the revision because the SJV was not enforcing a 35% reduction in primary fermentation emissions and therefore should not receive SIP "credit" for the reduction (EPA 2012 B).

The timeline in Table 1 depicts a struggle between industry and government. The wine industry, represented by the Wine Institute and Wine Growers of California, is hesitant to adopt VOC controls for wine fermentation because of the perceived financial and logistical uncertainty involved in doing so. Their stance on the issue is understandable, considering it is their role to ensure that the CA wine industry remains as competitive as possible with both domestic and global competition. Controlling the loss of VOC from fermentation is perceived to be a cost to business, one that the industry would prefer not to incur. On the other hand, the government, represented by Fresno County Air Pollution Control District (FCAPCD, which no longer exists and was subsumed into the SJVAPCD) and California Air Resource Board (CARB), is under legal obligation (based on the Clean Air Act) to decrease smog pollution in order to protect human health. Controlling the loss of VOC from wine fermentation represents a significant opportunity to improve air quality.

While the majority of the debate concerning the control of VOC from fermentation is directed towards the SJV, the SJV is not the only place where wine production plays a significant role in air quality. In CA, production caps currently exist for wineries in both the SJV and Santa Barbara County (ARB 2005, SBAPCD 2009). Additionally, ethanol emission inventory is monitored for wine production in the California counties of Monterey, San Francisco and San Luis Obispo, as well as in Australia (NPI 2003). In the US, if federal ozone standards become stricter, the California counties of Monterey, San Francisco and San Luis Obispo would face increased pressure to reduce ethanol loss from wine production and the existing restrictions for

SJV and Santa Barbara counties may become stricter. Note that SF does not have a county APCD. The Bay Area Air Quality Management District (BAAQMD) covers nine counties (or parts thereof): Alameda, Contra Costa, Marin, Napa, San Francisco, San Mateo, Santa Clara, southwestern Solano, and southern Sonoma counties.

#### Federal Ozone Standard

The Clean Air Act requires regular review of the federal ozone standards, also referred to as the National Ambient Air Quality Standards (NAAQS) for ozone. Since 2006, the Clean Air Scientific Advisory Committee (CASAC), which advises EPA on setting NAAQS standards, has unanimously advised lowering the allowable 8-hour ozone standard from 0.075ppm to between 0.060 to 0.070ppm, stating that there is,

““no scientific justification for retaining” the current primary O<sub>3</sub> (ozone) standard, and the current standard “needs to be substantially reduced to protect human health, particularly in sensitive subpopulations” (Henderson 2006, EPA 2006, EPA 2007).”

“Based on these considerations, the Administrator (of EPA) concludes that the current primary O<sub>3</sub> standard is not sufficient and thus not requisite to protect public health with an adequate margin of safety, and that revision is needed to provide increased public health protection (EPA 2008).”

As allowable ozone concentrations decrease, areas currently struggling to comply with existing standards face increased pressure to reduce associated emissions. This means that, for areas with significant wine production, additional interest will be directed to methods to control ethanol loss from wine fermentation.

#### Methods to Capture, Destroy or Offset Ethanol Loss

Methods currently available to wineries faced with air quality regulations include: decreasing wine production, purchasing emission offset credits or containing ethanol loss. Decreasing wine production has inherent drawbacks, while off-sets are not always available. Options for containing ethanol loss include: incineration, water scrubbing, carbon adsorption and condensation (Table 2).

Table 2. Methods to decrease ethanol loss from wine fermentation (SJVAPCD 2007).

Method	Mechanism	Capture efficiency
Incineration (Oxidation)	Converts VOC to CO <sub>2</sub> through the process of combustion	90%
Water Scrubbing	Transfers air born VOC to a liquid waste stream	90%
Carbon Adsorption	Transfers air borne VOC onto a solid substrate	90%
Condensation	Converts VOC from gas to liquid	90%

Incineration, water scrubbing and carbon adsorption were evaluated in 1987 when CARB was considering a Suggested Control Measure (SCM) for ethanol loss from wine fermentation in the SJV. Condensation was not evaluated. The demonstration, which was funded by the Wine Institute and Wine Growers of California and took place at CSU Fresno, found that all three methods were capable of reducing ethanol

loss by at least 90%. However, each method had feasibility concerns: high initial capital cost for incineration, disposal of ethanol laden water for water scrubbing and operational problems with carbon adsorption (Todd et al. 1988).

In addition to having a high initial capital cost, incineration requires the combustion of supplemental fuel, which creates nitrous oxides (an ozone precursor like VOC) therefore negating some of the VOC reduction (SJVAPCD 2007). Incineration also requires that the CO<sub>2</sub> be diluted with O<sub>2</sub> to enable combustion of VOC. Consideration needs to be given to the disposal of ethanol laden water produced from water scrubbing. Specifically, if above ground leach fields are used to dispose of ethanol laden water, a portion of the ethanol could evaporate to the atmosphere which would negate some of the VOC reduction. Additional concerns may be raised by water quality agencies with regard to the influence of ethanol laden water on soil and ground water. Carbon adsorption involves subsequent desorption of VOC from the carbon substrate by applying heat, decreasing pressure or reducing the hydrocarbon concentration around the adsorbent. Condensation is a relatively straightforward method for VOC collection. Its nondestructive nature is conducive to the separation and preservation of delicate ethanol and aroma compounds.

#### Composition of (Recovered) Fermentation Vapors

The loss of aroma that occurs during wine fermentation results in a decreased concentration of aroma compounds in the wine. Miller et al. (1987) found that up to 25% of ethyl esters and acetate esters produced during fermentation are lost to the



atmosphere. Esters are the primary source of fruity aromas in wine (Gurbuz, Rouseff and Rouseff 2006). While their contribution to the aroma and flavor of wine is tremendous, their concentrations are minute, generally not exceeding 100mg/L (Etiévant 1991). Most esters are present in wine at concentrations around their threshold detection values; esters present in concentrations below their threshold concentration impact wine flavor through synergistic interactions with other compounds (Sumby 2010). Thus, minute adjustments to ester concentrations can have significant effects on the flavor and aroma of wine. Bach (2001) found that collecting and adding back the otherwise lost volatile aromas significantly increased wine quality.

Condensing the vapors released during wine fermentation yields a clear solution of water, esters, up to 50% alcohol V/V and other compounds (D. Goldfarb, unpublished data, 2012). Compounds identified in the condensed vapors include ethyl acetate, isoamyl acetate, ethyl caproate, ethylbutyrate, 1-hexyl acetate, ethyl caprylate and ethyl decanoate (Table 3).

Table 3. Compounds identified in fermentation vapor condensate (A. Waterhouse, unpublished data, 2012) and descriptors of these compounds (K. Sumby et al. 2010).

Compound	Aroma Characteristic
Ethyl acetate	Fruity ( $\leq 100$ mg/ml), solvent, balsamic ( $> 100$ mg/L)
Ethyl butyrate	Floral, fruity, strawberry, sweet
Isoamyl acetate	Banana, fruity
Phenylethyl acetate	Flowery, rose
Ethyl caproate	Fruity, strawberry, green apple, anise
Hexyl acetate	Green, herbaceous, fruit, grape

Factors that affect ester production such as: ethanol formation (Rapp and Mandery 1986), temperature, pH (Simpson 1979, Ribereau-Gayon 1978, Van der Merwe and van Wyk 1981, Bertrand 1983), anaerobic conditions, SO<sub>2</sub> levels (Simpson and Miller 1984, Nykanen 1986), yeast strain (Takush and Osborne 2012), yeast nutrition (Francisco et al. 2008) and enzyme activity (Swiegers et al. 2007) may contribute to the extent of ester lost.

#### Quantification of Ethanol Loss From Wine Fermentation

While the scales, materials and conditions vary from study to study, four main methods have been used to quantify ethanol emissions from fermentation: gas chromatographic analysis (Fielder and Baumala, 1982), dichromate analysis (Wakentin and Nury 1963, Zimmerman et al. 1964) mathematical models (Wakentin and Nury 1963, Zimmerman et al. 1964, Williams and Boulton 1983) and flame ionization detection (Todd et al. 1988, Todd et al. 1990). Flame ionization detection is a common technique used by CARB to quantify VOC from various sources.

However, its application for wine fermentation is complicated by the high concentration of CO<sub>2</sub> present in VOC samples. CO<sub>2</sub> must be diluted with O<sub>2</sub> to enable combustion, which can decrease the accuracy of the method. Results of previous research on the quantification of ethanol loss from fermentations are given in Table 4 and graphed for illustration in Fig. 1. Results are presented in terms of “alcohol lost as a percent of total alcohol produced,” which adjusts for the variability in sugar metabolization and ethanol formation.

Since aroma and ethanol loss occur simultaneously, a better knowledge of ethanol loss has implications for aroma loss. Methods used to quantify VOC loss from wine fermentation (flame ionization detection, dichromate analysis, infrared detection) measure total hydrocarbons, which includes both ethanol and esters. However, given the relative scarcity of hydrocarbons other than ethanol in fermentation emissions, “ethanol” is used as an umbrella term for all wine VOC.

Table 4. Summary of Ethanol Loss Studies. Rows 1-14 (Fielder and Bumala 1982), Rows 15-30 (Todd et al. 1988).

	Year	Study	Alc. content of wine (%vol/vol)	Initial sugar (°B)	Fermentation Temperature (°C)	Alcohol lost as % of total alc. avail.
1	1938	Mathieu and Mathieu		18	35	1.5
2	1949	Flanze and Boudet		18.2	35	1.2
3	"	"		18.2	20	0.65
4	"	"		18.2	5	0.17
5	1963	Warkentin and Nury	4.6-10.6 range		30	1.17
6	"	"	(7.6 avg)		27	0.83
7	1964	Zimmerman, Rossi and Wick		21	26.5	0.84
8	"	"		16	26.5	0.7
9	1980	ARB (using Warkentin and Nury formula)	3.0-4.0 range		11	0.3
10	"	ARB (based on measured alcohol loss)			11	0.2
11	1982	EAL/UV Red Wine Madera	entire range	23	29	1.3
12	"	EAL/UV Red Wine Oakville	"	23.5	22	0.82
13	"	EAL/UV White Wine Madera	"	23	14	0.35
14	"	EAL/UV White Wine Oakville	"	22.4	17	0.2
15	1988	Todd, Castronovo and Ouchida White Wine I	10.3	20.1	15	0.18
16	"	"	10.3	20.1	15	0.2
17	"	"	10.3	20.1	14	0.09
18	"	"	8.6	20.1	15	0.09
19	"	Todd, Castronovo and Ouchida Red Wine I	14.11	24.1	25.5	0.83
20	"	"	14.17	23.9	25.5	1.3
21	"	"	14.3	23.9	26	0.31
22	"	"	13.65	24.3	24	0.2
23	"	Todd, Castronovo and Ouchida Red Wine II	11.83	25.4	26.5	0.9
24	"	"	13	24.8	25.5	0.77
25	"	"	12.44	25.1	24.5	0.66
26	"	"	12.54	25.5	27	0.44
27	"	Todd, Castronovo and Ouchida White Wine II	10.83	22.3	14	0.48
28	"	"	10.73	22.3	14	0.55
29	"	"	11.27	22.3	14	0.46
30	"	"	9.95	22.3	14	0.52

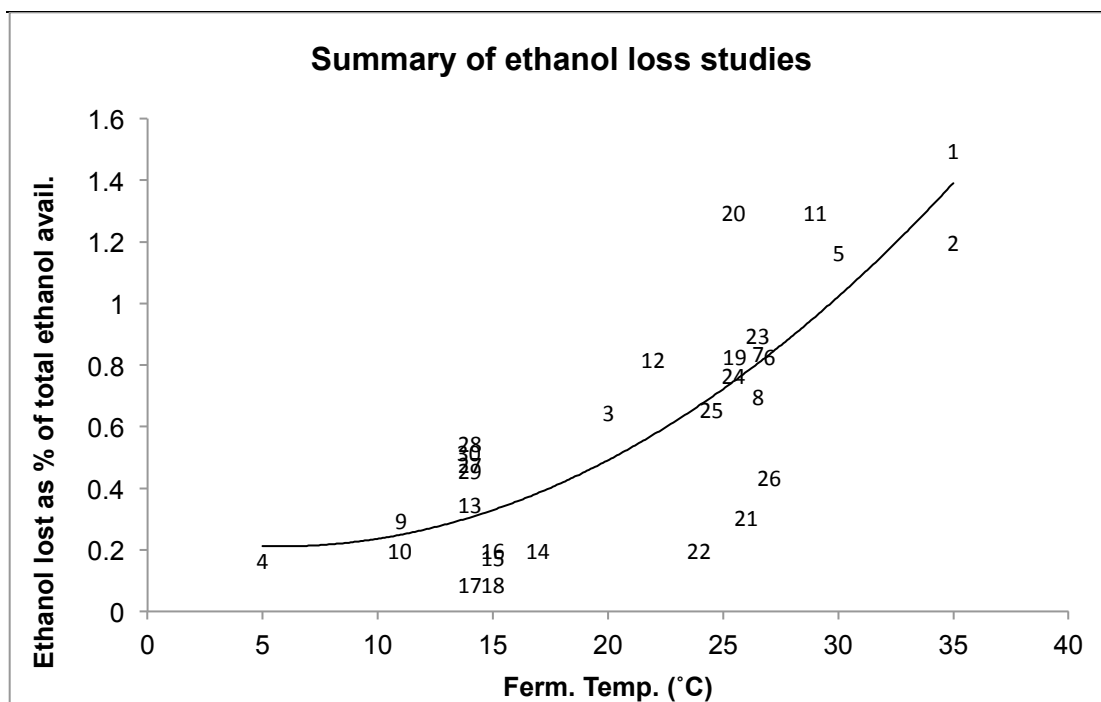


Fig. 1. Results of studies for ethanol lost as a % of total ethanol available with temperature (Fielder and Bumala 1982, Todd et al. 1988). Curve was created as best fit.

Ethanol loss has been observed to be from 0.2-1.5% of total ethanol available across a wide range of temperatures and conditions (Fig. 1). These losses increase exponentially with temperature (Fig.1). In order to mathematically predict ethanol loss across a range of must sugar contents and fermentation temperatures, the mechanism(s) responsible for these losses needed to be determined.

#### Mechanism for Ethanol Loss: Entrainment vs. Evaporation

In 1983, Lynn Williams employed kinetic and stoichiometric principals to determine if entrainment, evaporation or both mechanisms were responsible for ethanol loss from wine fermentation. The findings corrected a prevalent misunderstanding about

the role (or lack there of) of entrainment in volatile loss and laid the foundation for developing mathematical models to estimate these losses.

Bursting bubbles at the surface cause entrainment of liquid droplets by two mechanisms: film collapse and central jet. The film collapse mechanism produces droplets 20-150 $\mu\text{m}$  in diameter while the central jet mechanism produces droplets 200-1000 $\mu\text{m}$  in diameter. The size of the film collapse droplets are so small that their contribution to total volatile loss is negligible (Williams 1983). While the size of the central jet droplets are large enough to have a potential effect on total volatile loss, they do not travel beyond 10-20cm above the must surface (even when considering the added  $\text{CO}_2$  velocity of rapid fermentations and high volume to low surface area conditions of tall, slender tanks). The application of Stokes Law states that droplets greater than 15 $\mu\text{m}$  should fall back into must (Williams 1983). Thus, the only significant mechanism by which volatiles escape from fermentation is evaporation. Entrainment is not responsible for volatile loss from fermentation.

#### Modeling Ethanol Loss

Based on L.A. Williams's work, R. Boulton developed a model for predicting volatile loss from wine fermentation (Williams and Boulton 1983). Results are presented for a fermentation at 21.1°C with in initial sugar concentration and 20°B (Fig. 2). This model shows that the rate of  $\text{CO}_2$  loss peaks before that of ethanol loss. This model implicitly assumes that all  $\text{CO}_2$  produced during fermentation is released to the atmosphere.

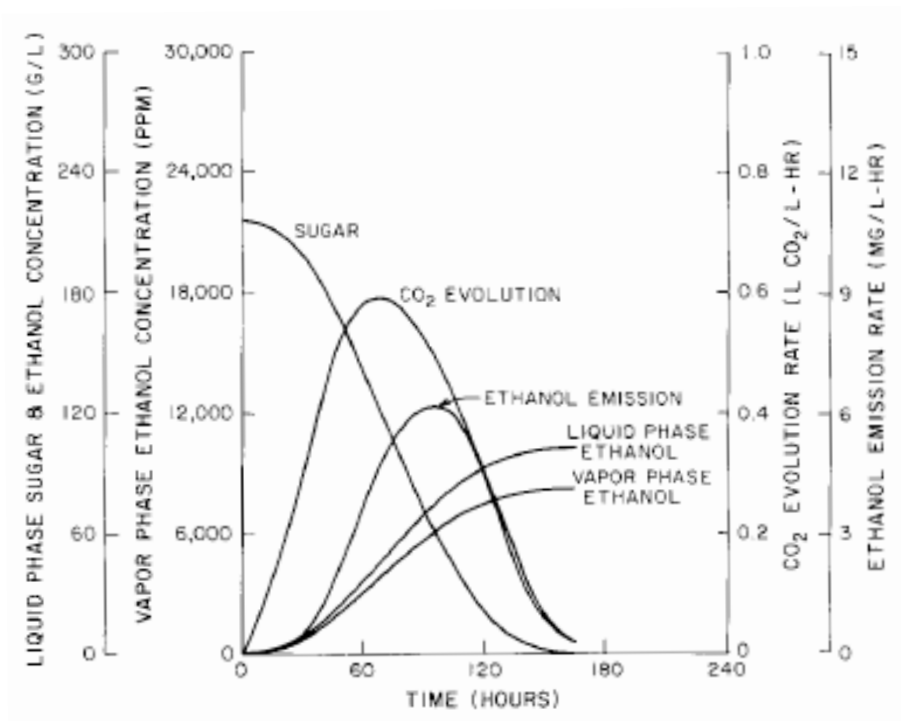


Fig. 2. Mathematical model for rates of sugar depletion, CO<sub>2</sub> evolution, ethanol emissions, liquid and vapor phase ethanol concentrations. Initial sugar=20°Brix, Temperature=21.1°C (Williams and Boulton 1983).

### Emission Factors for Ethanol Loss

Emission factors are multipliers used to estimate the mass of material emitted from various sources. Emission factors are developed and used by both CARB and EPA to estimate pollutant volumes for the purposes of monitoring and potentially regulating emission sources. Emission factors have been developed and imposed on wine production despite an incomplete understanding of these losses.

EPA emission factors for ethanol loss from red and white wine fermentation are 4.6lb/1000 gal wine and 1.8lb/1000 gal wine respectively. These emission factors

were based on available literature. The literature that these emission factors were based on was graded for perceived quality. Grades for data quality ranged: A, B, C, D (high to low). The quality of the resulting emission factor was also graded: A, B, C, D, E (range high to low). Both emission factors were given a rating of C (EPA 1995). These poor ratings demonstrate a lack of quality data resulting in an incomplete understanding of the subject and a need for better research.

CARB uses an emission factor of 6.2lbs EtOH/1000 gal reds and 2.5 lb EtOH/1000 gal whites. For both EPA and CARB emission factors, the volume is undefined: it could refer to finished wine or must, with or without a cap. When the SJVAPCD passed rule 4694 in 2005, they used the 6.2/2.5 factors from CARB and defined the liquid volume as must (ARB 2005). Emission factors attempt to be a “one size fits all” however, the variation in volatile loss is more dynamic than a simple distinction between red and white wine suggests.

#### Factors that Affect Ethanol Loss from Wine Fermentation

Greater ethanol loss occurs with larger volumes of must, larger surface areas and increased porosity of containers (Fielder 1982). On a per volume basis, the most significant factors that affect ethanol loss are total glucose/fructose metabolized and fermentation temperature. Ethanol loss increases with temperature and sugar metabolism (Nury and Warkentin 1963, Williams 1983, Williams and Boulton 1983). Temperature affects ethanol loss by influencing the partial pressure associated



with ethanol present in the must through two mechanisms: vapor pressure, as stated by the Antoine equation, and activity coefficient, as stated by solution thermodynamic relationships (Williams and Boulton 1983). In red wine, yeast adhere to the cap which can increase the temperature in the cap by 8°-11°C compared to the bulk liquid (Guymon and Crowell 1977). This increase in temperature causes an increase in ethanol loss (Williams and Boulton 1983).

While there is a correlation between temperature and rate of fermentation, the Williams-Boulton model indicates that, when rate is separated from temperature by adjusting yeast inoculum level, rate has no effect on ethanol loss (Williams and Boulton 1983). This finding is supported by modeling done by Williams (1983), who applied Stokes Law to determine that even the highest CO<sub>2</sub> flow rates (which are associated with the most rapid rates of fermentation) are insufficient to contribute to vapor loss by entrainment (Williams 1983).

Since ethanol is lost to the atmosphere with CO<sub>2</sub>, factors affecting CO<sub>2</sub> retention and loss can be expected to have an effect on ethanol loss. Specifically, the degree of must saturation with DCO<sub>2</sub> (unsaturation, saturation and super-saturation) has an effect on the volume of CO<sub>2</sub> lost to the atmosphere (Goldfarb 2010). This may have an effect on the amount of ethanol lost to the atmosphere. Also, factors affecting the portion of CO<sub>2</sub> retained by the must as DCO<sub>2</sub> affect the total volume of CO<sub>2</sub> lost, which may have a significant effect on the total volume of ethanol lost.

## Conclusion

Previous works consisted of non-continuous measurements and mathematical equations. Given the significant influence of temperature on volatile loss and the vast temperature gradient associated with wine fermentation (Guymon and Crowell 1977), and the wide variation in results of previous studies (Fig. 1), a better understanding of the relationship between temperature and volatile loss with continuous measurements is needed, though advancements can be made with discrete measurements.

In order to achieve this, new methods were developed to better understand the relationship between fermentation temperature and volatile loss. Temperature controlled, microscale fermentors were developed and infrared (IR) detection was adapted to quantify total volatile loss. A batch technique was developed for the low volume fermentors but the IR method used is applicable to continuous detection of EtOH loss at larger scales.

Before selecting the topic and experimental design that was pursued for this thesis, various topics and experimental designs were considered (Appendix 1).

## SECTION 2

### MATERIALS AND METHODS

#### Quantification of Volatile Loss with Temperature from Wine Fermentation

##### Introduction

This study looked at the effect of temperature (17°C, 22°C, 27°C, 33°C) on the release of ethanol from fermenting Syrah in a closed system. Microscale fermentations were used to conduct 4 replicates at each temperature. All emissions were collected in Tedlar bags and analyzed for concentration of ethanol and volume of gas (Fig. 3). The must was analyzed for % alcohol vol/vol and concentration of dissolved CO<sub>2</sub>.

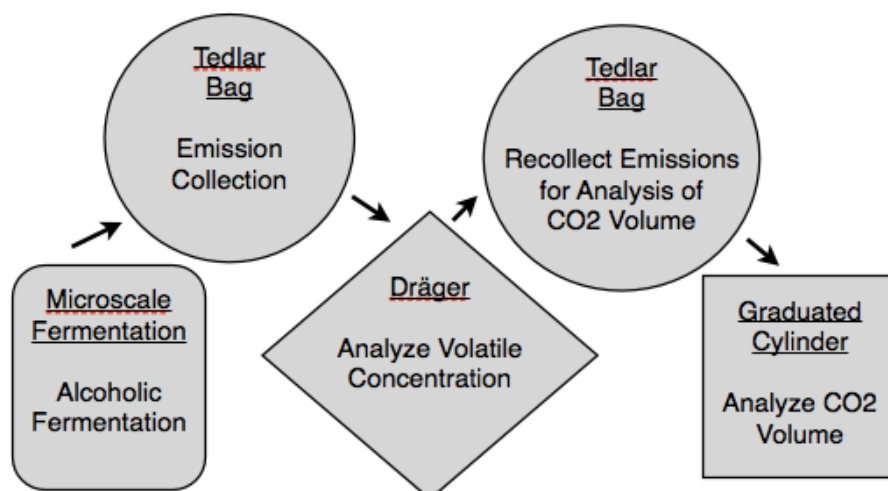
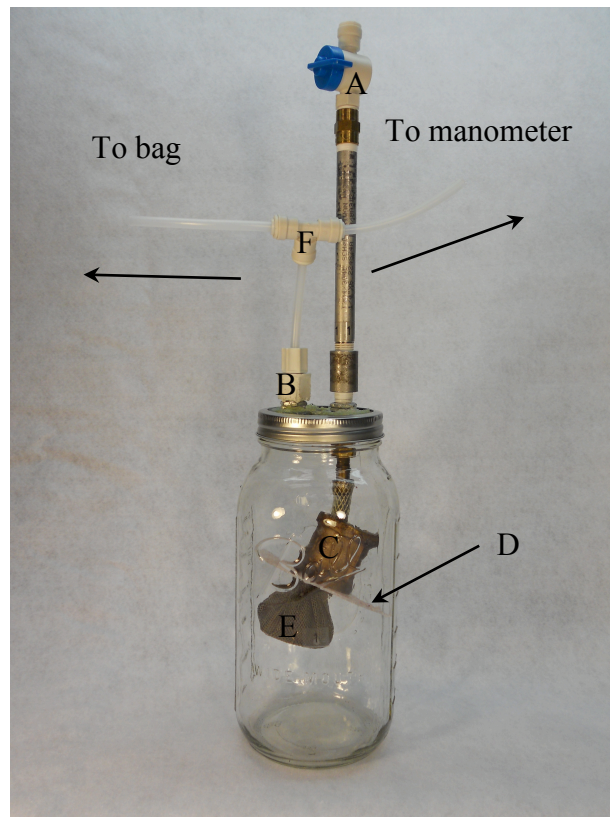


Fig. 3. Microscale fermentation exhaust was routed into Tedlar bags to be analyzed for VOC concentration and gas volume.

### Microscale Fermentor

The fermentor consisted of a half-gallon canning jar (Ball, Broomfield, CO) with a canning jar lid. A Blair Rotabroach (Blair Equipment Co. Flint, MI) was used to drill two holes in the canning lid which were used to create two ports. One port was designed to allow for intermittent Brix analysis by the Anton Parr densitometer. The second port routed fermentation emissions from the fermentor to the Tedlar bag (Fig. 4). A high-density polyethylene screen (McMaster-Carr, Sante Fe Spring, CA) and a one inch brass coupler (ACE, San Luis Obispo, CA) was used to submerge the cap, which eliminated the need for punch downs thereby decreasing potential sources of variability.

Fig. 4. Design of the microscale fermentor: (A) Brix port, (B) emissions route, (C) weight for submerged cap, (D) screen for submerged cap, (E) funnel wrapped in mesh-screen for must sampling, (F) “T” for emissions pathway and slack tube manometer



### Pressure Check for Loss of Gases

Each fermentor was pressurized to 762 mm H<sub>2</sub>O and sealed to monitor the rate of decrease in pressure. Jars 1,3,4 held 762 mm H<sub>2</sub>O for well over 24 hours. Jar 2 held 762 mm H<sub>2</sub>O for 9 hours and 12 minutes. These values indicate that any loss of volatiles would be negligible because volatiles travel the path of least resistance, which is into the Tedlar bags.

### Temperature Controlled Water-bath

The water-bath was composed of an 85L plastic storage bin (Sterilite Corp., Townsend, MA) that was filled with approximately 12 gallons of water. To increase the temperature of the water-bath, two 200W fish tank heaters (Hydor: Theo, Bassano Del Grappa, Italy) were placed on opposite sides of the water-bath. To ensure even heat distribution, two 195 gpm fish-tank pumps (Backyard Pro, San Jose, CA) were placed at opposite ends of the water-bath to circulate the water (Fig. 5). To decrease the temperature of the water-bath, water was circulated through 15.24m of 6.35mm OD copper tubing which was coiled to fit inside of a freezer/refrigerator (Absocold, Richmond, IN) (Fig. 6). Salt was used to depress the freezing point of the circulating water.

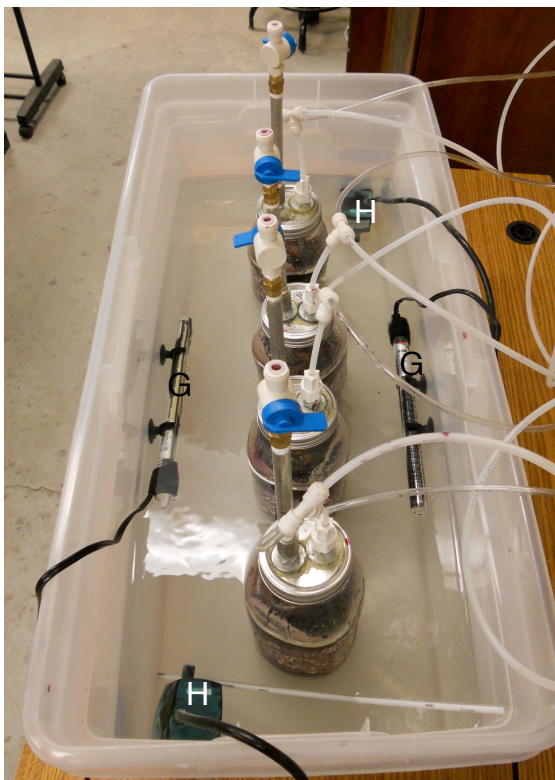


Fig. 5. Water-bath with heaters (G) and circulation pumps (H).



Fig. 6. Water chiller with water-bath water inlet (I) and water-bath outlet (J).

### Volatile Collection

All volatiles were routed through a combination of vinyl and polyethylene tubing into Tedlar bags which are made of polyvinyl fluoride (PVF) (Tedlar Bags SKC West Fullerton, CA). These materials are non-reactive with ethanol.

Once an adequate gas volume was obtained (6-10L), the full bag was replaced by an empty bag by closing both the valve of the Tedlar bag and the valve from the fermentation vessels. This allowed an empty Tedlar bag to be fitted to the emission tube with no loss of volatiles. Bag replacement had an elapsed time of no more than

7 seconds during which time, the pressure of the closed fermentation vessel never exceeded 762 mm H<sub>2</sub>O pressure.

The effectiveness of evacuating the contents of each bag was tested by filling bags in quadruplicate with lab grade dry CO<sub>2</sub> after removing fermentation emissions by the normal procedure described above. Analysis of the bag contents after being filed with dry CO<sub>2</sub> (see method below) indicated  $\leq 0.083\%$  remained of the ethanol that was previously in the bag.

### Grapes

*Vitis vinifera* L. cv. Syrah grapes were hand harvested from the California Polytechnic State University Trestle Vineyard (San Luis Obispo, CA). Clones: 383, 877, 174, 01, and 370. Rootstocks: Schwartzman, 5C, 101, 1023, 1017, 619B, and 5BB. One half ton of fruit was crushed, de-stemmed (Bucher X Pro 8, Sebastapol, CA.), treated with Potassium Metabisulfate at a rate of 40 mg/L, divided into freezer bags in 5.5lb quantities and placed in a freezer set to -26.11°C all within 4 hours of arrival to the winery. Care was taken to ensure that a similar juice to skin ratio was achieved for each bag of fruit. Initial analysis: pH=3.83, TA=6.43g/L and Brix=20.1°. All harvesting and handling of fruit took place on 28 October, 2010.

### Microscale Winemaking

Grapes were allowed to thaw at room temperature for a day. Grapes were double bagged to prevent leakage. Once mostly thawed, grapes were evenly allocated to the fermentors based on weight and visual assessment of the juice to skin ratio. The scale

(American Weight, Norcross, GA), which had a maximum capacity of 16 kg, was calibrated with NIST weights to be accurate  $\pm 5$ g. All fermentors were 1200  $\pm 5$ g prior to the addition of concentrate. The grape-filled fermentors were then placed in a water-bath at room temperature to accelerate the rest of the thawing process.

#### Additions Procedure

Once the must was fully thawed, additions were made (Table 5) and the must was thoroughly mixed prior to inoculation with yeast. For each ferment, Diammonium Phosphate (DAP) and Super Food were dissolved in 50mL juice and added to the must. GoFerm was dissolved in 10mL of the hottest faucet water possible (35°C at source). This GoFerm solution was then used to rehydrate the yeast for 10 minutes. During this time, the concentrate was added to the must and the must was thoroughly mixed. Next, the musts were inoculated with yeast, the fermentors were sealed, the valves were opened to allow volatiles to flow freely into the Tedlar bags and the desired water bath temperature was set.

Table 5. Must Additions

ADD	RATE	SOURCE
DAP	25g/hL	Beverage Supply Group, Napa, CA
Super Food	12.5G/hL	Beverage Supply Group, Napa, CA
Go Ferm	25g/hL	Lallemand, Zug, Switzerland
Grape Concentrate	Variable (Sufficient to raise Brix to 23.5°B)	California Concentrate, Lodi, CA
BDX Yeast	40g/L	Lallemand, Montreal, Canada



## ANALYSES

### Temperature

Fermentation temperatures were taken with an Antonn Par Density Meter DMA 35 (Ashland, VA) concurrently with every Brix measurement. Fermentation temperatures were also taken with a waterproof temperature data logger (Onset, Bourne, MA) hourly. The temperature logger was placed just below the screen for the submerged cap in the middle of the must. Information collected by the temperature loggers was analyzed with HOBOWare software (Onset, Bourne, MA).

### Brix

To take a representative sample for Brix and temperature analyses, the valve at the top of the Brix port of the fermentor was opened to allow the Anton Parr sample tube to reach the middle of the must, where there was a funnel with a fly screen to prevent clogging (E, Fig. 4). The Brix port valve was open for less than 45 seconds per Brix analysis. The Brix port extended below the must so there was no direct pathway for CO<sub>2</sub> or Volatile Organic Compounds (VOC) to escape during Brix sampling.

Brix were measured prior to fermentation and every time a bag replacement occurred. Hence, the drop in Brix that occurred during the collection of each bag of fermentation emissions could be calculated. Bag replacement and Brix analysis took place within 5 minutes of each other.

## Pressure

A manometer was made from 1/4"OD Vinyl tubing (Ace Hardware, San Luis Obispo, CA) and used to observe pressure throughout the fermentation (K, Fig. 7). A "T" connector was used to connect the manometer to the emissions pathway (F, Fig. 4).

Monitoring pressure throughout fermentation ensured that emission pathways remained unobstructed, allowing all gas and volatiles produced to flow freely into the Tedlar bags (L, Fig.7).

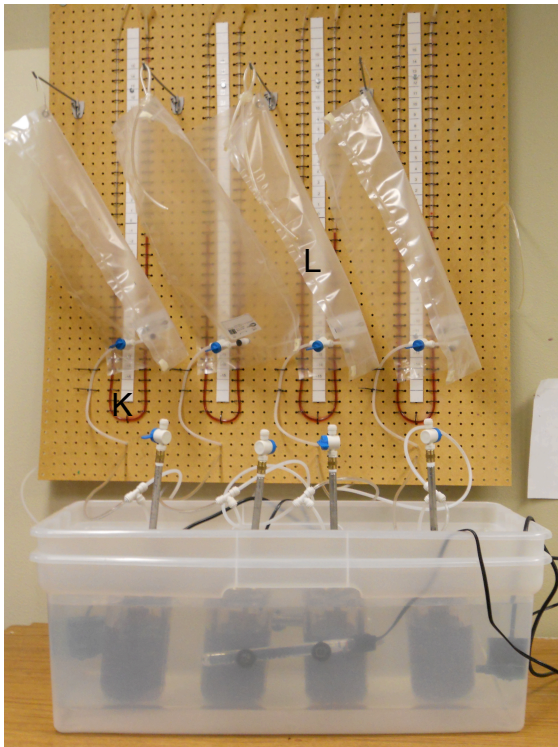


Fig. 7. Microscale ferments in temperature controlled water-bath attached to manometers (K) and Tedlar bags (L).

### Gaseous Ethanol Concentration

A Dräger Polytron IR (Pittsburgh, PA) was used to measure the concentration of ethanol in each bag. Dräger Polytron IR is an infrared gas detector for continuous monitoring of combustible gases and vapors that works based on the principal of infrared absorption. The Dräger was calibrated by standard nitrogen and methane gases (Enviro Safetech, San Jose, CA) following the protocol of Dräger to give the ppm of ethanol suspended in CO<sub>2</sub>. We can assume 96% VOCs quantified by the Dräger to be ethanol (Colome, pers. comm., 2012).

Weights were used to push the Tedlar bag contents into the Dräger to be analyzed. HOBOWare Software was used to analyze Dräger data. The Dräger outlet was connected to a second bag for subsequent quantification of CO<sub>2</sub> volume (Fig. 8).

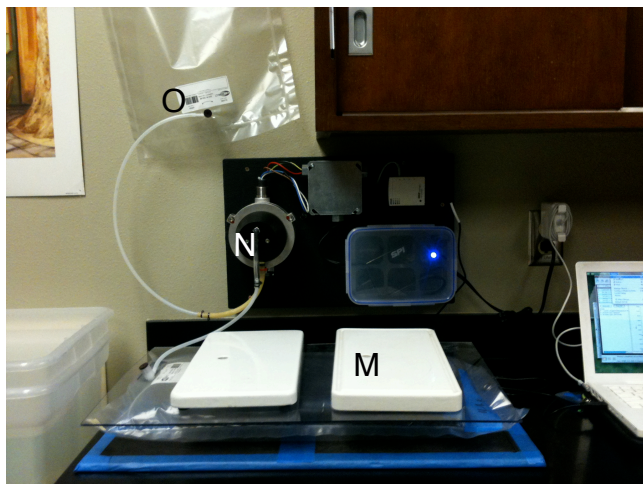


Fig. 8. Using weights (M) to express bag contents through the Dräger (N) for analysis of ethanol concentration, and into a second bag (O) for subsequent quantification of CO<sub>2</sub> volume.

### Gaseous CO<sub>2</sub> Volume

A 4000mL graduated cylinder was filled with water and inverted in a water bath so that no measurable amount of gas remained. Gas volumes of bags were expressed into the graduated cylinder (Fig. 9). The vacuum pressure created by the water column in the graduated cylinder allowed for all contents of the bags to be expelled. The collected fermentation gas was expressed through the Dräger input and collected in a secondary Tedlar bag through the Dräger output. Little gas remained in the bag that was expressed through the Dräger. Both the primary bag (pushed through the Dräger) and the secondary bag (used to collect gases from the Dräger output) were emptied into the graduated cylinder. All gas volumes were standardized to 20°C at 1 atmosphere of pressure.

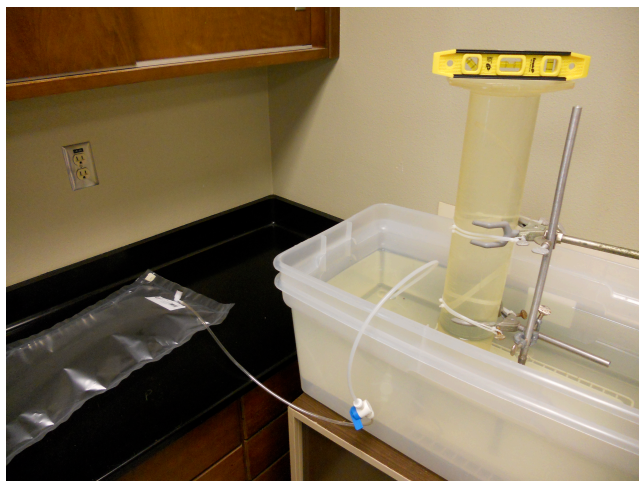


Fig. 9. Inverted graduated cylinder filled with water for quantification of CO<sub>2</sub> volume.

## SECTION 3

### RESULTS & DISCUSSION

Success of the developed temperature controlled microscale fermentor is expressed by presenting the minimal variation in rate of Brix depletion and consistent temperatures of the 4 replicates at 17, 22, 27 and 33°C. Results are presented for the effect of temperature on the rates and volumes of CO<sub>2</sub> and ethanol loss from fermentation. The effect that these losses have on wine composition is expressed by graphing the variation in final alcohol content (% vol/vol) of the resulting wines. Results are presented for the affect of temperature on ethanol vapor concentration through time. Observed volatile organic losses are compared to theoretical predictions.

#### Development of Microscale Fermentor

The somewhat inconsistent Brix depletion for the initial trial experiment (Fig. 10) was most likely due to the fact that the weights used to submerge the cap had a propensity to slip off the screen (Fig. 4 D) and sink to the bottom of the must. Jars had to be opened so that the weights could be adjusted. By the end of fermentation, weights had slipped again, some more than others. This initial trial experiment resulted in using different weights to submerge the cap (Fig. 4 C), which (Fig. 11 A-D), solved this issue.

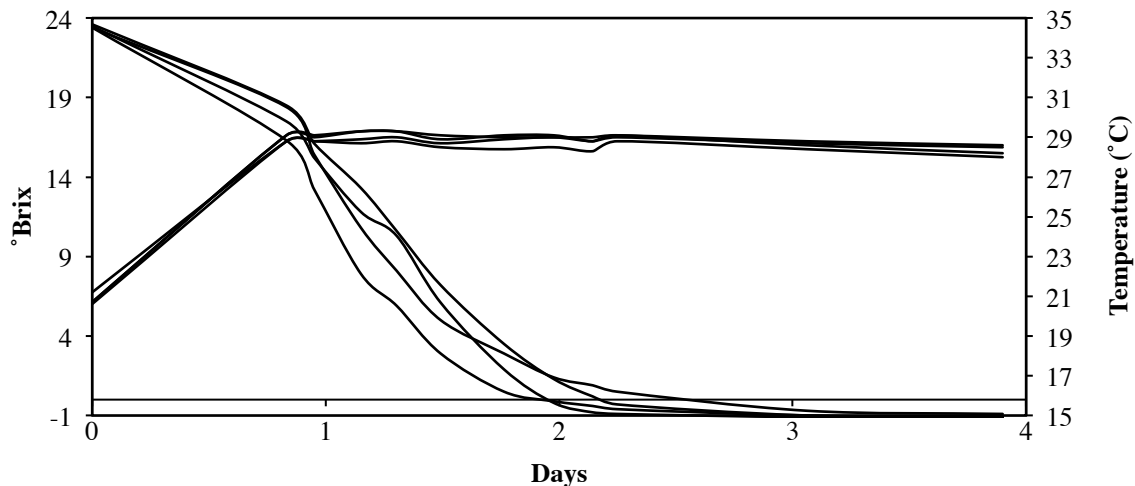


Fig. 10. Variation in °B and °C through time for four replicate Syrah fermentations at 28° using unbalanced weights to submerge the cap.

Results are presented for Brix depletion and temperature variation through time for four replicate Syrah fermentations at 17, 22, 27 and 33°C (Fig. 11 A-D). Brix and temperature values measured with an Anton Parr. Fermentation duration, which is dependent upon fermentation temperature, ranged from 3-9 days. Day 0 = inoculation. Average trial temperatures and variances were: 17.45°C  $\pm$  0.12, 22.17°C  $\pm$  0.04, 26.76°C  $\pm$  0.09, 33.28°C  $\pm$  0.21 (Table 2). The minimal variation in temperature and consistent rate of Brix depletion indicate a successful design and implementation of the microscale fermentation and temperature control techniques. Achieving consistent fermentation temperatures laid the foundation for a better understanding of the relationship between fermentation temperature and volatile loss.

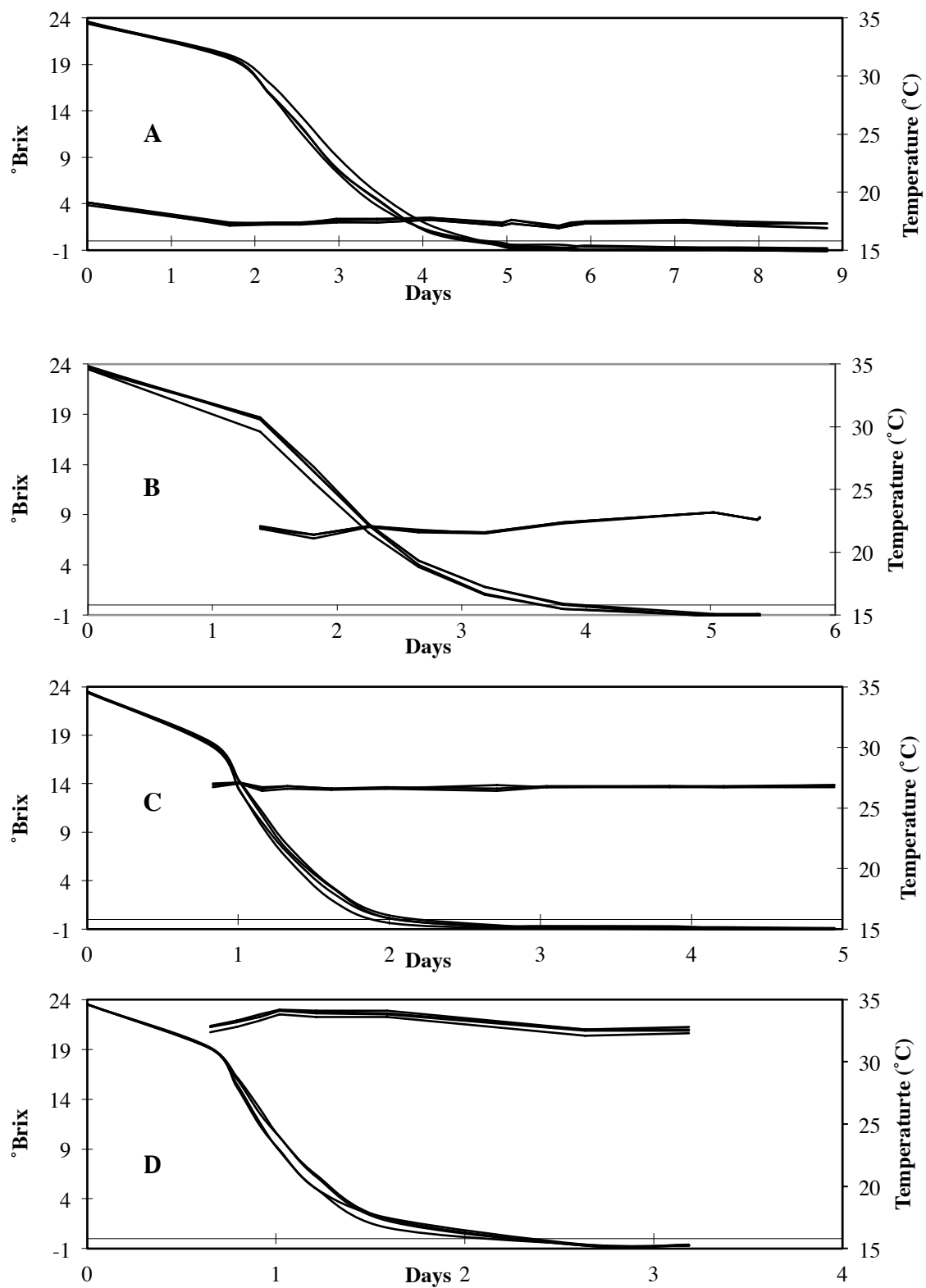


Fig. 11. Variation in °B and °C through time for four replicate Syrah fermentations at 17°C (A), 22°C (B), 27°C (C), and 33°C (D).

Trial Mean Temperatures and Variance				
Mean	Target Temp (°C)			
	17	22	27	33
Rep 1	17.58	22.1	26.6	32.99
Rep 2	17.53	22.2	26.8	33.31
Rep 3	17.35	22.2	26.8	33.35
Rep 4	17.35	22.2	26.8	33.48
Trial mean ± STDEV	17.45 ± 0.12	22.17 ± 0.04	26.76 ± 0.09	33.28 ± 0.21

Table 6. Mean temperatures (°C) per rep and mean temperature (°C) per trial ± variance.

#### Affect of Temperature on Volatile Loss from Wine Fermentation

Data was collected for °Brix, ethanol concentration and volume of CO<sub>2</sub> released intermittently i.e. every time a bag filled, which usually consisted of between 7 to 10 L of gas. An accurate mass flow meter was unavailable for microscale experiments so continuous data was not collected for direct comparisons and detailed modeling of fermentation losses. However, the batch data obtained did contain useful insights about the rates and volumes of both CO<sub>2</sub> and ethanol loss.

The rate of CO<sub>2</sub> loss clearly peaked before the rate of ethanol loss (Fig. 12 A-H). The findings confirmed current knowledge (Williams and Boulton 1983). The only trial in which the occurrence is not clearly visible was at 33°C, in which the highest values for CO<sub>2</sub> and ethanol loss were both measured on day 1.02 (Fig. 12 G, H). However, while the rate of ethanol loss distinctly peaked at 1.02 days (Fig. 12 H), the rate of CO<sub>2</sub> loss reached more of a plateau on days 0.91 and 1.02 (Fig. 12 D). The peak rate of CO<sub>2</sub> loss probably straddled the 0.91 and 1.02 day marks in Fig. 12 G. Therefore,



although the discrete measurements did not precisely detect when peak rate of CO<sub>2</sub> loss occurred for the 33°C trail, we can be confident that it preceded the peak rate of ethanol loss.

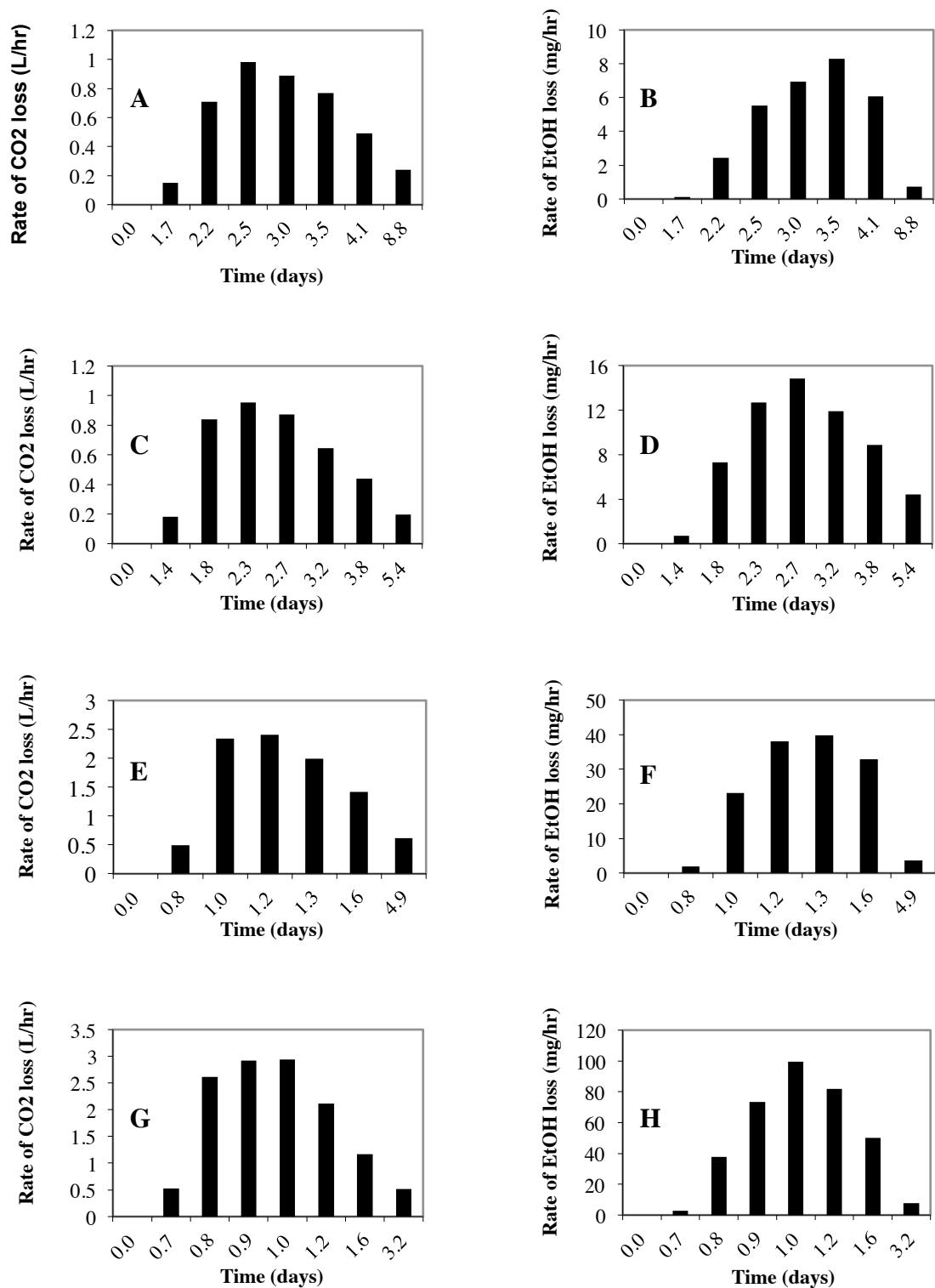


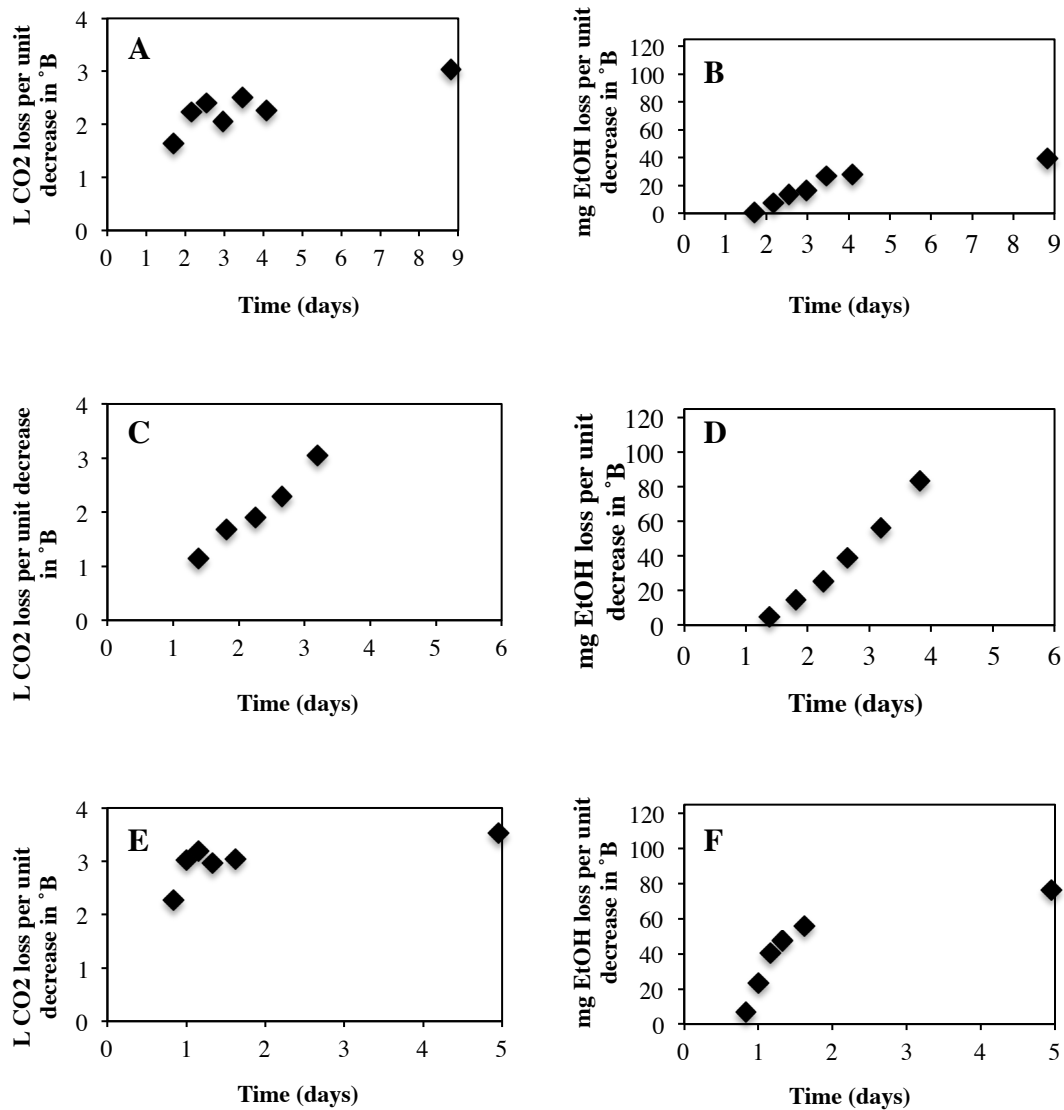
Fig. 12. Mean rates of CO<sub>2</sub> and ethanol loss during fermentation at 17°C (A, B), 22°C (C, D), 27°C (E, F) and 33°C (G, H).

The CO<sub>2</sub> loss per unit decrease in Brix was relatively consistent (Fig. 13 A, C, E, G) while ethanol loss per unit decrease in Brix increased with time (Fig. 13 B, D, F, H). On average, about 2.5L of CO<sub>2</sub> was released per unit decrease in Brix per ferment (Fig. 13 A, C, E, G). There was a slight trend of increased CO<sub>2</sub> release per unit decrease in Brix with time. A possible explanation for this is that the build up of alcohol in the must decreased its viscosity, which in turn, decreased its capacity to retain DCO<sub>2</sub>, causing greater volumes of CO<sub>2</sub> release with time.

The lowest value for volume of CO<sub>2</sub> lost per decrease in Brix occurred at the beginning of fermentation for all trials (Fig. 13 A, C, E, G). This was because, at the beginning of fermentation, the musts were unsaturated with DCO<sub>2</sub>. Much of the initial CO<sub>2</sub> produced was retained by the must as DCO<sub>2</sub>. CO<sub>2</sub> become more readily released from the musts once saturation or super-saturation of DCO<sub>2</sub> occurred (Goldfarb 2010).

To better understand how CO<sub>2</sub> and ethanol loss evolves throughout fermentation, average volume of CO<sub>2</sub> and ethanol loss per unit decrease in Brix were graphed with time (Fig. 13). For all trials, the volume of ethanol released per unit decrease in Brix increased with time (Fig. 13 B, D, F, H). The concentration of ethanol in the must increased with Brix depletion through time, which means that there is more ethanol available to lose. The 22°C trial (Fig. 13 C, D) is somewhat of an anomaly: it displayed the greatest variation/range in volume of CO<sub>2</sub> lost per unit decrease in Brix

(Fig. 13 C) and the last value for both CO<sub>2</sub> and ethanol loss per unit decrease in Brix were outliers that did not fall within the range of the y-axis (Fig. 13 C, D).



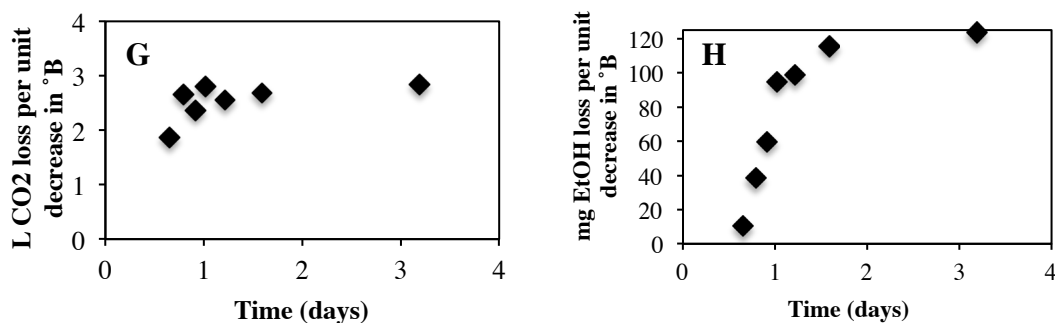


Fig. 13. Average volume of CO<sub>2</sub> (A, C, E, G) and ethanol loss (B, D, F, H) per unit decrease in Brix with time during fermentation at 17°C (A, B), 22°C (C, D), 27°C (E, F) and 33°C (G, H).

The  $\Delta^\circ\text{B}$  (change in °Brix) value associated with the outliers in the 22°C trial was lower than any other  $\Delta^\circ\text{B}$  value by a factor of 2. This uniquely low  $\Delta^\circ\text{B}$  value of 0.825 may have something to do with the associated outliers. The degree of variation in the volume of CO<sub>2</sub> released by each replicate per unit decrease in Brix for the 22°C trial (Fig. 13 C) appears to be related to the variation in volume of CO<sub>2</sub> released. Graphing the values for volumes of CO<sub>2</sub> released for each replicate from each trial (Fig. 14) shows that the greatest variation in volume of CO<sub>2</sub> released occurred in the 22°C trial. The variation in volume of CO<sub>2</sub> loss per  $\Delta^\circ\text{B}$  in the 22°C trial (Fig. 13 C, D) may be due to the variation in the measured volume of CO<sub>2</sub> released.

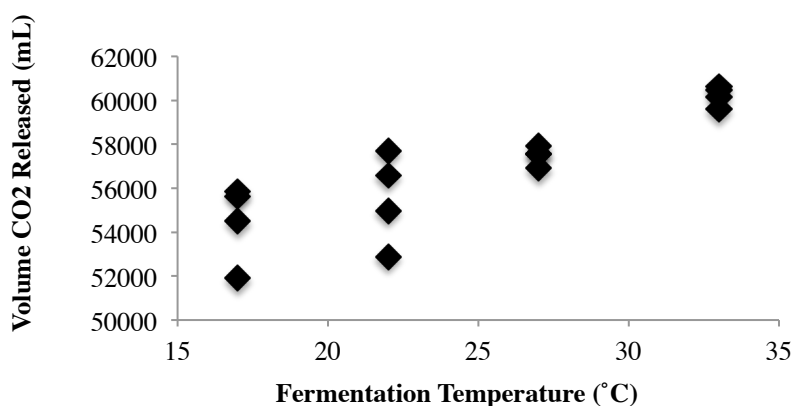


Fig. 14. Total CO<sub>2</sub> volumes released during fermentation for each of the four replicates at 17, 22, 27 and 33°C.

The 22°C trial, the first of the four trials to be conducted, may have had the greatest variability in CO<sub>2</sub> measurement because it was a new technique to the researcher and therefore needed to be practiced to increase accuracy.

Graphing the volumes of CO<sub>2</sub> released for each fermentation temperature (Fig. 14) illustrated a trend of increasing volumes of gas released with higher temperatures. ANOVA indicated that the trend of increased volumes of CO<sub>2</sub> released during fermentation was significant (Appendix 4). A reasonable explanation for this trend might be that higher temperatures decreased the solubility of DCO<sub>2</sub>, which caused greater volumes of CO<sub>2</sub> to be released. Supportively, graphing final DCO<sub>2</sub> concentrations for each fermentation temperature (Fig.15) illustrated a trend of decreased DCO<sub>2</sub> values with increasing temperatures. ANOVA indicated that this trend of decreased DCO<sub>2</sub> values with increasing temperature was significant (Appendix 5). Given that, as temperature increases, (1) CO<sub>2</sub> loss appears to significantly increase and (2) DCO<sub>2</sub> values appear to significantly decrease, it is

reasonable to propose that the increase in CO<sub>2</sub> loss is due to the decrease in DCO<sub>2</sub>. However, the average decrease in DCO<sub>2</sub> from 17°C to 33°C equaled 113.5 mL CO<sub>2</sub>, not enough to account for the average increase of 5744 mL CO<sub>2</sub> loss from 17°C to 33°C (Appendix 6). Thus, decreased concentrations of DCO<sub>2</sub> was only partially responsible for the observed increase in CO<sub>2</sub> loss. More research is needed to know if the balance of increased CO<sub>2</sub> loss with temperature was due to fermentation temperature, experimental error or other, yet to be identified, factors.

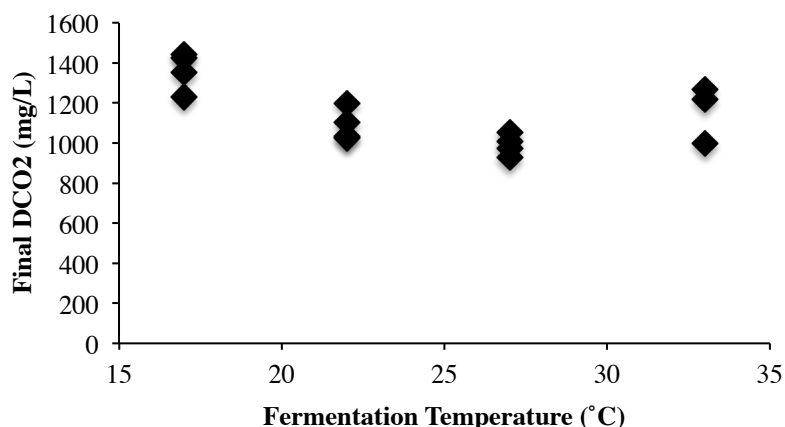


Fig. 15. Final DCO<sub>2</sub> concentrations for each of the four replicates at 17, 22, 27 and 33°C.

Graphing the volume of CO<sub>2</sub> released against the decrease in Brix shows that the volume of CO<sub>2</sub> released increased with greater Brix reductions (Fig. 16 A-D). At the beginning of fermentation, less CO<sub>2</sub> was released per decrease in °B. More CO<sub>2</sub> tended to be released per decrease in °B by the end of fermentation. By marking the first and last released gas volumes with the symbols: Δ and □ respectively, that general trend was apparent. The relatively imprecise relationship between decrease in °B and volume of gas released depicted in Fig. 16 A-D may be attributed to a

relatively small range in decrease in °B, a relatively small range in volume of gas released, the effect of the degree of must saturation (unsaturated, saturated, supersaturated) with  $\text{DCO}_2$  as well as other potential factors such as must viscosity.



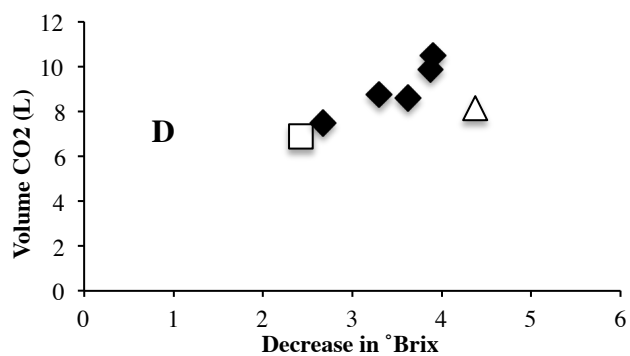
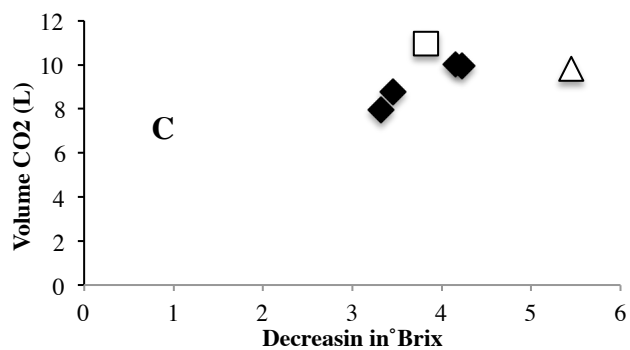
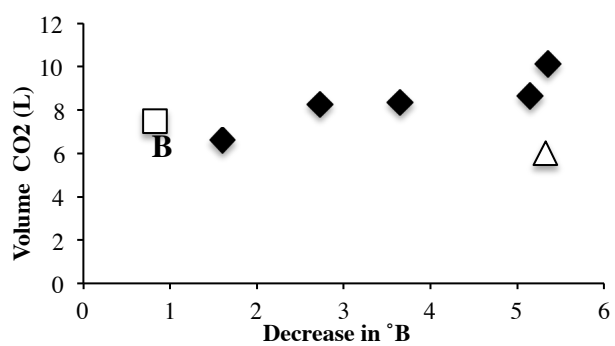
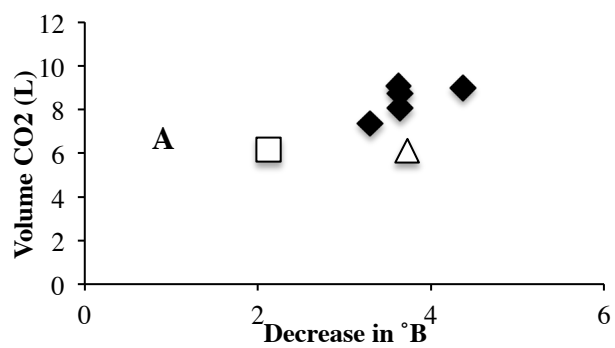


Fig. 16. Mean volume of CO<sub>2</sub> released per decrease in Brix for four replicate fermentations at 17°C (A), 22°C (B), 27°C (C) and 33°C (D).

Graphing ethanol concentration through time for each trial temperature shows that the concentration of ethanol in the CO<sub>2</sub> released from fermentation increases with time and temperature (Fig. 17). Points represent average values of aggregate data for each of the four replicates. Given the batch sampling method employed and the fact the ethanol concentrations increase with time, reported ethanol concentrations are affected by the volumes of gas collected. For example, for the final batch gas samples collected, which represent maximum ethanol concentrations for each trial (Fig. 17), the greater the volume of gas collected, the more dilute the reported maximum ethanol concentration becomes. Thus, when observing the relationships between maximum ethanol concentration and fermentation temperature in Fig. 17, it is helpful to bear in mind that average batch volumes of the final gas samples collected were 6.2, 7.5, 11.0 and 6.9 L for trials at 17, 22, 27 and 33°C respectively.

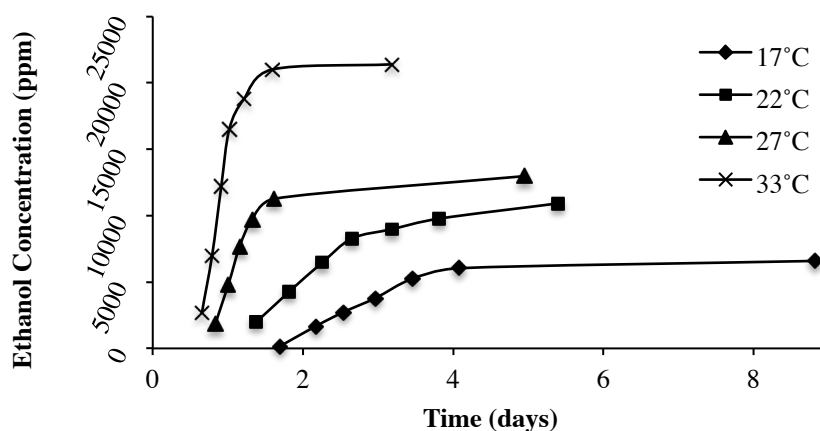


Fig. 17. Average ethanol concentrations through time for fermentations at 17, 22, 27 and 33°C.

### Comparison of Observed Ethanol Loss with Theoretical Predictive Model

Results for total ethanol loss from four replicate Syrah fermentations at 17°C, 22°C, 27°C and 33°C are presented and compared with values based on a theoretical predictive model (Fig. 18). The curves in Fig. 18 represent theoretical values generated by the Williams-Boulton model, which uses kinetic and stoichiometric principals to predict ethanol loss from wine fermentation (Williams and Boulton 1983). In this model, ethanol is released along with the CO<sub>2</sub> produced during fermentation. It is implicitly assumed that all CO<sub>2</sub> produced during fermentation is released to the atmosphere. That assumption is useful in developing a conservative estimation for CO<sub>2</sub> loss for the purposes of worker safety. For instance, if CO<sub>2</sub> loss were less than estimated, CO<sub>2</sub> exhaust systems would be more than adequate to protect against CO<sub>2</sub> asphyxiation. On the other hand, if CO<sub>2</sub> were greater than predicted, CO<sub>2</sub> exhaust systems could be inadequate, endangering human lives.

The temperature of the fermentations associated with observed ethanol loss values were derived from continuous data loggers. Circles represent mean temperature, whiskers express total range in temperature. A short heat spike accompanying an otherwise constant temperature profile accounted for the asymmetrical temperature whisker range for the 27°C trial. Ethanol loss values were calculated in aggregate by multiplying the vapor concentration of ethanol in CO<sub>2</sub> by the volume of CO<sub>2</sub> released from fermentation, as expressed in the equation below. Aggregate values were added together to obtain total ethanol loss.

$$EtOH \text{ loss}(mg) = \left( \text{Vapor concentration EtOH}(mg / m^3) \text{ in } CO_2 \right) \left( \text{volume } CO_2(m^3) \text{ released} \right)$$

The empirical results follow the general Williams-Boulton model trend and are very close to the lower boundary of the relationship. The discrepancy, between theoretical  $CO_2$  loss and actual  $CO_2$  loss observed in wine fermentation, accounts for the findings of this study being slightly lower than predicted by the Williams-Boulton model. For these trials, an average of 0.95% of total  $CO_2$  produced was retained as  $DCO_2$  at the end of fermentation (Appendix 2). These values do not account for the volume of  $CO_2$  retained as carbonic acid ( $H_2CO_3$ ) which may or may not be a significant fraction of total  $CO_2$  production.

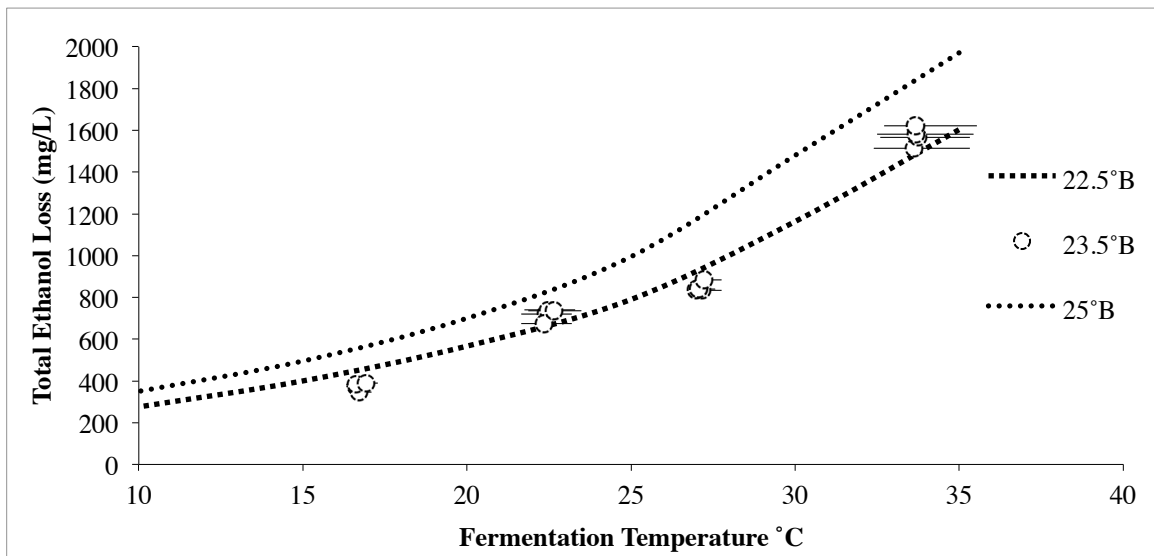


Fig. 18. Theoretical ethanol loss for fermentations at 22.5 and 25°B across a range of temperatures (10-35°C) compared to observed ethanol loss for fermentations at 23.5°B at 17, 22, 27 and 33°C.

Graphing the final alcohol content (%vol/vol) of each replicate fermented at each trial temperature (17, 22, 27 and 33°C) illustrated the significance that alcohol losses can

have on wine composition (Fig. 19). Four replicates are plotted for each trial except for at 17°C, which had 3 values. Identical values overlap at 27 and 33°C. Values were obtained with an ebulliometer. As fermentation temperature increased, the alcohol content of the wine decreased. Intuitively, that makes sense: if ethanol loss increases with temperature, then less ethanol remains in the wine. What may not be so intuitive is the extent of which ethanol loss can effect wine composition. In the study, final wine alcohol content ranged from 11.85 to 13.1% alc. vol/vol. That means that fermentation temperature was responsible for altering the alcohol content of the resulting wine by 10%. That has profound olfactory implications for wine when one considers the “sweet spotting” research conducted by Clark Smith and Dr. Fuglesang (Smith and Fugelsang 2001) which found that adjusting the alcohol content of wine by a mere 0.1% can have drastic affects on the consumers perception of bitterness, astringency, harmony and balance. That also serves as a potential explanation for why wineries observe variations in conversion ratios of sugar to alcohol from year to year despite identical yeast strains and must chemistry. A simple heat spike could be responsible for variations in observed conversion ratios.

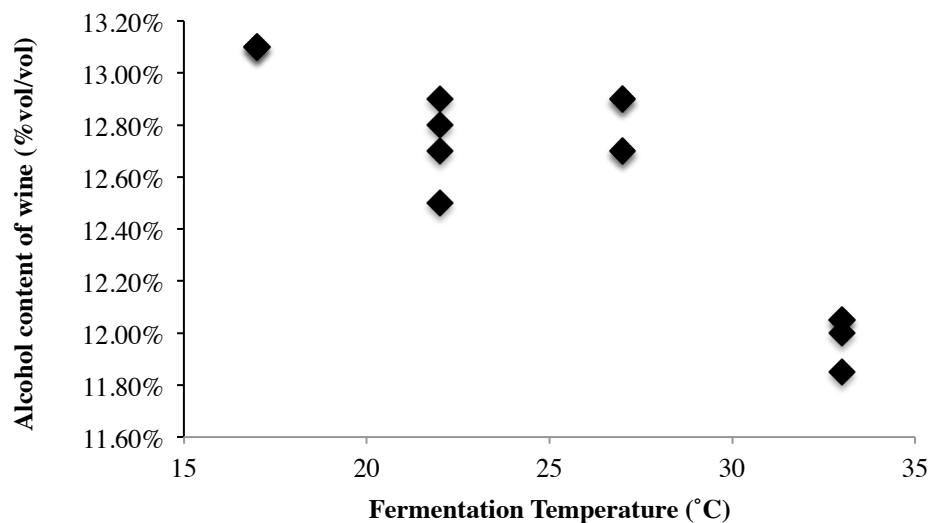


Fig. 19. Final alcohol content of wines with an initial sugar content of 23.5°B fermented at 17, 22, 27 and 33°C.

## OVERALL DISCUSSION

The loss of volatiles from wine fermentation represents a significant opportunity for ethanol and aroma recovery. A better understanding of how temperature affects these losses is relevant for calculating total potentially recoverable material and understanding the effect of volatile loss in wine composition. With increasingly strict air quality standards in many of California's wine producing regions, nondestructive recovery and reuse of these volatiles may represent the first pollution control solution with the potential for a positive ROI.

The IR detection method used in this research can be applied in future research efforts at both the lab and commercial scale for both batch and continuous measurements. Continuous measurements can be achieved by incorporating a CO<sub>2</sub> mass flow meter and data loggers. IR detection can also be used to quantify the capture efficiency of

ethanol and aroma recovery equipment. Specifically, IR detection can be used to measure the volatile concentration of the inlet and outlet gas of a given piece of equipment. Dividing the inlet vapor concentration by the outlet vapor concentration serves as a straightforward way to calculate % capture efficiency. When doing so, one must factor in that capture efficiency values are likely to vary with fluctuations in vapor concentration, CO<sub>2</sub> flow rates, and must and gas temperatures.

Future research efforts are needed to better understand how volatile loss from microscale fermentation compares to real world conditions. Non-isothermal conditions associated with a floating cap in typical red wine fermentation (Guymon and Crowell 1977) surely enable greater losses than suggested by estimates based on “target” fermentation temperature.

In consideration of air quality regulations that pertain to wine production, future research is needed to better understand what percent of VOC loss from tank headspace actually reaches the atmosphere. For example, a portion of the volatiles that exit the tank headspace may condense on various surfaces (walls, ceilings, equipment, etc.) enabling an opportunity for various microbes to metabolize the ethanol before it can reach the atmosphere. In fact, this phenomenon is so prevalent that the surrounding infrastructure in areas of spirit production and warehousing (Cognac France, Louisville Kentucky), are covered in an ethanol metabolizing biofilm that is so robust that it can be seen as a black growth by the naked eye. Research is needed to better understand the extent of this factor in spirits production

and warehousing as well as that of wine. Perhaps there is a distinction that needs to be made between how much ethanol escapes from fermentation compared to how much ethanol is likely to reach the atmosphere. Other research is needed to understand if the quantity of CO<sub>2</sub> retained by wine as carbonic acid (H<sub>2</sub>CO<sub>3</sub>) accounts for a significant portion of total CO<sub>2</sub> production. CO<sub>2</sub> retained as carbonic acid may account for part of the discrepancy between observed ethanol loss and values based on models in which ethanol loss is a function of CO<sub>2</sub> production.

### OVERALL CONCLUSION

A meaningful loss of compounds occurs during fermentation. When collected, these compounds have commercial applications; when allowed to escape, they can become a regulatory consideration. If efforts are made to preserve these otherwise lost compounds, the wine industry may unlock an economically viable world of resource recovery and reuse. Esters and other aroma compounds may be used to improve wine quality or applied in other aroma applications such as perfume manufacturing. Additionally, the ethanol may be separated for food grade applications or biofuel. Stripping the volatiles from the CO<sub>2</sub> released during fermentation serves as the first step in processing the CO<sub>2</sub> to be compressed for subsequent uses. CO<sub>2</sub> collection/compression/reuse systems are currently utilized in the beer industry where fermentation occurs year-round. Adapting such a system to an industry in which fermentation is seasonal would be challenging though, given that wine starts with about 3 times more sugar than beer, there is the potential to collect roughly 3 times more CO<sub>2</sub> per fermentation volume. Recovered CO<sub>2</sub> would be clean, food-grade and



reusable. This may prove to be a preferable option for wineries that want to decrease or eliminate their carbon footprint. Because wine production is considered carbon neutral (fermentation releases the CO<sub>2</sub> taken up by the vines during the growing season) subsequent collection, reuse or sequestration of the released CO<sub>2</sub> may enable wine production to become carbon negative.

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## APPENDICES

### A. Choosing a Thesis

Before settling on the question and experimental design for the thesis, “Methods and Observations for the Influence of Temperature on Volatile Loss from Wine Fermentation,” many other potential questions and experimental designs were considered. Below is a summary of the processes and considerations that were involved in arriving at the research question and associated experimental design that was eventually pursued.

The first idea that was considered as a potential direction for this thesis was to express capture efficiency of fermentation volatiles as a function of flow rate expressed by Reynolds number. The experimental design would involve a micro-scale fermentation, two mass flow meters and a Tedlar bag (Fig. A-1).

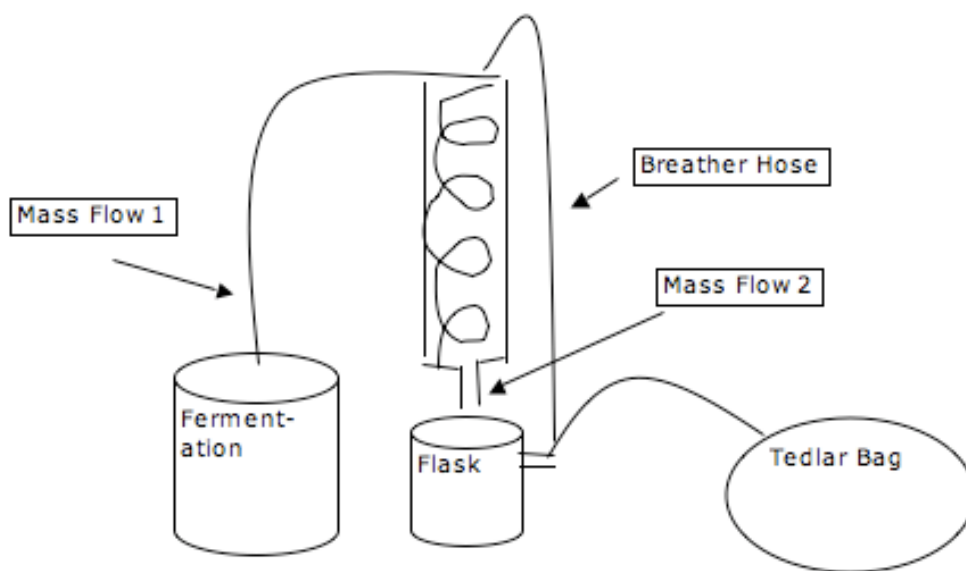


Fig. A-1. Design of experimental set-up that could be used to explore capture efficiency as function of Reynolds number.

It was hypothesized that capture efficiency would increase with flow, to a point, then decrease (Fig. A-2).

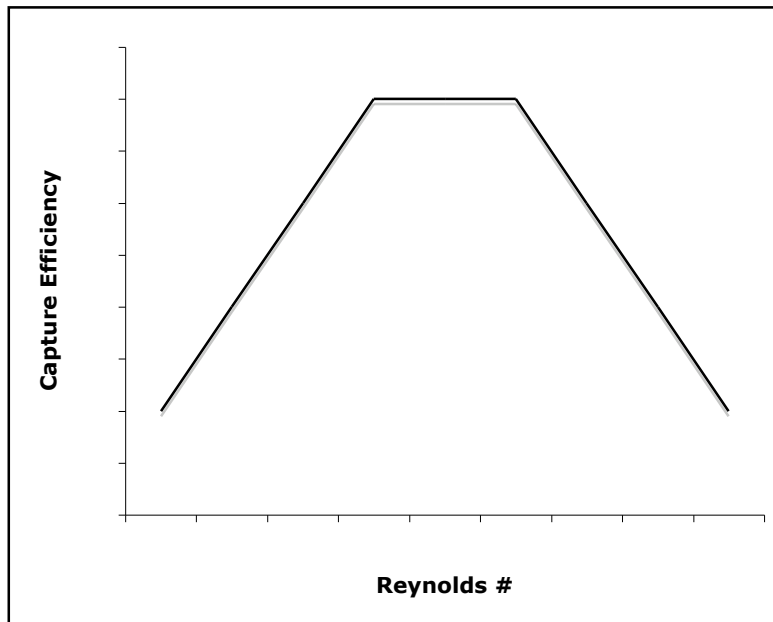


Fig. A-2. Hypothesis of the relationship between capture efficiency and Reynolds number.

The reason this subject was not pursued is because, on a laboratory scale, the difference between capture efficiencies would be too small to accurately detect and quantify. For example, total volatile loss for a one gallon ferment would be about 38 mL (see calculation below).

Total alcohol release for one gal fermentation  
0.5% of must volume  
 $(.005) (3.785 \text{ L}) = 0.019 \text{ L} = 19 \text{ mL}$   
 $(.005) (128 \text{ oz}) = 0.64 \text{ oz}$   
+equal parts H<sub>2</sub>O= 38 mL total volatile emission



If the average capture efficiency of the experimental set-up were 50%, and capture efficiency varied 15-20% experiment to experiment over the range of flow rates evaluated, then the difference from experiment to experiment would only amount to 2.85-3.8 mL, of which approximately 1mL would be stuck as residual condensate on the condenser walls (see calculation below).

$$(38 \text{ mL total volatile emission}) (50\% \text{ capture efficiency}) = 19 \text{ mL possible condensate}$$

Reasons for 50% capture efficiency:  
 Non-ideal condensers (straight not coiled)  
 Faucet water cool but not cold  
 Variable flow rate

$$\begin{aligned} &15\%-20\% \text{ difference ex to ex:} \\ &(.15) (19 \text{ mL}) = +/- 2.85 \text{ mL} \\ &(.20) (19 \text{ mL}) = +/- 3.8 \text{ mL} \\ &- 1 \text{ mL residual condensate on condenser walls?} \end{aligned}$$

Even if variation were measured based on weight, the variation from experiment to experiment would be in the noise range. With the resources on hand, I was not able to scale up the size of the experiment to overcome this obstacle. However, for future research efforts, it does appear that five gallon fermentations would be large enough to produce a significant result (see calculation below).

Total alcohol release for five gal fermentation  
 $(.005) (5 \text{ gal}) = 0.025 \text{ gal} = 3.2 \text{ oz} = 94.6 \text{ mL}$   
 + equal parts H<sub>2</sub>O = 189.2 mL total emission

$$\begin{aligned} 5 \text{ gal} &= 18.93 \text{ L} = 18,927 \text{ mL} \\ (18,927 \text{ mL}) (.005) &= 94.64 \text{ mL} \\ +\text{equal parts H}_2\text{O} &= 189.3 \text{ mL} \end{aligned}$$

$$(189.2 \text{ mL total emission}) (50\% \text{ capture efficiency}) = 94.6 \text{ mL possible condensate}$$

15%-20% difference ex to ex:  
(.15) (94.6 mL) = +/- 14.19 mL  
(.20) (94.6 mL) = +/- 18.92 mL  
\*Measure by weight

Major considerations:

Low total emissions

Condenser efficiency

Water temperature

Condenser design and size

Probable capture efficiency and total emissions great enough to yield significant  
measurable differences?

Change in condenser efficiency expressed in Reynolds number outside noise range?

Given the lack of viability of pursuing the above topic at the manageable one gallon ferment lab scale, I was forced to explore alternative directions. Instead of looking at the relatively low volume of the condensable emissions, sights were refocused towards total emissions. Instead of using a “middle-man” (condenser), all emissions could be routed into non-reactive Tedlar bags for subsequent analysis.

With that in mind the next question considered was, “How does fermentation temperature, duration, initial sugar level, nutrients, etc. affect total volatile ethanol production and total volatile CO<sub>2</sub> production throughout fermentation?” Besides needing to narrow down the number of variables, the logistic and financial feasibility would need to be assessed before moving forward.

The basic set-up seemed straightforward enough: simply route the fermentation volatiles into a Tedlar bag (Fig. A-3).

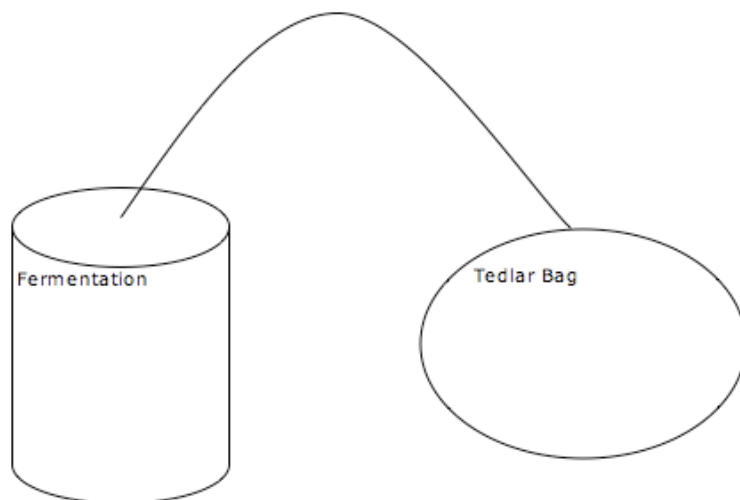


Fig. A-3. Preliminary experimental design for understanding the relationship between fermentation temperature and volatile loss: route emissions into bag for subsequent analysis.

An estimation of CO<sub>2</sub> production indicated that a 3.785 L (one gallon) ferment would yield about 227 L of CO<sub>2</sub> (see equation below).

$$\begin{aligned}
 &\text{Total gas release:} \\
 &(60) (\text{Vol Fermenting}) \\
 &(60) (3.785 \text{ L}) = 227.1 \text{ L} \\
 &227.1 \text{ L} / 7 \text{ days} = 32.44 \text{ L/day}
 \end{aligned}$$

Tedlar bags were only available in 10 L volumes thus, multiple Tedlar bags would be needed for each ferment. If each bag were filled 50%, by the end of fermentation, each ferment would have 46 bags that would need to be analyzed for volume of gas and concentration of volatiles.

A syringe could be used to displace the collected gas of each bag and measure the volume. This displaced gas would need to be collected in a separate bag for

subsequent analysis of VOC concentration. To save on lab fees associated with analyzing each bag, a proportional gas blend could be made after all bag volumes were known so that only one bag would need to be analyzed by a lab.

There were a lot of potential hurdles with the above design but the major obstacle was that bag costs were going to be over \$1000/ ferment (see calculation below).

Expenses:

10L Tedlar Bags at SKC are about \$27 each.

If each fermentation produces 227.1L of gas, and we fill bags 50%, we need about 46 bags/fermentation.

(46 bags/ferment) (\$27/bag) = \$1,242 in bags/ferment.

Multiply that by the number of reps (3 or 4?) and we arrive at a very large cost figure.

+ Lab analysis costs for each fermentation.

NOT FINANCIALLY FEASIBLE.

To overcome these hurdles, other methods were considered for measuring both volume and volatile concentration of the gas. A Dräger Polytron IR, an infrared gas detector that continuously measures combustible gases and vapors based on the principal of infrared absorption, could be used to measure the concentration of VOC. A mass flow meter could be used to measure the volume CO<sub>2</sub>.

I had access to borrow a Dräger from EcoPAS LLC. so that was feasible. However, I was unable to find a mass flow device that could operate in the range of flow associated with the micro-scale ferments with sufficient accuracy.

Thus, I could incorporate IR detection but I would have to use bags, at least in some capacity. Being able to analyze the VOC concentration of the collected gas in-house

meant that I did not need to collect the total volume of volatiles from each ferment before the concentrations could be analyzed. Once full, bags could be expressed through the Dräger for analysis of VOC concentration on the spot. With a closed system, the gas could be passed through the Dräger and into another bag for subsequent measurement of gas volume. Instead of using a syringe to measure the volume of CO<sub>2</sub>, an inverted graduated cylinder submerged under water would save time and reduce potential sources of error. Total ethanol content could be calculated once the volume and concentration of each bag was measured. 46 bags per ferment had been reduced to 3 bags per ferment or less and the costs associated with having to send gas samples to a third party lab to be analyzed were eliminated.

Consideration was given to various ways of testing different conditions (see notes below). However, based on equipment restrictions (pumps, heaters, outlets, human resources) it was decided to look at 1 condition per trial.

1st trial: 5 identical conditions, to understand variability.  
2nd-6<sup>th</sup> trial: 1 control, 4 variations  
=5 fold rep with experimental conditions, each time with a control.

5 vessels/ 75 ferments available = about 15 cycles available.

ANOVA can pull out mean shift if something happens like the power goes out, heat spike, heat drop.

Statistical Options:  
5 controls  
1 control, 1 each exp 1-4  
1 control, 1 each exp 5-8  
1 control, 1 each 1-4  
1 control, 1 each 5-8  
= 5 reps/ fermentation condition  
if too much variability, 4 conditions 10X

Mean and SD for 5 controls tight?

4 experimental conditions w/ 10 reps each or,  
8 experimental conditions with 5 reps each?

Once an appreciation was developed for the care needed to ensure minimal variation in fermentation condition (temperature) and it was realized that a critical pace & rhythm would be required to keep up with the analysis of continuous batch gas samples in real time, it was decided that testing one fermentation condition (temperature) per trial was most realistic.

The design passed initial financial and logistic feasibility checks so it was decided to move forward with exploring the influence of temperature on volatile loss during fermentation.

If any unforeseen hurdles emerged along the way, the backup research subject would be, “Is the pressure transducer an accurate means to monitor fermentation progress measured by the production of CO<sub>2</sub>?”

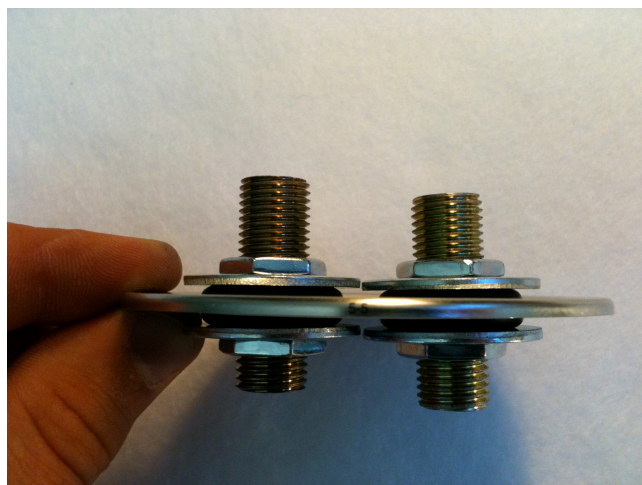
Conventionally, fermentation progress is monitored by measuring the decrease in brix. Since the decrease in brix is proportional to CO<sub>2</sub> production, it seems possible to monitor fermentation by monitoring and quantifying CO<sub>2</sub>. The benefits of this potential new monitoring technique would be real-time, automated fermentation data.

All hurdles that emerged while pursuing, “how fermentation temperature affects volatile losses,” were surmountable thus it was perused for this thesis, “Methods and Observation for the Influence of Temperature on Volatile Loss from Wine Fermentation.”

## B. Microscale Fermentor Components

Care was taken to select for the least reactive materials available for construction of the microscale fermenters: copper components were ruled out. Two trail fermentations served to saturate any sites of reactivity with ethanol vapor.

Microscale fermentor components included: stainless steel, brass, Cole-Palmer pipe adapters, polyethylene tubing, lock nuts, nipples, grommet, silicone cocking. The following pictures are provided to illustrate the construction of the microscale fermentor.







### C. Calculating DCO<sub>2</sub> as a Percent of CO<sub>2</sub> Released

Dissolved CO<sub>2</sub> (DCO<sub>2</sub>) as a fraction of total CO<sub>2</sub> produced was calculated with DCO<sub>2</sub>, CO<sub>2</sub> loss and press yield data collected from multiple trials.

Total CO<sub>2</sub> loss was quantified and standardized to 20 °C @ 1 atmosphere for all reps.

DCO<sub>2</sub> was quantified post fermentation with a Carbodoseur in triplicate and averaged for each rep (Table A-1).

Rep.		CO <sub>2</sub> Loss (mL)	DCO <sub>2</sub> (mg/L)
17	1	54512	1440
	2	51918	1350
	3	55630	1425
	4	55839	1230
	Mean	54475	1361
22	1	54982	1027
	2	57695	1103
	3	52884	1033
	4	56574	1198
	Mean	55534	1090
27	1	57543	975
	2	57529	1050
	3	56898	925
	4	57924	1005
	Mean	57474	989
33	1	59606	997
	2	60635	1218
	3	60146	1267
	4	60487	1185
	Mean	60219	1167

Table A-1. Total CO<sub>2</sub> loss (mL) and mean final DCO<sub>2</sub> (mg/L) levels for all reps. Mean DCO<sub>2</sub> values for each trial derived from post fermentation DCO<sub>2</sub> values taken in triplicate.

DCO<sub>2</sub> values were converted from units of concentration (mg/L) to units of volume (mL) thusly:

$$\left( \frac{\text{mg DCO}_2}{\text{L red wine}} \right) \left( \frac{1\text{g}}{1000\text{mg}} \right) \left( \frac{1\text{mol}}{44.01\text{g CO}_2} \right) \left( \frac{22.4\text{L CO}_2}{1\text{ mol}} \right) \left( \frac{1000\text{ml}}{1\text{L}} \right) = \frac{\text{ml CO}_2}{\text{L red wine}}$$

Hand pressing 4 reps from a trial run at 29 °C through a screen and funnel yielded an average press yield of 188 gal per ton.

$$(1200\text{g must}) \left( \frac{1\text{lb}}{454\text{g}} \right) \left( \frac{1\text{ton}}{2000\text{lb}} \right) \left( \frac{188\text{gal}}{1\text{ton}} \right) \left( \frac{3.7854\text{L}}{1\text{gal}} \right) = 0.93\text{L wine}$$

Multiplying (L CO<sub>2</sub>/L wine) values by the micro scale wine volume of 0.93L yields mL DCO<sub>2</sub> per micro scale ferment. Adding mL DCO<sub>2</sub> to mL CO<sub>2</sub> loss gives total mL CO<sub>2</sub>. Dividing mL DCO<sub>2</sub> by mL total CO<sub>2</sub> expresses DCO<sub>2</sub> as a percent of total CO<sub>2</sub> production (Table A-2).

Trial	DCO <sub>2</sub> (mL)	Total CO <sub>2</sub> (mL)	DCO <sub>2</sub> as % total CO <sub>2</sub>
17	644	55119	1.17
22	516	56050	0.92
27	468	57942	0.81
33	552	60771	0.91
Mean	545	57470	0.95

Table A-2. Mean DCO<sub>2</sub> (mL), total CO<sub>2</sub> (mL) and DCO<sub>2</sub> as % of total CO<sub>2</sub> for all trials.

For reference, average observed total CO<sub>2</sub> loss was 57.47 L compared to 50.02 L as predicted by theoretical calculation (Goldman 2007). Thus, emission factors used to estimate total CO<sub>2</sub> loss from wine fermentation appear conservative. Varying press yields will have an effect on total theoretical CO<sub>2</sub> production.

Total Theoretical CO<sub>2</sub> Production:

$$\left(\frac{882\text{lb } CO_2}{1000\text{gal red wine}}\right)\left(\frac{1\text{gal red}}{3.7854\text{L red}}\right)\left(\frac{453.59\text{g } CO_2}{1\text{lb } CO_2}\right)\left(\frac{1\text{mol}}{44.01\text{g } CO_2}\right)\left(\frac{22.4\text{L } CO_2}{1\text{mol}}\right) = \frac{53.79\text{L } CO_2}{\text{L red wine}}$$

(Goldman 2007)

$$\left(\frac{53.79\text{L } CO_2}{\text{L red wine}}\right)(.93\text{L wine}) = 50.02\text{L } CO_2$$

#### D. ANOVA: Volumes of CO<sub>2</sub> Released with Temperature

$F > F_{crit}$  thus, temperature appears to have a significant effect on CO<sub>2</sub> volume released from fermentation (Table A-3). Volumes of CO<sub>2</sub> released during fermentation significantly increase with temperature.

Analysis of Variance (One-Way) (alpha =5%)					
Summary					
Groups	Sample size	Sum	Mean	Variance	
17	4	217,898.71225	54,474.67806	3,245,503.045	
22	4	222,134.77283	55,533.69321	4,359,964.027	
27	4	229,894.02973	57,473.50743	180,583.126	
33	4	240,873.36214	60,218.34053	208,577.42	
ANOVA					
Source of Variation	SS	df	MS	F	
Between Groups	76,347,057.79828	3	25,449,019.26609	12.733	
Within Groups	23,983,882.87868	12	1,998,656.90656		
Total	100,330,940.67696	15			

Table A-3. ANOVA for Volumes of CO<sub>2</sub> Released with Temperature

### E. ANOVA: DCO<sub>2</sub> Values with Temperature

$F > F_{crit}$  thus, temperature appears to have a significant affect on final D CO<sub>2</sub> values.

DCO<sub>2</sub> values significantly decrease as temperature increases.

Analysis of Variance (One-Way) (alpha = 5%)					
Summary					
Groups	Sample size	Sum	Mean	Variance	
17	4	5,445.	1,361.25	9,206.25	
22	4	4,361.66667	1,090.41667	6,378.47222	
27	4	3,955.	988.75	2,756.25	
33	4	4,666.66667	1,166.66667	13,968.51852	
ANOVA					
Source of Variation	SS	df	MS	F	p-level
Between Groups	297,774.13194	3	99,258.04398	12.28841	0.00057
Within Groups	96,928.47222	12	8,077.37269		
Total	394,702.60417	15			

Table A-4. ANOVA for DCO<sub>2</sub> values with Temperature

#### F. Comparing the Decrease in DCO<sub>2</sub> with the Increase in CO<sub>2</sub> Loss with Temperature

Ave increase in mL CO<sub>2</sub> loss from 17 to 33°C = 5744

Ave decrease in mg of DCO<sub>2</sub> from 17 to 33 °C = -223

DCO<sub>2</sub> values were converted from units of concentration (mg/L) to units of volume (mL) thusly:

$$\left( \frac{\text{mg DCO}_2}{\text{L red wine}} \right) \left( \frac{1\text{g}}{1000\text{mg}} \right) \left( \frac{1\text{mol}}{44.01\text{g CO}_2} \right) \left( \frac{22.4\text{L CO}_2}{1\text{ mol}} \right) \left( \frac{1000\text{ml}}{1\text{ml}} \right) = \frac{\text{mL CO}_2}{\text{L red wine}}$$

-223mg DCO<sub>2</sub> = 113.50 mL CO<sub>2</sub>

DCO<sub>2</sub> values were converted from units of concentration (mg/L) to units of volume (mL) thusly:

Thus, the decrease in mg of DCO<sub>2</sub> from 17 to 33 °C (=113.50 mL CO<sub>2</sub>) does not fully account for increase in mL CO<sub>2</sub> loss from 17 to 33°C (=5744 mL CO<sub>2</sub>).