DETECTION AND REJECTION THRESHOLDS FOR *VITIS LABRUSCA* ASSOCIATED ODORANTS ARE DEPENDENT ON MATRIX AND REGIONAL EFFECTS

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by

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ABSTRACT

Pennsylvania is among the top 10 wine producing states in the nation, producing more than three-quarters of a million cases of wine each year (Wines and Vines, January 2016). In 2011, the state’s wine and grape industry had a net worth of nearly 2 billion dollars, with wineries generating over 40 million dollars of revenue for the state (Frank, Rimerman + Co, LLP 2013). Though some Pennsylvania wineries have benefited from wine’s increasing popularity, many still struggle to have their wines nationally recognized, due in part to Pennsylvania’s reliance on wines made from grapes of Vitis labrusca varieties. These grapes contain many odorants, with two of the most identifiable and highly researched compounds being methyl anthranilate (MA) and 2-aminoacetophenone (2AAP), which are described as having ‘grapy’ and ‘foxy’ notes (Apstein 1984). These compounds, while desirable in the context of grape jelly, often receive bad press in the wine world, particularly when reviewed by wine “experts”.

To verify the assumption that wine professionals prefer wines made from V. vinifera grapes over those made from V. labrusca grapes, I gathered survey data from wine professionals in Pennsylvania, New York, and New Jersey; three states with wine industries that are largely dependent on sales from V. labrusca wines. I argue that if the wine professionals, with careers that are partially dependent on the success of wines made from labrusca grapes, prefer vinifera wines, that their preferences would extend to the rest of the country where labrusca wine is less available and relatively unfamiliar.

While opinions of wine professionals can be influential on a wine’s prestige, of equal importance are opinions of wine consumers, who are the driving force behind sales. Interestingly, analyzing data for consumption habits revealed that about half of wine consumers in Pennsylvania, New York, and New Jersey enjoy consuming labrusca wines. Perhaps as a result, total dollar sales of labrusca wines in the state of Pennsylvania rival those of vinifera wines.
In addition to gaining an understanding of consumer and professional opinions of
*labrusca* wines, the large body of my thesis work involved investigating the sensory perception of
the two active aromatic compounds in *labrusca* grapes, methyl anthranilate (MA) and
2-aminoacetophenone (2AAP). The first objective of this work was to determine the hypothesized
matrix effects on detection thresholds for MA and 2AAP in water, model wine, and wine. A
group of untrained participants who reported consuming white wine, but not that which was made
from *labrusca* varieties, was used to determine the detection threshold according to the procedure
outlined in ASTM-E679-04. Six levels with increasing concentrations of either MA or 2AAP
were used to test the thresholds in water, model wine, and a white wine (Riesling). The study was
a two-day counterbalanced design to account for possible learned task effects. These values were
then compared to values previously published in the literature based on ranking tasks, as opposed
to the 3 alternative-forced choice task presented in this study. In addition to evaluating matrix
effects on detection thresholds, I also used the detection threshold studies to assess the differences
between the thresholds for MA and 2AAP reported in the literature, using the same participants to
evaluate both compounds using the same methodology.

The second objective of my work was to determine how the concentration levels of
methyl anthranilate and 2-aminoacetophenone affect consumer and wine expert preference by
assessing rejection threshold for each of the two compounds in an unoaked Chardonnay.
Rejection threshold is the concentration at which a participant prefers the control sample versus
the treated sample. To accomplish this, I investigated how demographics, specifically regional
location, may affect rejection threshold. While wine consumers that live in Pennsylvania are
likely familiar with consuming wines of a grapey nature, it is equally likely that consumers in
California are not, as *V. labrusca* wines are not as ubiquitous in California. I therefore expected
that the rejection threshold for the two compounds would be lower in the CA population than in
the PA population. In other words, a lower concentration of each compound would be necessary to get the participant to reject the spiked sample and instead prefer the control, untreated wine.

Within the CA population, I hypothesized that wine experts would have a lower rejection threshold than regular non-expert wine consumers. Just as trained panelists are more attuned to the attributes to which they have been trained on a panel for a sensory task, so too are wine professionals more likely to pick up defects or off-odors in wine. I partnered with the University of California-Davis to conduct a rejection threshold study comparing the concentrations of MA and 2AAP necessary to elicit a clear preference for the control wine versus the wine with added amounts of MA and 2AAP.

The results of this research provide critical information about the detection of the potentially undesirable odors of methyl anthranilate and 2-aminoacetophenone in wine. The proven matrix effects suggest that at low concentrations, the influence of the native varieties’ graply aromas may depend on the potency of the native aromatics of the wine variety. With the knowledge that levels of MA and 2AAP in their wines are detectable, but not necessarily of consequence to purchase decisions, PA winemakers can save time and money and propel the PA wine industry forward, well past the negative reputation provided by some who equate PA wines with ‘alcoholic grape juice.’
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Chapter 1

Literature Review

1.1 Introduction

The aroma of wine can be characterized by a large number of descriptors. Some aromas, like hydrogen sulfide (rotten eggs), acetic acid (vinegar), and ethyl acetate (nail polish), are considered wine faults while others, such as whisky lactone (woody), isoamyl acetate (fruity), and cis-rose oxide (floral) are seen as unique aromas that contribute to the bouquet of a wine. Wines made from grapes of the *Vitis labrusca* species are known to smell grapy and/or foxy, which is commonly attributed to the compounds of methyl anthranilate and 2-aminoacetophenone (Sale & Wilson 1926; Acree et al. 1990). While these two compounds are not typically thought of as occurring in wines from *vinifera* grapes, untypical aging (UTA) of *vinifera* whites (Rapp et al. 1993) and scalping of these odorants from equipment used to process *labrusca* grapes (Smith 2014) can potentially increase their concentration to levels in *vinifera* wines that are perceptible.

Methyl anthranilate and 2-aminoacetophenone are structurally similar, as both are a derivative of anthranilic acid, with MA being an ester of the acid. Prior reports have suggested there is a 2-log difference in detection thresholds between the two volatile aromatics, with values for MA being reported around 300 µg/L (Nelson 1977) and those for 2AAP being closer to 1-2 µg/L (Fan 2007). Given the structural similarity, such a large difference in perception may seem surprising. Significant differences in perception due to molecular structure, however, have been demonstrated previously with work showing differences in aromatic perception between carvone enantiomers (Brookes et al. 2009), perhaps as a result of receptor sensitivity. Additionally, there are substantial differences in the psychophysical methods used here for assessing detection
thresholds compared to earlier studies (Nelson 1977), especially in comparison to standard approaches that use a forced-choice paradigm (e.g. ASTM E679-04).

Reports of detectable concentrations in *vinifera* and *labrusca* wines are well documented, but the effect of the matrix, odorant masking, potential synergistic effects, and combined physiochemical effects on perception have yet to be investigated to fully elucidate how *labrusca* wines are perceived by wine experts and non-expert consumers.

### 1.2 Wine Composition

#### 1.2.1 Ethanol

Next to water, ethanol is the most prevalent molecule in wine, accounting for 10-14% v/v of table wines, and up to 21% in fortified wines (Berg et al. 1955). It is produced naturally by yeast, typically *Saccharomyces cerevisiae*, during fermentation by the conversion of glucose to ethanol. Final ethanol concentration is dependent on sugar conversion, as well as initial Brix in the grapes pre-fermentation. Chaptalization, a process developed by French chemist Jean-Antoine-Claude Chaptal, may sometimes be used to increase the starting sugar concentration of juice (or must) in order to increase the final concentration of ethanol in the wine. This process is often performed in wine regions that have cooler climates and shorter growing seasons that prevent the grapes from fully ripening on the vine before harvest (Gough 1998).

Ethanol has been shown to affect the perceived astringency and bitterness of grape seed tannin oligomers (Fontoin et al. 2008) and have a moderate effect on perceived viscosity and density in model white wine solutions (Nurgel & Pickering 2005). Ethanol concentration and “fullness” of a wine are often associated anecdotally, and this was confirmed by Pickering et al. (1998) in controlled experiments, where they found the relationship between ethanol
concentration and perceived viscosity and density could in fact be correlated. Specifically, the maximum viscosity occurred at 10% v/v ethanol and maximum perceived density occurred at 12% v/v ethanol. There is also some evidence that ethanol may enhance the perceived sweetness of sucrose (Berg et al. 1955), particularly relevant to Rieslings that have remaining residual sugar post-fermentation.

1.2.2 Organic Acids

While wine is primarily a water/ethanol solution, there remains a small percent of the wine that is composed of organic acids, and these acids have a significant influence on the sensory properties of the wine. The most important non-volatile organic acids that contribute to wine flavor are tartaric (0.15-0.49 g/100 mL), lactic (0.046-0.338 g/100mL), malic (0.109-0.280 g/100 mL), succinic (0.05-0.20 g/100 mL), and potassium acid tartrate (0.10-0.50 g/100 mL), with other acids being present in much lower quantities (Berg et al. 1955). Like ethanol, organic acids have also been shown to affect the perception of sweetness in a wine (Berg et al. 1955).

Model wines often include organic acids because they contribute to mouthfeel and wine perception, in addition to regulating the system’s pH and titratable acidity to mimic that found in real wine. Nurgel and Pickering (2005) crafted a model wine using 100 mg/L of malic acid and 200 mg/L potassium bitartrate while Fontoin et al. (2008) used various levels of tartaric acid. Fontoin et al. (2008) found that tartaric acid concentration did not affect perceived bitterness or astringency of grape seed tannin oligomers when the model wine was held at a constant pH. In this same study, however, the pH was shown to influence the astringency perception of the tannin oligomers in model wine solutions, with a higher pH leading to a lower perceived intensity of astringency.
1.3 Wine Aroma of *Vitis labrusca*

There is a species of grape native to North America that grows particularly well in the states of Pennsylvania, Ohio, New York, and New Jersey; and, along with other native American species, ultimately saved the French wine industry from ruin after the infestation of *Phylloxera* in the late nineteenth century (Banerjee et. al 2010). These native species are commonly used as rootstock in *Phylloxera* infested areas, to which *Vitis vinifera* vines have been grafted. This grafting is performed so the *vinifera* vine is imparted with *Phylloxera* resistance. Of particular interest to this work is the cold-tolerant species, known as *Vitis labrusca*. While *labrusca* vines readily produce ripe grapes that are well suited for eating, they also contain two impact odorants that most wine experts consider unsuitable for wine. Methyl anthranilate (MA) and 2-aminoacetophenone (2AAP) have been known to impact the aroma of *V. labrusca* grapes, exhibiting a “grapy” and “foxy” odor (Sale & Wilson 1926; Acree et al. 1990).

1.3.1 Methyl Anthranilate

Methyl anthranilate has long been considered to be a character impact compound for Concord grapes, as well as other *labrusca* varieties (Power 1921). Robinson et al. (1949) reported that the ester develops in New York Concord grapes during the last stage of maturation and declines slightly when the berries become overmature. The compound is synthesized in the berry from a reaction between anthraniloyl-coenzyme A and methanol, which is catalyzed by alcohol acyltransferase (Wang et al. 2005). MA, naturally occurring in Concord grapes, has long been recognized as a bird repellent (Kare 1961). It is for this reason that it is believed that the berry synthesizes MA in order to deter birds from the fruit, thereby protecting them from damage by acting as an irritant (Avery et al. 1992). In recent decades, solutions of isolated MA have been
utilized as a bird repellent to protect various agricultural produce and horticultural crops from bird damage (Mason et al. 1991).

Methyl anthranilate is known to have a grape odor to humans (Furia & Beltanca 1975) that is sometimes described as being floral. Nelson & Acree (1977) investigated MA in American wine by surveying the amount of MA in a number of vinifera and labrusca varieties. They found Pinot Blanc, Sylvaner, and Riesling to be free of MA, with levels below the limit of detection achievable in that era. In contrast, they found MA to be present in the labrusca varieties, depending on the vintage. Levels in Niagara ranged from 0.617 to 3.102 ppm, 0.699 to 1.752 ppm in Concord, 0-0.102 ppm in Delaware, and around 0.178 ppm in Catawba. More modern data suggests MA is not typically detectable instrumentally in vinifera wines, though one study by Moio and Etievant (1995) did find some trace levels of MA (0.6-4.8 µg/L) in Pinot Noir.

Figure 1-1: Chemical structure for methyl anthranilate.

MA is a phenolic ester (see Fig. 1-1) that is by nature nonpolar, with a low volatility in both its pure state and in water (Weast, 1975). Roberts & Acree (1995) determined that the octanol:water partition coefficient (log P) of MA was equal to 2.23. Another study assessed volatility using centrifugation and HPLC to calculate a value of 84 for the octanol:water partition coefficient (Aronov & Clark, 1996). The low volatility observed by Roberts & Acree (1995) may explain why MA so often added has to be added at such high concentrations as a flavorant in food products such as candy (20-50 mg/L) and chewing gum (2200 mg/L), despite having a reported detection threshold of 300 µg/L, as measured in white wine (Nelson et al. 1977).
1.3.2 2-Aminoacetophenone

More pertinent to *vinifera* wines is the “foxy” compound, 2-aminoacetophenone, which has been known to contribute foxiness even in the absence of MA (Acree et al. 1990). To humans, 2AAP has been described as having a scent of acacia blossoms and mothballs, as it pertains to atypical aging defects (Schneider 2014). Since the discovery of atypical, or untypical aging (UTA) in *vinifera* wines, it has been relevant to wine production regions that do not grow *labrusca* grapes which have prompted researchers to identify impact compounds that may be responsible for UTA. 2AAP was identified as one of these compounds (Rapp et al. 1993; Sponholz & Huhn 1996), and though 2AAP does not naturally occur in *vinifera* grapes, it has been found in measurable quantities in some old German Rieslings (Schneider 2014).

![Chemical structure for 2-aminoacetophenone.](image)

The mechanism for the formation of 2AAP is not fully understood but is known to occur post-fermentation and appears to be formed primarily during wine storage after final additions of sulfur dioxide (Hoenicke et al. 2002a,b). 2AAP is believed to form as a result of the degradation of indole-3-acetic acid (IAA), but the mechanism has yet to be elucidated. The occurrence of UTA has not been reported for red wines, even when these wines have been spiked with IAA, presumably because of the presence of naturally occurring phenolics that act as radical scavengers, which prevent superoxide radicals from mediating the reaction of IAA to 2AAP (Christoph 1998).
The formation of UTA also seems to be partially dependent on viticultural practices. Hoenicke et al. (1999) found that incidences of UTA increased with vine stress caused by low rainfall or increased solar radiation and nutrient deficiencies. They also found that grape maturity and ripeness was influential, with grapes harvested early in the season showing greater levels of UTA post-fermentation. Premature harvest, in combination with high yielding vines, led to the highest levels of UTA (Hoenicke et al. 1999). Additional vineyard practices like overcropping and reduced nutrient supplementation, along with environmental conditions like drought that caused water stress, also led to higher reported levels of UTA (Schneider 2014).

While the studies by Hoenicke et al. and Schneider concentrated on UTA formation in German Rieslings, there has also been work done in the Finger Lakes region of New York, which also specializes in the production of Riesling. It was found that the formation of UTA in NY Rieslings correlated with hot, dry growing years. In a study by Henick-Kling et al. (2008), the effects of irrigation and nitrogen fertilization on UTA formation were investigated. During dry years, vines that were irrigated produced fruit that resulted in wine with less ‘dishrag’ and ‘furniture polish’ notes. Oddly, whereas 2AAP increases with UTA occurrences in German Rieslings, no correlation of 2AAP concentration and UTA off-flavors was found in NY Rieslings, suggesting that 1,1,6-triethyl-1,2-dihydronapthalene (TDN) may be more of an indicator compound, instead.

Kosmerl & Zlatic (2009) also studied how storage conditions of white wines may impact the formation of UTA. In their study, some wines were stored under normal, recommended conditions (low cellar temperature, high relative humidity) while others were stored under stressed conditions (high ambient temperature, low humidity, ultraviolet-light exposure). Under normal storage conditions, the wines had a maximum concentration of 0.72 µg 2AAP/L, averaging 0.23 µg/L. Under stressed conditions, the wines had a maximum concentration of 0.71 µg 2AAP/L, with an average of 0.42 µg/L, which was higher than the average 2AAP
concentration found for wines stored under normal conditions. Older vintages tested, on average, had higher concentrations of 2AAP, with the 2005 vintages sampled averaging 0.48 µg/L 2AAP and the 2006 vintages averaging 0.15 µg/L 2AAP. Of the wines tested, Malvasia saw the highest concentration of 2AAP with levels ranging from 0.04-0.72 µg/L, Chardonnay measuring 0.07-0.57 µg/L, and Riesling having 0.08-0.42 µg/L.

Finally, prior data suggest the detection threshold for 2AAP may vary depending on the wine used for evaluation. Fan et al. (2007) reported a threshold value for 2AAP of 0.5 µg/L in Chardonnay and 1.0 µg/L in Pinot Gris using trained panelists who were assigned a ranking task. The study by Hoenicke et al. (2002b) found the detection threshold in Riesling to be 2 µg/L.

1.4 Matrix Effects

1.4.1 Partition Coefficients

To be perceived, aroma molecules must be released from a food or beverage matrix and reach the olfactory epithelium, located at the back of the nasal cavity (Plug and Haring 1993). These molecules must be volatile in order to separate into the air layer above the food or beverage and subsequently pulled into the nasal cavity via the orthonasal or retronasal route. There are many factors that affect the volatility of an aroma molecule; including ethanol, sugar, and other wine components (Conner et al. 1998), saliva and temperature in the oral cavity (Roberts and Acree 1995), as well as the chemical structure of the compound and polarity of the matrix (Roberts and Acree 1995; Weast 1975).

These aroma compounds, even when perceived intensely, are typically present in extremely low concentrations, and can be approximated as an ideal state of infinite dilution. This allows Henry’s law to be used to model the physics of the partition coefficient. Assuming the
samples are contained and can reach a rough state of equilibrium, the partial pressure of the aroma compound in the air is proportional to its concentration in the liquid phase (e.g. wine). Therefore, the air/liquid partition coefficient often reported in volatility studies is defined as the ratio of the concentration of the volatile in the gas phase to the concentration in the liquid phase (Taylor 1998). Depending on the route the aromatic molecule takes to the olfactory epithelium, certain factors will be more of an influence on its release from the matrix.

**1.4.2 Orthonasal versus Retronasal Olfaction**

Overbosch et al. (1991) concluded the driving force for release of aromatic volatiles in the oral cavity is the divergence from the thermodynamic equilibrium between the liquid phase of the matrix and the air phase in the mouth. The importance of temperature in the oral cavity has been widely acknowledged, and this was emphasized in a study by Burdach & Doty (1987), which showed changes of temperatures in the mouth could modify volatility and therefore cause changes in flavor perception. Genovese et al. (2009) analyzed salivary effects and found that a white wine treated with human saliva led to a 32-80% decrease in the headspace concentration of esters. In contrast, this same effect was not found in red wines, which Genovese and colleagues speculated might be due to the interaction of red wine polyphenols with saliva mucin and esterase, inhibiting their activity on aroma volatiles.

Roberts & Acree (1995) investigated the extent to which conditions in the oral cavity affect retronasal aroma by looking at variables like saliva and temperature. Compared to the acidic pH of wine, the neutral pH of saliva can shift the relative equilibrium between odorants. MA, with a pKa of 2.32 (Buckingham 1994) and 2AAP, with a pKa of 2.44 (Sykulski 1979) both exist in their neutral and anionic forms at wine pH (~3.5). As neutral saliva increases the pH of the wine solution in the mouth, more of the volatiles are converted to their conjugate bases, which
can affect their volatility. It is therefore plausible that different salivary flow rates can affect volatilization of wine aromatic compounds. Roberts & Acree (1995) looked at the concentration of MA and 2AAP in the gas phase upon simulation of shearing and temperature conditions that would occur in the mouth, finding that both conditions led to a significant increase in the volatilization of these two compounds. With regards to the effect of pH, their starting grape beverage pH was 2.6. They saw an initial increase in volatility of MA and 2AAP as pH was increased by saliva from 2.6 to 3.0, but then a decrease as they reached a pH of 3.78 and an even further reduction at a pH of 5.11.

Clearly, then, there are multiple factors that affect volatilization of aromatic compounds depending on whether the study assesses orthonasal or retronasal olfaction, which in turn affect perception of odor–active compounds. Voirol & Daget (1986) compared perception after sipping, sniffing, or retronasal inhalation and found that threshold values for solutions of citral and vanillin were lowest for sipping and highest for retronasal inhalation. Later, Voirol & Daget performed a similar study in 1989 and found sipping a meat flavoring led to improved performance for concentration discrimination compared to sniffing. Pickering et al. (2007) compared detection thresholds for 2-isopropyl-3-methoxypyrazine evaluated by both orthonasal and retronasal olfaction. They found that the group threshold for retronasal evaluation (1.147 +/- 0.461 ng/L) was lower than the group threshold for orthonasal evaluation (1.563 +/- 0.531 ng/L) in a Gewürztraminer. In a red wine blend, however, the opposite occurred with the group threshold for retronasal evaluation (2.29 +/- 0.543 ng/L) being higher than that of orthonasal evaluation (1.031 +/- 0.314 ng/L). The observation in red wine agrees with an earlier study performed by Diaz (2004), who found the intensities for a series of ethyl esters to be higher at lower concentrations when detected by sniffing rather than retronasal sampling. Comparing intensity ratings with water-air partition coefficients led him to conclude that the physicochemical
properties of the flavor compounds will determine the extent of flavor release in the mouth, thereby changing the intensity by retronasal evaluation.

Since sniffing, or orthonasal olfaction, is affected by ambient conditions, the matrix of the solution itself is more of the determinant for the volatility of aroma compounds in the matrix. Because ethanol is a significant volume of wine, next to water, prior work has looked at the effect of ethanol on headspace concentrations of aromatics. Conner et al. (1998) assessed the volatility of ethyl esters of varying chain length to evaluate the extent to which ethanol affects partitioning of the esters between the air and liquid phases. The study mentions previous work had found odor thresholds of ethyl hexanoate to vary widely from 0.037 mg/L in model wine and water and 0.056 mg/L when sugar was added to the solution, to 0.85 mg/L in a dry white wine. To investigate this range of values, Conner et al. created solutions of 5, 10, and 17% ethanol, all of which failed to change the headspace concentration for ethyl hexanoate. This information suggests that for alcoholic beverages less than 17% alcohol v/v, there are no significant differences in activity coefficients of ethyl esters as a result of ethanol concentration. The authors conclude that differences in the aroma thresholds noted previously must therefore result from a sensory phenomenon or a chemical interaction between the aroma volatiles and other beverage components. Although not offered as an alternate explanation, threshold differences may also vary as a result of sniffing variations. Mainland and Sobel (2006) reviewed work investigating the impact of sniff frequency and strength on perceived odor intensity, concluding that sniffs affect both intensity and identity perception of odorants.

1.4.3 Odorant Masking

Ethanol is a critical component of wine, responsible for approximately 12% of a wine’s composition, with most of the remaining composition being water. Ethanol is therefore an
important molecule to consider in the context of partitioning of aroma molecules. Ethanol has been shown to have a limited effect on partitioning of select ethyl esters at concentrations below 17% (Conner et al. 1998), but play a more prominent role in masking mixtures of wine odorants. The perception of two impact odorants important to wine, whiskey lactone and isoamyl acetate, was assessed in varying concentrations in water and in a 12% ethanol aqueous solution (Berre et al. 2007). Participants were asked to rate the odor intensities of “fruity-banana” (isoamyl acetate), “woody-coconut” (whiskey lactone) and “alcohol.” The perceived intensity of isoamyl acetate was not found to be a function of concentration in the aqueous and 12% alcohol matrices, and showed no significant differences in volatility between the two solutions. Woody odor intensity increased in the alcohol solution despite the whiskey lactone partition coefficient measured between the liquid and gas phases being lower in the dilute alcohol solution compared to the water solution. This is consistent with the higher solubility of whiskey lactone in a wine model solution found by Barrera-Garcia et al. (2006). Overall, the highest concentrations of isoamyl acetate and whisky lactone were rated as more intense in the alcohol solution compared to the aqueous solution. This observation agrees with the additive effect of ethanol on aroma intensity found by Pet’ka et al. (2003) but contrast the finding by Grosch (2011) that ethanol concentration in a complex model wine system and intensity of fruity and floral odors are inversely related (i.e. higher ethanol lowers perceived intensity of fruity and floral odors).

1.5 Detection Threshold Evaluation Methods

A detection threshold can be defined as the “lowest concentration of a substance in a medium relating to the lowest physical intensity at which a stimulus is detected as determined by the best-estimate criterion” (ASTM E679).
1.5.1 Ranking Tasks

The evaluation of methyl anthranilate in white wine by Nelson et al. (1977) was performed using two series of sample sets in which participants were asked to rank the samples in increasing order of MA concentration. This protocol followed the belief that the compound would be perceived as more intense at increasing concentrations above detection. The two sets contained four samples each (0.00, 0.01, 0.03, 0.1 and 0.1, 0.3, 1.0, 3.0 ppm). Seven panelists experienced in wine evaluation could consistently rank the samples correctly in the second set, but could not rank the first set of lower concentrations correctly. Nelson et al. therefore concluded that the 0.1 ppm sample was probably only ranked correctly in the set of higher concentrations by process of elimination. Since the 0.3 ppm sample was the lowest concentration consistently ranked correctly, 0.3 ppm (300 µg/L) was labeled as the detection threshold.

Fan et al. (2007) also utilized ranking tasks to evaluate the detection threshold for 2-aminoacetophenone in white wines. Six samples (0, 0.02, 0.05, 0.1, 0.5 and 1 µg/L) were presented to eight panelists with prior sensory training and experience. Panelists were asked to smell the samples in the first session and create a list of descriptors, refining that list in the second session. The following three sessions were replicates where panelists were instructed to smell the samples in random order and rank the samples in order of the intensities of the descriptors they had previously defined. Detection threshold was determined as the point at which descriptors shifted from Cluster 1 (e.g. green apple, vanilla, fruity) to Cluster 2 (e.g. mothball, shoe polish, wet hay). This value was 0.5 µg/L in Chardonnay and 1.0 µg/L in Pinot Gris.
1.5.2 Forced-Choice Methods

Some more recent studies have used 3 alternative forced-choice (3AFC) methods, defined as “a set consisting of one test sample and two blank samples” (ASTM E679) to evaluate detection thresholds for aromatic compounds, both for orthonasal and retronasal evaluation (Pickering et al. 2007). The procedure for this method is outlined in ASTM standard method E679. There are four steps involved with the procedure. The first step entails preparation of the concentration sets such that there are three samples in each set, two samples of which are the blank or control sample and one of which is spiked with the odorous compound. The standard protocol also dictates that the concentration of the added compound increase in geometric increments with each subsequent set. The panelists then receive the sets, starting their evaluation at the lowest concentration, which should be 2-3 steps below the estimated threshold. Panelists then indicate which of the three samples is different than the other two, much like they would in a traditional triangle test.

Individual-level best-estimate thresholds (BETs) are then derived from their response patterns of correct/incorrect evaluations of the samples. The a priori criterion for BET states that it is the geometric mean of the last missed concentration and the next adjacent higher concentration of the sample correctly identified. In cases where an individual evaluates all samples in the series correctly, the BET is the geometric mean of the lowest concentration in the series and the concentration that would be the next geometric increment below that concentration. If an individual evaluates all samples in the series incorrectly, the BET is defined as the geometric mean of the highest concentration in the series and the concentration that would be the next geometric increment above that concentration. To estimate threshold for the entire group, the mean of the individual BETs is calculated using a geometric mean, which is defined as “the nth root of the product of n terms” (ASTM E679). For example, the geometric mean of 60 and 120 is
sqrt(60 x 120), or 85. Geometric means are used because they are more robust to outliers than arithmetic means.

1.5.3 Graphical Analysis

Lawless (2010) presented a useful modification to the analysis approach and calculation of the threshold values once data have been acquired using the E679 method. He pointed out the potential shortcomings of the individual and group BET calculation methods. For one, the previous method did not have any consideration factored in for correct evaluation due to chance. Secondly, while previous work (Stevens et al. 1988) had shown that temporary signal loss could occur where an individual has the signal but then temporarily loses it, the ASTM method did not have a way to account for this phenomenon. Also, there was no way to prevent individuals that had become adapted, fatigued or overwhelmed at the highest levels, or had failed to correctly identify any of the different samples in the series, from skewing the resulting values calculated for the threshold. Lawless alleviated some of these potential shortcomings of the ASTM calculation by correcting for chance performance using Abbott’s formula (Eq. 1).

Equation 1: Abbot’s Formula.

\[ P_d = \left( P_{obs} - P_{\text{chance}} \right) / \left( 1 - P_{\text{chance}} \right) \]

For a 3-AFC test, the probability of correctly identifying the sample due to chance \( P_{\text{chance}} \) is 1/3. The traditional detection threshold criterion is set at the level where half of the population correctly identifies the different sample \( (P_d = 0.5) \). Therefore, when corrected for chance performance, \( P_{obs} \) needs to be greater than or equal to 2/3 for significance. Therefore, instead of calculating individual BETs that may be high or low estimates and not truly representative of the population, the correct number of responses at each concentration should be counted. The proportion of participants correct for each concentration should then be calculated.
Using an appropriate regression model to fit the data, the value at which 2/3 of the participants correctly identified the different sample can then be interpolated to determine the threshold value for the tested compound.

1.6 Rejection Threshold

Perhaps one of the first and most influential studies to evaluate how an impact odorant may negatively affect a wine, thereby leading to its rejection by a consumer, was the study done by Prescott et al. (2005) where the extent to which cork taint in wine acts as a deterrent was investigated. Cork taint is produced by 2,4,6-trichloroanisole (TCA), and is described as having musty odors that most consumers, and all experts, generally find unacceptable in their wine. Until this study, prior work had focused on the detection of TCA rather than the level at which consumers no longer preferred the sample containing TCA over a blank control. While intuitive, the approach taken by Prescott and colleagues was very novel, and highlighted the importance of assessing preferences of consumers, rather than trained panelists and/or experts, as it was more generalizable to the typical wine consumer.

To determine the consumer rejection threshold (CRT) for TCA, a paired preference test, which is one standard method for assessing preference, was utilized. A replicate series of eight paired comparison tests for each TCA level was presented in ascending concentration such that participants started with the lowest TCA level of 0 and concluded with the highest. Interestingly, to explore the possibility of carry over effects from previous samples, as well as adaptation and fatigue, a second experiment was conducted where the samples were randomized and concentrations were not presented in ascending order. The CRTs for each of the experiments, calculated from binomial distribution tables for paired comparison, were similar with the
ascending presentation resulting in a CRT of 3.1 ppt (n = 58) and randomized presentation resulting in a CRT of 3.7 ppt (n = 30).

This method used to evaluate CRT for TCA by Prescott in 2005 has since been used to evaluate CRT for 1,8 cineole (eucalyptol) in an Australian dry red wine blend (Saliba et al. 2009), ethyl phenylacetate and phenylacetic acid in a Spanish red wine blend (Campo et al. 2012), and sweetness (glucose) in Semillon (Blackman et al. 2010). Of particular relevance to this thesis is the study that evaluated CRT of 1,1,6-triethyl-1,2-dihydronaphthalene (TDN) in 1-year-old Riesling wines (Ross et al. 2014), as TDN is often considered to be an indicator compound for untypical aging (UTA) in Rieslings (Henick-Kling et al. 2008). In the study by Ross et al. (2014), the CRT for TDN was 82.4 µg/L for the 2011 Riesling and 157.4 µg/L for the 2010 Riesling, suggesting that the difference in CRT values observed was due to variation of the wine composition depending on the vintage.

The relationship between detection threshold and rejection threshold can be complex. Work by Harwood et al. (2012) evaluated detection and rejection threshold values for a chocolate product that contained varying concentrations of an added bitterant. While detection threshold values did not vary significantly for milk chocolate versus dark chocolate consumers, the rejection threshold for the bitterant among participants who preferred dark chocolate was more than 2 times higher than those preferring milk chocolate. This finding suggests that an individual’s liking, or rejection, of chocolate bitterness may be independent from their ability to detect the bitterness in the chocolate milk.
1.7 Expert versus Non-expert Consumer Evaluation

1.7.1 Defining an “Expert”

The term “expert” is often used in sensory evaluation, but despite its wide usage, there has been little done to standardize the term’s meaning. Lawless (1984) suggested that industry experts could be sorted into three categories: (1) trained panelists who have completed a standardized and guided training program, (2) individuals with an abundance of experience with a certain product that allows them to use their familiarity with the product and its attributes to serve as an “expert” evaluator, and (3) individuals whose work revolves around the development of products that are based on sensory attributes (e.g. flavor chemists). Additionally, Byrnes et al. (2015) noted that often in sensory, the terms “expert” and “trained panelist” are used interchangeably, attributing this imprecise usage to the varied sources of expertise described by Lawless.

1.7.2 Sensory Assessment Differences

Hayes and Pickering (2011) suggested that individuals who are considered “experts” in their respective fields may have self-selected their fields because of differences in innate ability. Lawless (1984) noted that the use of experts in sensory evaluation suggests that training or exposure to particular stimuli or products can develop perceptual abilities in individuals who have reasonable innate sensory acuity, who are motivated to attend to the task, and who are able to focus their attention on specific sensory stimuli. Additionally, he summarized the possible changes in sensory abilities that could occur through the process of becoming an “expert”. Such relevant transformations included (1) an increase in sensitivity, or a decrease in respective thresholds, (2) an increase in discrimination ability for suprathreshold concentrations of differing
attributes or varying intensities of the same attribute, and (3) a developed ability to consistently and accurately apply verbal descriptors to different sensory attributes or characteristics.

Since these observations for potential changes in perceptual abilities were documented, there has been more work done comparing the sensory acuity and performance of “experts” versus naïve consumers. The sweetness acceptance study by Blackman et al. (2010) evaluated the rejection threshold for sweetness (reflected by glucose concentration) as assessed by novice consumers, experienced consumers and winemakers on the basis that some level of residual sugar appeals to the majority of wine consumers, particularly American. Those authors found that winemakers preferred wines with less added sugar than did experienced consumers, who preferred wines with less added sugar than novice consumers. They concluded that preferred sweetness levels were impacted by the individual’s knowledge, experience and involvement in the wine industry. They added that this difference highlighted not only the difference in preferences between consumers and experts, but also differences within a consumer group that was controlled for experience level.

In contrast to the findings of the sweetness rejection study that found differences between experts, experienced consumers and novice consumers, work by Pickering et al. (2007) failed to find a difference in detection thresholds for 2-isopropyl-3-methoxypyrazine in wine between winemakers and non-winemakers after testing anecdotal claims that winemakers are more sensitive to the compound than non-winemakers. This same study, however, found a direct correlation between familiarity with ladybug taint (LBT) and sensitivity to the compound, as indicated by detection threshold. That is, the greater familiarity an individual had with LBT, the lower their detection threshold. The researchers noted that a potential bias in their study existed as many of the non-winemakers in the study not only had a high degree of involvement with wine, but also a high familiarity with LBT. They conclude that this may have lessened the difference in thresholds determined by the winemaker and non-winemakers, noting that if a difference were to
be found in future studies, it would likely be due to odor familiarity and not sensory acuity, as is
often attributed to winemakers and experts.

Other work, originally done in 2005 by Prescott and colleagues, led to finding a similar
inverse correlation between knowledge of a compound and identification as both the detection
threshold (DT) and consumer rejection threshold (CRT) of cork taint caused by TCA were
evaluated. The best estimate thresholds for both the CRT and DT were significantly positively
correlated ($r_s = 0.34; p < 0.05$) while there were significant negative correlations between
knowledge of cork taint, measured by number of questions correctly answered about cork taint,
and threshold values. Cork taint knowledge correlated negatively with both DT ($r_s = -0.31; p <
0.05$) and CRT ($r_s = -0.38; p < 0.05$) (Prescott et al. 2005). Non-expert consumers were used to
detect TCA, identifying the TCA sample at 2.1 ppt in a New Zealand Vidal Chardonnay. Prior to
this study, estimates for the DT of TCA ranged from 2-5 ppt in both white and red wines
(Liacopoulos et al. 1999; Amon et al. 1989) to 210 ppt in Sauvignon Blanc (Suprenant et al.
1997). These widely varying threshold values prompted a closer investigation by Prescott et al.,
but also consideration of the evaluators. Inexperienced tasters determined the value of 210 ppt
found by Suprenant and Butzke in 1997. When the samples were re-evaluated by a more
experienced panel, the DT was 17.4 ppt. Trained/experienced panelists were also used to arrive at
the value of 2-5 ppt (Liacopoulos et al. 1999) and 4 ppt (Amon et al. 1989).

Other work on detection thresholds assessed differences in detection threshold of $n$-
butanol in indoor air evaluated by a trained sensory panel and untrained participants (Polednik et
al. 2008). Panelists were recognized as trained once they had participated in more than two odor
sensory assessing sessions. The study found that participation in subsequent sensory sessions
lowered the detection thresholds for participants, especially those who had participated in a
second session compared to just the one, therefore leading the authors to conclude that the
evaluation practice of the panelists lowered their threshold concentration values. Experience-
induced changes have also been found with sugar taste discrimination (Gonzalez et al. 2008) and taste identification of monosodium glutamate (MSG) (Kobayashi et al. 2006). Both studies saw an increase in taste discrimination following brief periods of exposure, but a decline and return to baseline if the exposure was not sustained, with sensitivity to MSG significantly declining after 10 days (Kobayashi et al. 2006) and increased sensitivity to sugar reversing within 33-34 days (Gonzalez et al. 2008).

Early work suggesting familiarity of odors can increase performance with repeated testing for the recognition of volatiles was first published decades ago when olfaction sensory perception emerged as an active field of research (Engen 1982; Desor and Beauchamp 1974; Cain 1979). In 1984, Lawless investigated the difference between expert and non-expert wine consumers for descriptive analysis of white wine. While experts used more terms to describe the aroma of the wines compared to the non-experts (2.2 versus 0.9), the number of terms used to describe general flavor was more similar (2.0 versus 1.7). Of these descriptors, the experts wrote more concrete descriptors (e.g. lychee, peppery) than abstract descriptors (e.g. dry, chemical, drinkable). Descriptors listed by experts were 55% concrete and 45% abstract while 39% of descriptors listed by non-experts were concrete and 61% were abstract.

Siegrist and Cousin (2009) explored how expectations, or positive and negative information, can influence a wine taster’s sensory experience. Participants received either positive information (a rating of 92/100 from Robert Parker, the wine critic) or negative information (a rating of 72/100 from Robert Parker) before or after evaluating a red wine from Argentina that Parker had, in fact, rated at 92/100 points. Participants who received positive information about the wine before evaluating it gave a significantly higher hedonic rating than participants who had received negative information about the wine prior to tasting. Additionally, those participants who had received positive information prior to the tasting were also willing to pay more for the wine they evaluated compared to those who received negative information prior
to the tasting. These differences, however, were not found between participants who received negative or positive information about the wine after tasting the wines. This finding was consistent with the conclusions drawn from the work by Lee and colleagues in 2006, in which the authors found that disclosing the identity of an added secret ingredient (balsamic vinegar) to beer significantly decreased preference, but only when the information was disclosed prior to the tasting. When the information was presented to consumers after the tasting and before the evaluation, there was not a significant difference in preference compared to the blind tasting. There has not yet been work involving the extent to which these findings would influence expert tasters, who may have firmer beliefs and expectations that influence top-down processing, versus non-expert consumers, who may rely more on their bottom-up processes, like their sensory experiences. Expectation influences, important to top-down processing, on a wine were investigated by Wansink et al. (2007) who found that the stated origin of a wine can influence consumer ratings of the wine, with the same wine being less highly rated by consumers when labeled as being from North Dakota as compared to California.

1.8 Purpose and Significance

In 2015, Americans purchased over 37 billion dollars worth of wine in the United States (Wines & Vines, 2016). The most recent published economic impact report, for 2011, listed Pennsylvania as producing the fifth highest amount of grapes in the nation. With over 13,500 acres of grape-bearing land, PA produced over 90,000 tons of grapes, 13% of which were wine grapes (Frank, Rimerman + Co. LLP 2013). With a growing market, the Pennsylvania wine industry has seen a substantial increase in wineries and an increase in total production, as a result. While PA produces wines made from *Vitis vinifera* grapes, it also heavily relies on revenue generated from the sale of *Vitis labrusca* wines (USDA, 2013). These grapes that are native to
Pennsylvania, as well as neighboring states, contain two impact odorants, methyl anthranilate (MA) and 2-aminoacetophenone (2-AAP), which are often described as “grapy” and “foxy” (Apstein, 1984). While many residents of Pennsylvania are familiar with these compounds, be it in wine, grape jelly, or juice, some wine consumers in other countries along with wine experts find these aromas to be atypical of certain wine varieties, bordering on offensive. How much of each compound is necessary to elicit rejection, however, has not previously been systematically investigated.

While the detection threshold for methyl anthranilate has been reported as 300 µg/L (Nelson et al. 1977), it is not known at what concentration individuals reject the compound, and whether there may be significant differences between individuals who are accustomed with the compound versus those to whom wines with grapy smells are unfamiliar. Likewise, while the detection threshold for 2-aminoacetophenone ranges from 0.5 µg/L in Pinot Gris and Chardonnay (Fan et al. 2007) to 2 µg/L in Riesling (Hoenicke et al. 2002), its influence on acceptability in wine has not previously been evaluated in detail. Therefore, formal, well-controlled studies are necessary to evaluate the concentrations necessary to elicit rejection of wines with added MA and 2AAP across different populations.

Individual differences in sensory perception, as well as differences in thresholds due to matrix effects, also need to be addressed for this type of research. Although a handful of studies have been done to evaluate detection thresholds for the two compounds of interest, no study to date has compared both while using the same individuals. Therefore, it is possible or even likely that some of the reported difference between the perceptions of the two compounds may be due to inherent differences between individuals. Additionally, we also want to assess how detection threshold varies across matrices, as wine has a more acidic pH than water, as well as a mix of ethanol, organic acids, sugars, and other components which may affect partitioning of volatiles and/or act as masking agents.
Hypothesis: I hypothesize that there will be a significant effect of the matrix on detection thresholds; that the threshold value for methyl anthranilate and 2-aminoacetopheneone will be lower in water than in the model wine and wine matrices. Additionally, I hypothesize that the native variety impact odorants will be rejected by the California population, but not by the Pennsylvania population.

Specific Aims:

1. To compare reported consumption of *Vitis labrusca* wines and *Vitis vinifera* wines by wine experts and non-expert consumers.

2. To determine the effect of the matrix on detection threshold.
   a. To determine the detection threshold for methyl anthranilate in water, model wine and *V. vinifera* wine.
   b. To determine the detection threshold for 2-aminoacetophenone in water, model wine and *V. vinifera* wine.

3. To assess the reported differences in detection thresholds for methyl anthranilate and 2-aminoacetophenone.

4. To determine the rejection threshold for native wine impact odorants added to *V. vinifera* wine.
   a. To determine the rejection threshold for methyl anthranilate in wine, as evaluated by California wine experts, California non-expert consumers, and Pennsylvania non-expert consumers.
   b. To determine the rejection threshold for 2-aminoacetophenone in wine, as evaluated by California wine experts and California non-expert consumers.
Chapter 2

The Pennsylvania Wine Industry: A Survey of Statistics, Consumption and Beliefs

The importance of *V. labrusca* grapes to the wine industry of Pennsylvania, as well as New York and New Jersey, cannot be underestimated. A 2012 survey by the US Department of Agriculture (2013) reported *labrusca* varieties (Concord and Niagara) accounting for 61% of wine grape production in the state of Pennsylvania. This report also listed *labrusca* wines as accounting for nearly one-third of dollar sales in the Pennsylvania wine industry. Compiled USDA reports from 2007 to 2012 show an interesting comparison of production of wine grapes for the *V. vinifera* and *V. labrusca* species, compared to the resulting sales (Fig. 2-1). Although production of *V. labrusca* wine grapes are well in excess of *V. vinifera* grapes, total sales for *labrusca* are only slightly above *vinifera* in most years, reflecting higher margins for *vinifera*, though not incorporating the higher cost associated with growing *vinifera* grapes.

Figure 2-1: Production of *vinifera* and *labrusca* grapes and dollar value of sales from 2007 to 2012, in Pennsylvania. Columns represents the quantity of *V. labrusca* and *V. vinifera* grapes produced by the ton from 2007 to 2012, with the lines following the dollar value of sales.
2.1 The Pennsylvania Wine Industry

The wine industry in Pennsylvania has seen a marked increase over the last five years, growing rapidly since 2010. In 2010, there were 159 bonded wineries in the state of Pennsylvania whereas there were 287 wineries by then end of 2015 (TTB 2015). Figure 2-2 shows the growth of wineries in Pennsylvania, relative to its neighbors, Ohio and New York. The growth of Pennsylvania’s western neighbor, Ohio, has mirrored that of the growth Pennsylvania. This is likely due to the similar climates and growing regions in both states. While Pennsylvania is much larger than Ohio, the main growing regions for *labrusca* in both states are along the shores, and slightly inland, of Lake Erie while most *vinifera* varieties are grown in the milder climate of southern Pennsylvania.

![Graph showing growth of bonded wineries in NY, PA, and OH](image)

Figure 2-2: Bonded wineries in NY, PA, and OH. (TTB, 2015)

Total wine production in Pennsylvania has also seen a rapid increase in the past five years. In 2010, Pennsylvania bottled 2,902,664 gallons of still wine. By 2014, production increased an order of magnitude, as the state bottled 29,218,025 gallons of still wine (TTB 2007-2014). Figure 2-3 illustrates the increase in production relative to the increase in the number of wineries in the state.
Despite the growth of Pennsylvania’s wine industry, there is still a considerable difference between Pennsylvania and the West coast’s leading producer, California. While Pennsylvania saw record numbers for production in 2014, bottling 29,218,025 gallons of still wine, California bottled 545,880,684 gallons of still wine in 2014, a 14% increase from 477,130,626 gallons in 2007. The production totals of Pennsylvania and California, relative to the entire country of the United States, are shown in Figure 2-4.

Figure 2-3: Bottled gallons of still wine in Pennsylvania relative to the number of PA wineries. (TTB 2007-2014).

Figure 2-4: Bottled gallons of still wine in Pennsylvania compared to California and the entire United States. (TTB 2007-2014)
There is clearly a large divide in the wine industry between Pennsylvania and California, with Pennsylvania producing only 5.4% of that produced by California and only 4.2% of total production in the United States. These differences in production are also reflected in the number of designated wine grape-growing areas, known as American Viticultural Areas (AVAs), which are contained in each state. While California has over 120 AVAs, Pennsylvania currently has five: Central Delaware Valley (shared with New Jersey), Cumberland Valley (shared with Maryland), Lake Erie (shared with New York & Ohio), Lancaster Valley, and Lehigh Valley AVA (TTB, 2016).

2.2 Consumption of \textit{V. labrusca} Wines by Consumers

It is not always the quantity of wine that is produced that is the distinguishing statistic for a state’s wine industry; the quality of the commodity is also critical for a state’s success in the industry. Simply put, a market cannot be competitive if the supply outweighs the demand. To understand the consumption patterns and attitudes of \textit{labrusca} and \textit{vinifera} wines by consumers, survey data were collected by Dr. Kathy Kelley, Professor of Horticultural Marketing and Business Management at The Pennsylvania State University, and subsequently analyzed. Of particular interest here is the subset of respondents who consume native varieties (\textit{V. labrusca}), which is the part of the population that will be discussed further.

2.2.1 Consumption Data

Survey participants were given a list of 18 varieties and asked to indicate which of the varieties they consumed when a) they are entertaining and/or celebrating a special occasion and when b) they are \textit{not} entertaining and/or celebrating a special occasion. The 18 varieties included
11 *vinifera* wines (Chardonnay, Pinot Gris, Grüner Veltliner, Lemberger, Sauvignon Blanc, Muscat/Moscato, Riesling, Gewurztraminer, Cabernet Sauvignon, Cabernet Franc, Merlot), 4 *labrusca* wines (Concord, Niagara, Catawba, Delaware) and 3 wines made from hybrids (Traminette, Vidal Blanc, Chambourcin). Respondents who reported consuming any of the *labrusca* wines in either of the scenarios listed above were classified as “native variety” consumers. Of 846 respondents, 405 indicated that they consumed *labrusca* varieties. The following analyses focus on these consumers.

Of the 405 *labrusca* consumers (34% male and 65% female), 46% were permanent residents of New York, 42% were from Pennsylvania and 12% from New Jersey. The distribution of average household incomes is shown in Figure 2-5.

![Figure 2-5: Distribution of annual household income for *labrusca* consumers. (Data collection and initial analysis by Kelley, K. and statistics tabulated and graphed by Perry, D.)](image)

Census data from 2014 indicates the average household income for Pennsylvania was $53,234, compared to $58,878 for New York and $71,919 for New Jersey. Data for annual household income of *vinifera* consumers were not analyzed here, but it would be an interesting comparison to see if the income distribution is skewed higher for *vinifera* consumers, as the majority of wines made from *labrusca* grapes are cheaper than *vinifera* wines.
Consumption frequency data were collected from the survey respondents and the breakdown of consumption habits for each of the four *labrusca* varieties is illustrated in Figure 2-6.

![Consumption frequency for each of the labrusca varieties. (Data collection and initial analysis by Kelley, K. and statistics tabulated and graphed by Perry, D.)](image)

Concord was the most widely consumed of the four *labrusca* varieties, followed by Niagara, Catawba, and Delaware. Of the native variety consumers, 24.7% reported consuming Concord wines, 14.6% Niagara, 8.6% Catawba, and 9.4% Delaware at least once a week.

The occasion in which *labrusca* wines were reportedly consumed varied slightly depending on whether the respondent was entertaining and/or celebrating a special occasion versus if they were not. Of the 846 total survey respondents reporting on everyday drinking (i.e. not entertaining and/or celebrating a special occasion), 30% consumed Concord, 19% Niagara, 9% Delaware, and 11% Catawba. Compared to reporting on when they entertained and/or celebrated a special occasion, 27% consumed Concord, 16% Niagara, 8% Delaware, and 10% Catawba. A chi-squared test of significance for consumption of Concord and Cabernet Sauvignon for everyday drinking versus entertaining revealed that there was a significant difference (p <
0.05) between the situational appropriateness of consuming each variety. Likewise, there was also a significant difference (p < 0.01) in consumption of Niagara and Chardonnay for everyday and entertaining situations. In general, consumption of labrusca wines decreased when native variety consumers were entertaining or celebrating a special occasion compared to not celebrating, while consumption of vinifera wines increased when celebrating a special occasion. This was true for all vinifera varieties, which is statistically significant based on a sign test (two-sided, p < 0.05). Representative varieties for labrusca (Concord, Niagara) and vinifera (Chardonnay, Pinot Gris, Cabernet Sauvignon, Merlot) and their situational appropriateness for consumption as selected by the 405 native variety drinkers are shown in Figure 2-7. These patterns suggest that even among consumers who enjoy labrusca wines, they may still be likely to consider serving or consuming vinifera wines in special situations.

![Figure 2-7: Consumption of wine varieties when entertaining and/or celebrating a special occasion (entertaining-wine) versus when not entertaining and/or celebrating a special occasion (everyday-wine). (Data collection and initial analysis by Kelley, K. and statistics tabulated and graphed by Perry, D.)](image)
2.3 Wine Professionals Opinions of *V. labrusca* and *V. vinifera* Wines

The premise for the work of this thesis operated under the observation that wines made from *V. vinifera* grapes are more popular, as *labrusca* wines are rarely grown outside the United States, and the assumption that *vinifera* wines are better regarded than those made from *V. labrusca* grapes. Evidence for this assumption is scattered throughout the wine literature and in books intended to educate the general public and wine enthusiasts, alike. Most mass market wine books do not cover *labrusca* wines, with those that do simply writing them off as much more scarce in the market, especially internationally, because “the flavor of those grapes is less popular in wine” (e.g. McCarthy and Ewing-Mulligan, 2012) with others not mentioning *labrusca* by name but simply referring to them as part of a group of “other species that by themselves are worthless for wine” (Wagner, 1976). To better document this anecdotal bias, I distributed a survey with questions regarding consumption and liking of wines made from *V. labrusca* and *V. vinifera* grapes to individuals who work in the wine industry in the states of Pennsylvania, New York, and New Jersey; that is, the three states where wine made from *V. labrusca* grapes is most important to the regional wine market. Complete data are provided in Appendix A.

2.3.1 Consumption Data

Consumption frequency data were collected for wines made from both *V. labrusca* and *V. vinifera* grapes. The results show a strong preference for *vinifera* wines among wine professionals. Wine professionals were defined here as members of the wine industry who had received formal training and/or a viticulture/enology degree, as well as those who worked in the industry and made tasting decisions that had financial consequences for the winery. The distribution of intake frequency is presented in Figure 2-8. While 87.6% of respondents reported
consuming *labrusca* wines 1-3 times a year or less, 94.8% of respondents reported consuming *vinifera* wines more than 1-3 times year. Instead, 79.4% of wine professionals reported consuming *vinifera* wines at least once a week, with 25.9% reportedly consuming them every day. Comparatively, only a single respondent reported consuming *labrusca* wines daily.

Figure 2-8: Consumption frequency of *labrusca* and *vinifera* wines among wine professionals (data collected and analyzed by Perry 2016).

Wine professionals were also more likely to pay a higher premium for *vinifera* wines compared to *labrusca* wines. 87.9% of respondents were not willing to pay more than $14.99 for a standard size (750 mL) bottle of a *labrusca* wine, whereas 81% were willing to pay in excess of $15.00 for a bottle of *vinifera* wine. Additionally, none of the respondents were willing to pay more than $25 for a bottle of *labrusca* wine, while 19% reported they were willing to pay that much for a *vinifera* table wine. These data confirm that if and when wine professionals do choose to consume *labrusca* wines, they are not willing to pay nearly as much as they would for a bottle of *vinifera* wine; this suggests *labrusca* wines simply aren’t as valuable to wine professionals.
2.3.2 Likert Scale Data

To understand possible differences in opinions between *V. labrusca* and *V. vinifera* wines held by wine professionals, eight different questions were asked, using a seven point Likert scale for each. Each Likert scale ranged from “strongly disagree” to “strongly agree”. Questions were balanced in favor of *vinifera* and *labrusca* to avoid survey bias, which may orient respondents to a specific answer and therefore skew results. Of the 58 wine professionals who took the survey, four chose not to answer all the questions, leaving a total of (n = 54) ratings for the Likert scale questions.

There is often an assumed association between sweet wines and *labrusca* wines: to test the strength of this association, wine professionals were asked how much they agreed with the statement “wines made from *labrusca* grapes are sweet.” While 31.5% of respondents were unsure (answered “neutral”), almost 52% agreed or strongly agreed that *labrusca* wines are sweet with only 5.6% strongly disagreeing. Interestingly, wine professionals were also asked their preference for sweet table wines by indicating how much they agreed with the statement “I like sweet table wines.” Figure 2-9 shows the results, with the majority (76%) of respondents either strongly disagreeing or disagreeing with the statement, suggesting that the perceived sweetness of *labrusca* wines may account for some of its disliking.
Survey participants were also asked about occasion-specific consumption of *vinifera* and *labrusca* wines, answering to statements about opening a bottle of either species for everyday drinking and for celebrating. When asked how much they agreed with opening a bottle of *labrusca* for celebrating a special occasion, only 5.6% agreed or strongly agreed, with 87% disagreeing or strongly disagreeing. When asked about *labrusca* consumption for everyday drinking, the statistics were almost identical, reflecting the fact that most wine professionals simply do not regularly consume *labrusca* wines. This is further supported by the fact that 89% of wine professionals agree or strongly agree to purchasing a *vinifera* wine when buying as a gift for a wine enthusiast.

While it is clear that wine professionals do not often consume *labrusca* wines or gift them to others, there is more disagreement within the professional community with regards to quality and how that may differ between the *labrusca* and *vinifera* wines. When asked how much they agreed with the statement “Assuming the wines are clear of defects, a wine made from *V. vinifera* grapes is superior in quality to a wine made from *V. labrusca* grapes”, 65% of respondents agreed or strongly agreed, but 17% disagreed or strongly disagreed, with 18%
indicating they were unsure. Additionally, when asked how much they agreed that quality wine can be made from *labrusca* grapes, 76% of respondents agreed or strongly agreed and only 6% disagreed or strongly disagreed. It therefore appears that while wine professionals believe that quality wine can be made from *V. labrusca* grapes, they prefer not to consume it.

Quality is an ambiguous term often used in sensory evaluation and yet not clearly defined. On a broad scale, there are four approaches for what and who defines quality: (1) consumer satisfaction versus evaluations by experts judges, (2) conformance to product specifications, (3) product consistency, and (4) purpose of fit (Garvin 1984). Consistent with the first approach, quality may be dictated by consumer appeal, which may vary from ratings and opinions from connoisseurs, or experts. Opinions may be further influenced by a product’s adherence to specifications and consistency (i.e. a product delivers on what it advertises). Importantly, quality may be transient, dependent on the circumstance. While quality may have different meanings to different participants or in different contexts, it is clear from the survey of experts that no matter how clean and fault-free a wine is, if it tastes like Concord grapes, the majority (84%) of wine professionals are not going to like it.
3.1 Abstract

Conceptually, a detection threshold represents the lowest concentration at which an individual or a group of individuals can reliably perceive a given stimulus, with a commonly used operational definition of 50% performance above chance. Estimated measures of detection thresholds (DTs), however, are often reported in the literature with little attention given to the matrix in which the stimuli were evaluated. Here, we highlight the impact of matrix effects on DTs for two odor-active compounds commonly found in *Vitis labrusca* wines. Differences in orthonasal DTs for methyl anthranilate (MA) and 2-aminoacetophenone (2AAP) in water, a model wine system, and wine were demonstrated using a within-subject design and forced choice (i.e. criterion free) psychophysical methods. Six sample triads, each containing 2 blanks and 1 spiked sample, were presented to participants with the instructions to choose the “different” sample, and this was repeated in different matrices (water, model wine, and wine). The estimated DTs for both compounds were significantly lower in water versus the model wine system and wine. This finding recapitulates the strong need to carefully consider the nature of the delivery matrix when determining and comparing threshold estimates. Additionally, data from prior reports have suggested DTs for MA and 2AAP may differ by two orders of magnitude in spite of their structural similarity. We failed to confirm this difference here: although 2AAP thresholds were somewhat lower than MA thresholds, differences were much smaller than what had been
suggested previously. This, again, emphasizes the need to make comparisons within the same individuals, using appropriate methods with sufficient numbers of participants.

### 3.2 Introduction

Aroma is an influential factor of flavor perception. Two distinct types of olfaction, typified by different routes of exposure, are known to impact sensory aspects of food products. These are orthonasal olfaction, where odorants reach olfactory receptors via the nostrils, and retronasal olfaction, where volatiles released during mastication and swallowing pass through the back of the throat to reach the same receptors (Pierce & Halpern 1996). The concentrations of odorants that reach these receptors can range from levels well below threshold where no sensation is perceived, to levels well in excess of threshold, where the sensation is clearly perceived. Within the flavor chemistry literature, the concentration of a specific odorant found within a food is often expressed in terms of an odor activity value (OAV), which is defined as the ratio of the concentration of the compound in the food over some estimate of the detection threshold for that compound (Guth 1997).

Often, the literature reports detection thresholds for odor active compounds without emphasis on the matrix in which the value was estimated. This practice makes it difficult to evaluate the suitability of the threshold value for applications, such as the calculation of OAVs. This odor activity value must be assessed based on threshold values that were evaluated in the same matrix of interest for the OAV. For example, an air quality report listing OAVs should use threshold values where air is the diluent. Conversely, a fragrance company focused on product formulation would likely be more interested in threshold values based on assessments of the chemicals in water, water/alcohol matrices, or possibly oil. This consideration must be made to
prevent reporting OAVs that may be off by orders of magnitude, which may then be perpetuated through the literature.

Beverages are complex systems, chemically and perceptually. Given the same concentration of a compound, it may be well pronounced in one application and subtle in another. Potential masking effects have been studied with relation to aroma and taste perception, but the extent to which these effects influence detection thresholds remains largely unanswered. To investigate differences in thresholds evaluated in diverse matrices, we used methyl anthranilate (MA) and 2-aminoacetophenone (2AAP), two odor-active compounds in *V. labrusca* wines. Previous studies with 2AAP have suggested that detection thresholds may change with respect to the wine varietal in which the study is performed. These studies suggest a detection threshold for 2AAP of 0.5 µg/L in Pinot Gris and Chardonnay (Fan 2007) and 2.0 µg/L in Riesling (Hoenicke 2002b). The more complex aroma profile of Riesling (e.g. floral, petrol) versus the more neutral aroma profile of a typical Pinot Gris or Chardonnay suggests that perceptual masking effects may be an important part of understanding the underlying cause of matrix dependence of detection thresholds.

Prior reports suggest there may be a two-log difference in detection thresholds for MA and 2AAP, despite their structural similarity. While the detection threshold for MA in Riesling has been reported as 300 µg/L (Nelson 1977) and 2AAP in Riesling as 2 µg/L (Hoenicke 2002b), a direct comparison of the two compounds has never been investigated using the same group of participants. Consequently, a direct comparison of these compounds using a forced-choice procedure in a within-subjects design was performed here with a relatively large sample (n > 40) to elucidate potential differences in the detection thresholds for these compounds. This was done to mitigate differences that may have arisen in previous studies of the compounds from small numbers of participants and/or differences in psychophysical methodology.
Accordingly, the matrix dependency for detection thresholds was analyzed using methyl anthranilate and 2-aminoacetophenone as the volatile odor-active compounds of interest. Here, we investigated differences in threshold values as a function of the matrix, using water, model wine and wine as the matrices. Additionally, we explored the magnitude of the previously reported differences in detection thresholds between MA and 2AAP, two similarly structured compounds. This innovative study is novel in that: (1) it relies on ascending 3-alternative forced choice methods for threshold evaluation (2) the evaluators are untrained participants, and (3) the large sample sizes allow for segmentation of the data and graphical methods of analysis.

### 3.3 Materials and Methods

#### 3.3.1 Overview

A total of five experiments were executed to study the matrix effects on detection thresholds for MA and 2AAP. See Table 3-1 for a summary. The specific concentration series for each was adjusted as needed, depending on published literature and informal pilot testing (details are given below).

Table 3-1: Overview of Experiments (see supplemental table B-1 for additional details such as sample size, date of each experiment, etc.)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Water</th>
<th>Model Wine</th>
<th>Wine</th>
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<tbody>
<tr>
<td>Experiment 1</td>
<td>MA</td>
<td>MA</td>
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<tr>
<td>Experiment 2</td>
<td>MA vs. 2AAP</td>
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<tr>
<td>Experiment 3</td>
<td>2AAP</td>
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<tr>
<td>Experiment 4</td>
<td>MA</td>
<td>MA</td>
<td></td>
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<tr>
<td>Experiment 5</td>
<td>2AAP</td>
<td>2AAP</td>
<td></td>
</tr>
</tbody>
</table>
3.3.2 Stimuli

**Odorants.** Stock solutions were made using methyl anthranilate (99.5% purity, FG, Sigma-Aldrich, Milwaukee, WI, USA) and 2-aminoacetophenone (99.3% purity, FG, Sigma-Aldrich, Milwaukee, WI, USA).

**Wine.** Bulk Riesling (Franz Reinhart, Production Date July 10, 2014. Lot No. L-14191, Packed by Bingen am Rhein, Serial 4002301431350) was purchased from a retail store run by the Pennsylvania Liquor Control Board (PLCB). Riesling was selected as the base wine, consistent with past work that has evaluated the detection thresholds for methyl anthranilate (Acree 1977) and 2-aminoacetophenone (Hoenicke et al. 2002b).

**Model Wine.** A model wine system was created to match the pH, alcohol, and titratable acidity of the Franz Reinhart Riesling, which were measured using standard analytical techniques. The alcohol of the Riesling was measured using ebulliometry (Iland 2004). Reverse osmosis water was used as the starting base for the model wine, and a sufficient volume of ethanol (190 proof, KOPTEC, King of Prussia, PA) was added to reach a final ethanol concentration of 9.65% v/v. The titratable acidity was adjusted to 8.15 g/L using tartaric acid (MA): 99.7% purity, FG, Sigma-Aldrich, Milwaukee, WI, USA; study 2 (2AAP): 99.8% purity, FCC, Spectrum Chemicals, Gardena, CA, USA, consistent with Goodstein et al. (2014) who modeled the titratable acidity of white wine using tartaric acid. The pH was adjusted to 3.10 using a solution of 5N sodium hydroxide (NaOH pellets, FCC, 97.8% purity, Macron Fine Chemicals, Sweden).

3.3.3 Concentration Ranges

Within each experiment, participants received stimuli that covered 2.5 orders of magnitude in concentration, in half log steps. Concentration ranges were selected based on
benchtop testing and values scattered throughout the literature. The concentrations listed for each experiment below are nominal. Like the work performed by Green and Hayes (2003), we did not control for variable volatility rates for the two stimuli across solutions. We did, however, strictly follow protocol for sample preparation and sample storage and are therefore confident that the relative concentrations for the samples are stable across participants and across experiments.

**Experiment 1.** MA in Water versus Wine. Concentration series of (0.056, 0.18, 0.56, 1.8, 5.6, 18) µg/L in water and (0.18, 0.56, 1.8, 5.6, 18, 56) µg/L in wine were used to evaluate the detection threshold of MA.

**Experiment 2.** MA versus 2AAP in Water. Concentration series of MA (0.18, 0.56, 1.8, 5.6, 18, 56) µg/L and 2AAP (0.018, 0.056, 0.18, 0.56, 1.8, 5.6) µg/L were used to evaluate the detection thresholds of the two stimuli in water.

**Experiment 3.** 2AAP in Wine. A concentration series of (0.056, 0.18, 0.56, 1.8, 5.6, 18) µg/L was used to evaluate the detection threshold of 2AAP in wine.

**Experiment 4.** MA in Water versus Model Wine. Concentration series of (0.56, 1.8, 5.6, 18, 56, and 180) µg/L in water and (1.8, 5.6, 18, 56, 180, 560) µg/L in model wine were used to evaluate the detection threshold of MA.

**Experiment 5.** 2AAP in Water versus Model Wine. Concentration series of (0.056, 0.18, 0.56, 1.8, 5.6, 18) µg/L in water and (1.8, 5.6, 18, 56, 180, 560) µg/L in model wine were used to evaluate the detection threshold of 2AAP.

**Sample Preparation.** Solutions were stored in a refrigerator (34°F) until the day of the sensory test. Samples were prepared no more than seven days in advance of administering to participants for the sensory test. Solutions were removed from the refrigerator and allowed to come to room temperature before being administered to participants for sensory testing.
3.3.4 Data Collection and Analysis

**Sensory Methodology.** For determination of detection thresholds, participants received 30 mL samples served in standard ISO wine tasting glasses, each covered with a paper cap labeled with a three digit blinding code. All glasses were prepared one hour before serving to allow for temperature and headspace equilibration. The samples were presented and evaluated in individual booths at room temperature (21°C) under red light (100 W Sylvania 115-125V red flood light) in the Sensory Evaluation Center located in the Rodney E. Erickson Food Science Building at the Pennsylvania State University.

For each experiment, 48 participants were recruited from an existing pool of interested individuals who had previously opted-in to be contacted about taste and smell experiments in our facility. Following screening, scheduling was handled using software from SONA systems (Tallinn, Estonia). Healthy, non-smoking individuals with no known taste and smell defects who consumed white wine at least once every six months were invited to participate in the study. Individuals under the age of 21, as well as currently enrolled undergraduate students, regardless of age, were excluded from the study. The screening also asked individuals to indicate their consumption of real and fictitious wine varietals: participants who indicated they drank the fictitious varietal (“Grebenheim”) were excluded from the study.

With the exception of the experiment determining the detection threshold for 2AAP in wine (Experiment 3), all other experiments were two-day studies in which participants who completed day 1 of testing were asked to come back to complete the second part of the experiment within two days of initial testing in a crossover design. Sample presentation was counterbalanced such that half of the participants received one series of samples (e.g. MA) the first day and the second series (e.g. 2AAP) the second day while the other half received the
second series the first day and the first series the second day. This was done to account for possible learning effects across repeated testing (Lawless & Heymann 2010).

A standard ascending concentration series using a 3 alternative forced choice (3AFC) task was used to estimate detection thresholds (ASTM E679-004). Participants were presented with six sets, containing three samples each, and asked to evaluate them based on orthonasal olfaction. Each set contained two samples of the control (untreated sample of water, model wine, or wine) and one sample of the diluent spiked with either 2AAP or MA. The triads were presented so that the spiked sample increased in concentration, with the subject receiving the lowest spiked concentration for their first set (triplet) of samples. Within each triplet, the order of samples within each was counterbalanced across individuals.

For each sample, participants were asked to swirl the glass three times before removing the paper cap, remove the cap, and smell the sample. Within the triplet, they were told to identify which of the three was the “different” sample. Responses were collected electronically using Compusense five software (Guelph, ONT). Between each set of samples, subjects waited 90 seconds before sampling their next set of samples, after which time they were presented with a screen instructing them to sample their next set. This break between triads was enforced via software.

**Data Analysis.** Data were exported from Compusense, sorted and tabulated at the end of each experiment. For the two-day studies, data were excluded for subjects that failed to return for the second day. Data were also excluded for non-responders, which we defined as subjects who were unable to complete the task successfully at least once (i.e. individuals who failed to correctly identify the different sample in at least one of the last three triads containing the three highest concentrations of stimulus). As described in ASTM E-679, individual best estimate thresholds (BETs) were calculated as the geometric mean of the highest concentration missed and the next highest concentration in which the different sample at all subsequent higher
concentrations was selected correctly. Group BETs were calculated as the geometric means of the individual BETs. Separately, we also calculated group detection thresholds graphically based on sigmoidal fits of the group proportions of correct responses. We fit the threshold functions using the four parameter logistic nonlinear regression function in GraphPad Prism 5.0C for OSX (GraphPad Software, San Diego CA). In this analysis, detection threshold was defined as the concentration at which the resulting performance was 50% greater than chance (see Lawless, 2010). That is, because the chance of guessing correctly in a triangle test is 1/3, and perfect performance is 1 (100% correct responses), a chance-adjusted proportion of 2/3 was used as the threshold criterion; this proportion was used to calculate the threshold concentration directly in software.

3.4 Determining Detection Threshold for Methyl Anthranilate in Water versus in Wine

3.4.1 Results

Data for 38 participants who evaluated the methyl anthranilate samples in water and for 36 participants in wine were analyzed using a 4-parameter non-linear regression function modeled after the Hill equation (Fig. 3-1). Widely used in pharmacology, the four-parameter logistic regression equation (Eq.2) describes the top of the curve (max), the bottom of the curve (min), the spot halfway between min and max (classically EC50), and the slope of the curve (the Hill coefficient).

Equation 2: Hill Equation.

\[ Y = Min + \left[ \frac{(Max - Min)}{1 + 10^{(EC_{50}-X) \times HillSlope}} \right] \]
The bottom constraint was fixed at 33.33% (performance due to chance) and top constraint at 100% (perfect performance). While identification of the compound in the first four samples for water (0.056-1.8 µg/L) and wine (0.18-5.6 µg/L) fluctuated around chance performance, the criterion for detection threshold (67%) was met, giving detection threshold values of 7.51 µg/L in water and 45.0 µg/L in wine.

Figure 3-1: Evaluated by the same group of participants using ascending 3-AFC methodology, the detection threshold (DT) for methyl anthranilate (MA) in water was 7.51 µg/L, which was significantly lower \([F(1,8)=8.463, p=0.0196]\) compared to the DT of 45.0 µg/L when evaluated in wine.
3.5 Determining Detection Threshold for Methyl Anthranilate versus 2-aminoacetophenone in Water

3.5.1 Results

Data for 43 participants were analyzed (Fig. 3-2) to evaluate the detection threshold of 2-aminoacetophenone and methyl anthranilate in water, resulting in detection thresholds of 1.00 µg/L for 2AAP and 8.10 µg/L for MA.

Figure 3-2: Evaluated by the same group of participants using ascending 3-AFC methodology, the detection threshold (DT) for 2-aminoacetophenone (2AAP) in water was 1.00 µg/L, which was significantly lower [$F(1,8)=35.53, p=0.003$] compared to the DT of 8.10 µg/L found for methyl anthranilate (MA).
3.6 Determining Detection Threshold for 2-aminoacetophenone in Wine

3.6.1 Results

To assess matrix effects on detection threshold, the detection threshold for 2-aminoacetophenone was evaluated in wine to compare to the value previously determined (see Experiment 2) for the compound in water. Data from 38 participants were analyzed (Fig. 3-3) to result in a detection threshold for 2AAP of 10.54 µg/L.

![Experiment 3: DT$_{2AAP}$ in Wine](image)

Figure 3-3: Detection Threshold (DT) for 2-aminoacetophenone (2AAP) in Wine.
3.7 Determining Detection Threshold for Methyl Anthranilate in Water versus Model Wine

3.7.1 Results

Data from 42 participants evaluating the detection threshold for methyl anthranilate in water were analyzed and compared to data from 40 of the same participants who also evaluated the detection threshold for MA in model wine. The shapes of the curves, seen in Figure 3-4, varied between matrices, resulting in detection threshold values of 7.57 µg/L in water and 89.4 µg/L in model wine.

Figure 3-4: Evaluated by the same group of participants using ascending 3-AFC methodology, the detection threshold (DT) for methyl anthranilate (MA) in water was 7.57 µg/L, which was significantly lower \([F(1,8)=8.097, p=0.0216]\) compared to the DT of 89.4 µg/L when evaluated in model wine (MW).
3.8 Determining Detection Threshold for 2-aminoacetophenone in Water versus Model Wine

3.8.1 Results

The detection threshold for 2-aminoacetophenone was also evaluated in water versus model wine with 44 participants who evaluated the samples in water and 43 participants in model wine. The detection threshold for 2AAP in water was 1.17 µg/L and 5.56 µg/L in model wine. The value in water agrees well with the value calculated from Experiment 2, 1.00 µg/L, showing the consistency of the method across different individuals and time.

Figure 3-5: Evaluated by the same group of participants using ascending 3-AFC methodology, the detection threshold (DT) for 2-aminoacetophenone (2AAP) in water was 1.17 µg/L, which was significantly lower [F(1,8)=9.58, p=0.0148] compared to the DT of 5.56 µg/L when evaluated in model wine (MW).
3.9 Results and Discussion

3.9.1 Matrix Effects on Detection Thresholds

Classically, the detection threshold for a chemical is defined as the lowest concentration at which 50% of the population can identify its presence. While matrix effects are widely assumed to influence perception, there is a surprising paucity of work that systematically characterizes the extent to which the matrix affects the estimated detection threshold. Table 3-2 provides the detection thresholds for MA compiled from experiments 1, 2 and 4, which were determined graphically using a chance correction method similar to Lawless (2010). The values calculated using the group BET method outlined in the ASTM E-679 method were generally consistent with the values determined graphically (see Table B-1).

Table 3-2: Detection Thresholds (µg/L) for Methyl Anthranilate. Values for detection threshold (DT) are listed with the 95% confidence intervals (CI) generated by GraphPad Prism software.

<table>
<thead>
<tr>
<th></th>
<th>Water DT</th>
<th>95% CI</th>
<th>Wine DT</th>
<th>95% CI</th>
<th>Model Wine DT</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>7.51 (n=38)</td>
<td>[3.1-17.9]</td>
<td>45.0 (n=36)</td>
<td>[21.2-95.5]</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>8.10 (n=43)</td>
<td>[3.82-17.2]</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>7.57 (n=42)</td>
<td>[2.30-25.0]</td>
<td>–</td>
<td>–</td>
<td>89.4 (n=40)</td>
<td>[28.2-283]</td>
</tr>
</tbody>
</table>

Two results are clear from Table 3-2. First, with appropriate psychophysical methods, the detection thresholds for MA in water are consistent and reproducible across experiments, suggesting the methods used here to determine the detection threshold were robust. Second, the effect of the matrix is clearly demonstrated between water and wine, and water and model wine, as there are statistically significant differences for both comparisons, given that the confidence intervals do not overlap. Finally, we caution that the apparently higher value for model wine
versus the wine should not be over interpreted, as the two confidence intervals for this pair shows substantial overlap.

Table 3-3: Detection Thresholds (µg/L) for 2-aminoacetophenone

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th></th>
<th>Wine</th>
<th></th>
<th>Model Wine</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DT</td>
<td>95% CI</td>
<td>DT</td>
<td>95% CI</td>
<td>DT</td>
<td>95% CI</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>1.00</td>
<td>[0.685-1.47]</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(n=43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 3</td>
<td>–</td>
<td>–</td>
<td>10.5</td>
<td>[3.79-29.3]</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(n=38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 5</td>
<td>1.17</td>
<td>[0.614-2.24]</td>
<td>–</td>
<td>–</td>
<td>5.56</td>
<td>[2.94-10.5]</td>
</tr>
<tr>
<td>(n=44)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(n=43)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-3 summarizes the detection thresholds for 2AAP in water, model wine and wine. Experiment 5, a within-subjects design, showed a statistically significant difference (p < 0.05) in 2AAP detection thresholds in water versus model wine: the detection threshold for 2AAP in water was 1.17 µg/L compared to 5.56 µg/L for model wine. Also, a between-subjects comparison of water and wine detection thresholds for 2AAP (Experiment 2 versus 3) shows a statistically significant difference: 1.00 µg/L versus 10.5 µg/L. Table 3-4 summarizes the detection thresholds for both compounds across all matrices, with the sigmoidal fits for the evaluations shown in Figure 3-6.

Table 3-4: Mean Detection Thresholds (µg/L) Across All Matrices

<table>
<thead>
<tr>
<th></th>
<th>Water*</th>
<th></th>
<th>Wine</th>
<th></th>
<th>Model Wine</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DT</td>
<td>95% CI</td>
<td>DT</td>
<td>95% CI</td>
<td>DT</td>
<td>95% CI</td>
</tr>
<tr>
<td>MA</td>
<td>7.73</td>
<td>–</td>
<td>45.0</td>
<td>[21.2-95.5]</td>
<td>89.4</td>
<td>[28.2-283]</td>
</tr>
<tr>
<td></td>
<td>(n=36)</td>
<td></td>
<td></td>
<td></td>
<td>(n=40)</td>
<td></td>
</tr>
<tr>
<td>2AAP</td>
<td>1.09</td>
<td>–</td>
<td>10.5</td>
<td>[3.79-29.3]</td>
<td>5.56</td>
<td>[2.94-10.5]</td>
</tr>
<tr>
<td></td>
<td>(n=38)</td>
<td></td>
<td></td>
<td></td>
<td>(n=43)</td>
<td></td>
</tr>
</tbody>
</table>

*values reported here were determined by calculating the mean of values generated from individual sigmoidal fits across the appropriate experiments with varying sample sizes
3.9.2 Investigating Reported Differences in Detection Threshold Between Methyl Anthranilate and 2-aminoacetophenone

Extant literature fails to directly compare the detection threshold for MA to that of 2AAP using the same participants. To eliminate the possibility of confounding factors such as individual differences in perception, which is of particular concern for studies using small sample sizes, this study utilized a larger group of participants, based on a power calculation (see Appendix D for calculation) that suggested a minimum sample size of n=22, to evaluate detection thresholds for both MA and 2AAP using a constant method.

The existing literature reports a detection threshold for MA of 300 µg/L (Acree et al., 1977) and 0.5-2 µg/L for 2-aminoacetophenone (Hoenicke et al., 2002a; Fan et al., 2007; Hoenicke et al., 2002b) in various white wines, suggesting a two-log difference in thresholds. Here we used a within-subjects design in Experiment 2 to directly compare the detection threshold for MA versus 2AAP in water. The detection threshold for 2AAP was 1.0 µg/L [95%
CI of 0.7 – 1.5 µg/L] compared to 8.1 µg/L for MA [95% CI of 3.8 – 17.2 µg/L]. Present data suggest the apparent two-log difference reported previously may be an artifact of variation in testing methods and/or small sample sizes.

![Graph of DT<sub>MA</sub> vs. DT<sub>2AAP</sub> in Water](image)

**Figure 3-7:** Evaluated in water, the detection threshold (DT) for methyl anthranilate (MA) of 8.1 µg/L was significantly higher [F(1,8)=35.53, p=0.0003] than the DT value (1.0 µg/L) evaluated for 2-aminoacetophenone (2AAP).

Previous studies looking at detection thresholds for MA and 2AAP have used ranking methods to quantify the threshold values. Published values were based on evaluations from small groups (n < 10) of trained panelists. In contrast, this study used the forced-choice methodology outlined in ASTM method E-679. Notably, the E-679 method differs slightly from both standard 3-AFC tests (which asks participants to identify the strongest or weakest sample) and the triangle test (which asks participants to identify the different sample). Moreover, a standard triangle test would typically be counterbalanced, such that half of participants receive two of sample A and
one of sample B, while the other half of participants receive two of sample B and one of sample A. The ASTM threshold method intentionally blends these two methods: participants always receive 1 spike and 2 blanks (like a 3-AFC test) to minimize exposure to reduce adaptation, but participants are instructed to pick the different sample (like a triangle test), thereby requiring a different cognitive strategy on the part of the participant (differencing versus skimming).

Additionally, the objective of this study was to estimate detection thresholds as they might occur in the general population; therefore untrained panelists (n=35-45) were used to evaluate detection threshold rather than trained panelists. These methodological differences may explain the deviation of our estimates from previously published values regarding the detection threshold for MA in wine. The stability and consistency of our results across multiple studies suggests the present values may be more accurate than previously published values for these compounds.

In this study, we found the detection threshold concentrations for MA and 2AAP to be matrix dependent. Additionally, we found that the differences between MA and 2AAP were not as great as might be expected from prior literature. We speculate this was due to the use of larger numbers of participants here as well as use of a consistent psychophysical method (a forced-choice task) for MA and 2AAP across all matrices. Accordingly, variability that would normally arise as a consequence of innate individual differences across participants, as well as methodological variation between studies, was substantially reduced. Finally, we propose use of sigmoidal fits to model the participant responses using the chance-corrected factor described elsewhere by Lawless (2010) as a more appropriate method that should be used. Finally, given the matrix dependency shown here, this recapitulates the need to use an appropriate delivery system for psychophysical studies on aroma perception, as well as flavor chemistry studies that express concentrations in terms of odor-activity values.
3.10 Study Limitations

A model wine system was included here as an attempt to differentiate perceptual masking effects from physical partitioning effects as a potential mechanism to explain the observation that detection thresholds for both compounds were consistently higher in wine than in water. However, ethanol, a key component of model wine, is also odor active at the concentrations used here (see Leonardo et al. 1969), so it remains unknown whether differences in detection threshold result as an effect of perceptual masking or partitioning phenomena. Given this, it is not possible to directly answer the question of masking versus partitioning using solely psychophysical testing, as we cannot deconfound the odor activity from ethanol from its role in partitioning. That said, we did not find any evidence suggesting that DTs were higher in wine than in model wine, which might be anticipated due to the presence of many additional odor active chemicals in real wine. Tentatively then, this suggests either a) the shift in threshold is due to simple partitioning, or b) that the olfactory sensations from ethanol alone are sufficient to cause this shift (i.e. additional odorants are not required to shift the thresholds for 2AAP and MA upward).

Also, this study focused solely on orthonasal detection thresholds. It is unknown how this work may translate to retronasal delivery of these odorants, as saliva (Ruth et al. 2001), warming and agitation in the mouth may also influence partitioning (see Genovese et al. 2009). Additional considerations would include the role of cross modal mixture suppression from chemesthetic or tactile input (e.g. ethanol burn or viscosity changes).
Chapter 4

Determining Rejection Thresholds for 2-aminoacetophenone and Methyl Anthranilate in Wine Non-Expert Consumers and Wine Experts

4.1 Abstract

Wines made from *Vitis labrusca* grapes contain two key aromatic volatiles that are commonly and widely characterized as ‘grapy’, or “foxy”. These two compounds, methyl anthranilate and 2-aminoacetophenone, while structurally similar, occur naturally in wine at different concentrations. Rarely found in *V. vinifera* wines, they can occur at concentrations well above detection threshold, and impart an undesirable grapey odor to wines vinified from *labrusca* grapes. Wine experts, often with extensive experience in evaluation of *vinifera* wines, may consider these notes to be a fault in wine; conversely, consumers, who may have had greater exposure to *labrusca* wines, may find these aromatics acceptable. To investigate the concentration of the two compounds necessary to elicit rejection, a 2 alternative forced-choice task was utilized to compare a control unoaked Chardonnay against the base wine with added methyl anthranilate or 2-aminoacetophenone in an ascending concentration series. To explore the role of location on rejection, data were collected in both California and Pennsylvania. Consistent with the underlying assumption that wine experts are less accepting of *labrusca* compounds, wines with high concentrations of methyl anthranilate were rejected by wine experts. However, consumers in California rejected these same wines less frequently, while consumers in Pennsylvania never reached a rejection threshold. Strikingly, 2-aminoacetophenone failed to elicit rejection in both California wine experts and consumers. These results suggest that while *vinifera* wines may occasionally have trace amounts of methyl anthranilate and 2-aminoacetophenone,
which are more characteristic of *labrusca* wines, the presence of these compounds at their naturally occurring levels are not enough to reject wine experts or consumers, regardless of their state of residence.

### 4.2 Introduction

An increasingly competitive marketplace is driving researchers to understand the many factors that influence customers’ decisions to purchase and consume products. While many of these variables are more related to marketing aspects, there are intrinsic characteristics of the product itself that influence repeated purchase. Additionally, with an expanding wine industry, it is increasingly difficult for small businesses to break into a saturated market. Therefore, it is critical that when opportunities arise for these small businesses to give their wines more exposure, their wines are well received and not rejected. The Pennsylvania wine industry is rapidly expanding, due, in part, to the revenue generated from wines made from *Vitis labrusca* grapes. As stated previously, however, such wines are not highly regarded or considered in the wine literature written by trained Masters of Wine. Two aromatics in these wines that give them their characteristic “grapy” and “foxy” aromas are methyl anthranilate (MA) and 2-aminoacetophenone (2AAP). While a few prior studies have addressed questions regarding human ability to detect these compounds in wine, there has been little to no work done evaluating the extent to which varying concentrations of these compounds affect wine acceptability.

Multiple studies have investigated differences in product evaluation and liking by experts versus non-expert consumers (e.g. Roberts and Vickers, 1994). Additionally, while experts may report not liking *labrusca* wines, it remains true that these wines are highly prevalent in the tasting rooms of many wineries in Pennsylvania, Ohio, New York, and New Jersey. Therefore, there appears to be a prominent divide between experts and non-expert consumers in preference
for, or against, *labrusca* wines. To evaluate this supposed divide, we evaluated two odor impact compounds from *labrusca* – MA and 2AAP – in an unoaked Chardonnay at concentrations that one would likely encounter if consuming a standard *labrusca* wine, or a *vinifera* wine that had been processed on equipment also used to process *labrusca* grapes (Smith, 2014). Wine experts from California, defined as those who had a formal education in viticulture and/or enology as well as those who worked in the industry and held tastings that had financial consequences for their business, were asked to evaluate six sets of samples in an ascending series using a 2 alternative forced-choice (2AFC) method. Non-expert consumers in California were also asked to do the same. These data were used to determine the rejection threshold of each of the compounds added to the wine in each group. To investigate potential regional differences, the same approach was used in Pennsylvania with non-expert consumers.

Prior work has shown increased exposure to a food product can increase liking (Hartvig et al. 2014), and familiarity with a product can influence situational appropriateness (Giacalone et al. 2015). To determine whether *labrusca* associated aroma compounds would influence consumer rejection of wine, methyl anthranilate was evaluated for its rejection threshold in an unoaked Chardonnay by non-expert consumers in both California and Pennsylvania. A previous pilot study failed to find a rejection threshold for 2-aminoacetophenone among non-expert wine consumers in Pennsylvania (Smith 2014). Pennsylvania consumers have more exposure to *labrusca* wine since it accounts for nearly half of Pennsylvania wine sales, while California consumers do not often have a chance to evaluate these wines, as *labrusca* varieties like Concord and Niagara are rarely sold as single variety wines in California. Though native grapes are available across the entire country in the form of jellies and jams, the results collected across rejection threshold experiments showed that Californians, on average, prefer jellies and jams that were not grape-flavored. Therefore we hypothesized wine consumers in California would have a lower rejection threshold for methyl anthranilate in the white wine compared to wine consumers
in Pennsylvania. To address our secondary aim, both California and Pennsylvania consumers were asked about their consumption habits, and wine interest levels, to estimate familiarity/exposure effects that may carry over from other food products (e.g. grape juice and grape jelly made from concord grapes). These data were used as a segmentation variable for the Pennsylvania consumers, but a parallel analysis was not possible for the California consumers, as very few of them reported consuming grape jelly.

4.3 Materials and Methods

4.3.1 Stimuli

**Odorants.** Stock solutions were made using methyl anthranilate (99.5% purity, FG, Sigma-Aldrich, Milwaukee, WI, USA) and 2-aminoacetophenone (99.3% purity, FG, Sigma-Aldrich, Milwaukee, WI, USA).

**Wine.** Yellowtail unoaked Chardonnay (marketed as ‘tree free’) was purchased in 1.5 L bottles from retail stores near Davis, California and State College, Pennsylvania for the California and Pennsylvania testing sites, respectively. Chemical analysis of the wine, conducted previously by Smith (2014) on previous vintages of the same wine, indicated there was not a measureable level of methyl anthranilate or 2-aminoacetophenone in the wine.

**Sample Preparation.** Six solutions of methyl anthranilate were prepared in Chardonnay; with concentrations of (3.91, 61.3, 154, 387, 972, and 2440) µg/L. An additional six solutions of 2-aminoacetophenone were prepared in Chardonnay, with concentrations of (0.250, 0.630, 1.56, 3.91, 9.77, and 24.4) µg/L. These concentrations were decided based on the levels likely to be found in wines made from both *V. vinifera* and *V. labrusca* grapes (Fan 2007; Kosmerl and Zlatic, 2009; Moio and Etievant, 1995; Nelson, 1977; Panighel, 2010). Solutions were stored in a
refrigerator (~1°C) until the day of testing. Samples were prepared no more than five days in advance of the sensory test. Wines were removed from the refrigerator and allowed to come to room temperature (~20°C) before being presented to participants. As with the experiments for detection threshold evaluation, the concentrations provided above are nominal; solutions were not quantitatively analyzed before samples were delivered to participants. The sample preparation and storage protocol was strictly followed on both college campuses, however, and therefore the relative concentrations received by participants across experiments should be stable.

4.3.2 Data Collection and Analysis

Data collection from California consumers and experts were performed at the University of California-Davis, in collaboration with Professor Hildegarde Heumann and Dr. Nadia Byrnes in the Department of Viticulture & Enology. These sensory tests were conducted in the Robert Mondavi Institute for Wine and Food Science (RMI) sensory facility on the UC-Davis campus. Data from Pennsylvania consumers was conducted in the Sensory Evaluation Center at Penn State (SEC), located in the Rodney E. Erickson Food Science Building on the main Penn State campus, in University Park, PA.

Testing Facility and Evaluation – California. For determination of rejection thresholds in the California participants, individuals received 30 mL samples served in ISO type black wine tasting glasses (ISO 3591:1977), each labeled with a three digit blinding code and capped with a watch glass to preserve the headspace equilibrium. Panelists were provided with drinking water for rinsing and unsalted crackers for consumption between samples to prevent sample carryover. Sessions took place at the RMI sensory facility, equipped with individual sensory booths lit with daylight lamps. Responses were collected using a computerized system stationed in each booth (FIZZ, Biosystems, Counternon, France).
Testing Facility and Evaluation – Pennsylvania. Pennsylvania consumers received 30 mL samples served in clear ISO wine-tasting glasses (ISO 3591:1977), each covered with a tightly fitting paper cap labeled with a three digit blinding code. Panelists were provided with drinking water and instructed to rinse their mouth between samples to prevent sample carryover. Sessions took place in the Sensory Evaluation Center at Penn State, equipped with individual testing booths, illuminated by daylight lamps, with digital data collection stations. Responses were collected using a computerized system stationed in each booth (Compusense Cloud, Guelph, Ont).

Screening Criteria for California Participants. For each experiment, up to 48 participants were recruited. California participants were recruited from an existing pool of interested individuals who had previously opted-in to be contacted about experiments in the facility. Additional recruiting was also done by word-of-mouth to local residents, staff and visitors to the University of California (Davis, CA) attending wine-focused events hosted by the Department of Viticulture and Enology; this second method provided most of the wine experts and professionals who participated. Participants were asked to describe their interest in wine via a multiple-choice questionnaire. Those who endorsed responses indicating they were wine experts or wine professionals (i.e. ‘I know a great deal about wine. I have formal training and/or education.’ and ‘When I drink/taste wine, there is a financial consequence of the tasting.’) were considered ‘experts’ for our purposes here, and their data was analyzed separately from wine ‘consumers’, most of whom indicated their interest in wine as “casual enjoyment” (“I enjoy consuming wine, but do not know much about wine”), “novice/beginner” (I have a basic understanding of wine), or “wine enthusiast” (I know much more than the basics and am interested in learning more about wine; however, there is still much for me to learn”). Following screening, scheduling was handled using software from SONA systems (Tallinn, Estonia). All participants provided written consent and proof of age before testing. All procedures were
conducted with approval of the University of California, Davis Institutional Review Board (#729234-1).

**Recruitment of Wine Consumers in Pennsylvania.** Participants were recruited from an existing pool of interested individuals who had previously opted-in to be contacted about taste and smell experiments in the Penn State SEC. Following screening, scheduling was handled using software from SONA systems (Tallinn, Estonia). Healthy, non-smoking individuals with no known taste and smell defects who consumed white wine at least once a month were invited to participate in the study. Individuals under the age of 21, as well as currently enrolled undergraduate students, regardless of age, were excluded from the study. The screening also asked individuals to indicate their consumption of real and fictitious wine varietals: participants who indicated they drank the fictitious varieties (“Seville Vioge” and “Petite Chableau”) were excluded from the study. Individuals who consumed wine made from *labrusca* grapes once a month or more frequently were also excluded from the study. To ensure we had only motivated, non-expert wine consumers, those who indicated they had no interest in wine and those who indicated they were wine experts were not invited to participate in the study. Before testing, participants gave written consent and proof of age. All procedures were done with the approval of The Pennsylvania State University Institutional Review Board (#37365).

**Sensory Methodology.** An ascending concentration series using a 2 alternative forced choice (2AFC) task was used to estimate rejections thresholds. Participants were presented with six sets, containing two samples each, and asked to evaluate them. Each set contained one sample of the control (untreated sample of wine) and one sample of the same wine spiked with either 2AAP or MA. The sets were presented so that the spiked sample increased in concentration, with the participant receiving the lowest concentration for their first set of samples. Within each set, the order of samples was counterbalanced across individuals.
All glasses were prepared one hour before serving to allow for temperature and headspace equilibration. The samples were presented and evaluated in individual booths at room temperature (21°C) under white lighting. For each sample, participants were asked to taste the sample, swirl it around their mouth, and expectorate. Within the pair, they were instructed to indicate which of the two samples they preferred. Between each set of samples, subjects waited 90 seconds before moving on to the next pair; this break between sets was enforced via software.

**Data Analysis.** Data were exported from FIZZ and Compusense into Microsoft Excel. For each concentration, the number of participants who selected the control sample was counted. The rejection proportion for a pair was defined as the number of participants who preferred the control sample divided by the total number of participants. We estimated thresholds across the concentration series in two ways, using both the linear regression function and the four-parameter logistic nonlinear regression function in GraphPad Prism 5.0C for OSX (GraphPad Software, San Diego CA). Again, the four-parameter logistic regression equation (Eq.3) describes the top of the curve (max), the bottom of the curve (min), the spot halfway between min and max (classically EC$_{50}$; called RjT$_{50}$ here), and the slope of the curve (the Hill coefficient).

**Equation 3:** Adapted Hill Equation. RjT$_{50}$ has been substituted for the classic EC$_{50}$.

\[
Y = Min + \left[ \frac{(Max - Min)}{1 + 10^{(LogRjT_{50} - X) \times HillSlope}} \right]
\]

Here, rejection threshold was defined as the concentration at which the resulting performance was 50% greater than chance (see Lawless, 2010). That is, because the chance of choosing randomly in a 2AFC preference test is 1/2, and perfect performance is 1 (100% preference for one sample), a chance-adjusted proportion of 0.75 was used as the threshold criterion; the concentration corresponding to this proportion was calculated directly in software.

Given four free parameters in the regression equation, we faced a choice as whether the lower and upper bounds of the nonlinear function should be constrained to 0.5 and 1, respectively.
(see Harwood et al. 2012). Because the observed data here never reached complete rejection (1.0), we decided to leave the min and max parameters free, with one exception: the methyl anthranilate data for the California experts was fit with a constrained equation, where min and max were fixed to 0.5 and 1. The California data were also fit using linear regression to compare to the Pennsylvania data, which were fit using linear regression due to the clustering of the data around chance performance (0.5).

An additional post-hoc analysis of rejection threshold values was performed on the data, excluding participants that failed to reject the highest concentration in the series, presented in the last set of samples. The non-linear regression, modeled after the Hill equation, was then used to fit the remaining data with the bottom limit constrained to 0.5 and the top constraint set to 1.0.

4.4 Determining the Rejection Threshold for Methyl Anthranilate in California Wine Consumers versus Wine Experts

4.4.1 Results and Discussion

A total of 37 wine experts and 48 wine consumers participated in the study to estimate a rejection threshold for methyl anthranilate in an unoaked Chardonnay. Both groups failed to reach total rejection (proportion of 1.0), but both met the criterion for rejection (proportion = 0.75) to varying degrees.

Table 4-1: Rejection Thresholds (µg/L) in Wine for Methyl Anthranilate among California Wine Experts and Consumers.

<table>
<thead>
<tr>
<th></th>
<th>Experts</th>
<th>Consumers</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-parameter regression fit</td>
<td>5.20</td>
<td>-</td>
</tr>
<tr>
<td>*2-parameter regression fit</td>
<td>123</td>
<td>-</td>
</tr>
<tr>
<td>Linear regression</td>
<td>130</td>
<td>1700</td>
</tr>
</tbody>
</table>

*Hill equation with the bottom and top values constrained to 0.5 and 1.0, respectively.
In this 2-AFC task, participants were forced to select the sample that they prefer. This means that there was a 50:50 chance that the participant would select a sample at random. A rejection threshold is defined as the concentration where 50% of participants select the control sample (and therefore “reject” the other sample), so to adjust for random chance in the observed data, 75% of participants must choose the control. While both the experts and the consumers did have at least one sample set with 75% rejection, no pair elicited complete rejection (i.e. 100% of the participants preferring the control sample). This complicated our analysis, as reducing the number of free parameters by constraining the min and max values to 0.5 and 1 led to interrupted sequences or low goodness-of-fit ($R^2$) values; thus, these analyses are not shown in Figure 4-1 or reported for the bulk of the data. Conversely, the four-parameter (unconstrained) regression models for the consumer and expert data had acceptable $R^2$ values of 0.9233 and 0.8826, respectively. Finally, given the shape of the functions shown in Figure 4-1, we also used linear regression to model these data, resulting in $R^2$ values of 0.8452 and 0.4610 for consumers and expert data, respectively.
Though the work presented here looks at the odor of methyl anthranilate to the extent that it is undesirable in wine, there is a distinct possibility that it can add fruity notes to the aroma that are desirable to a portion of the population. Therefore, data from participants who failed to reject the sample with the highest concentration (2440 µg/L) of MA were excluded from the data set that was analyzed a second time using the Hill equation (see Fig. 4-2), with the bottom constraint set to 0.5 and top constraint confined to 1.0. Of the wine experts, 76% rejected MA in the last set, resulting in a rejection threshold of 15.4 µg/L. Within the wine consumers, 73% rejected MA at its highest concentration, resulting in a rejection threshold of 119 µg/L.

Figure 4-1: Rejection threshold for methyl anthranilate in unoaked Chardonnay among wine experts and consumers in California. The 4-parameter nonlinear regression (“uncon”) fit to the data is shown, along with the linear regression fit to model rejection threshold data.
Participants were also asked to self-report their interest level in wine. Those who selected the options of “I enjoy consuming wine, but I do not know much about wine” or “beginner - I have a basic understanding of wine” were sorted into a Low Interest group. A second High Interest group was formed by those who selected “wine enthusiast - I know more than the basics and I am interested in learning more; however, there is still much to be learned” or “knowledgeable in wine-I know a good deal about wine; however, I have yet to reach an expert level” (“high” wine interest group).

Figure 4-3 suggests that the rejection function for non-expert consumers with a high interest level for wine may approach the value from the wine experts. Above the range for detection threshold, indicated in the figure, rejection data of methyl anthranilate among experts and high interest level consumers begin to converge.
The linear regression for the experts, high interest and low interest consumers are shown in Figure 4-4. While it is intuitive that low interest consumers may have an elevated rejection threshold compared to their high interest counterparts, and the data would seem to support this, the relatively low sample size (n=17) after segmentation means these data should be interpreted cautiously. Additional studies screening for consumers with varying wine interest levels would provide more information about this apparent split in rejection threshold between consumers with high wine interest levels and low interest levels.
4.4.2 Conclusion

While the expert group reached the rejection threshold at a lower concentration of 130 µg/L, compared to the consumers at 1700 µg/L, according to the linear regression models, both groups remained around a 75% rejection response once reaching threshold, failing to ever reach complete rejection of wine with concentrations of methyl anthranilate that an individual would ever potentially encounter in any kind of *vinifera or labrusca* wine. Collectively, these data suggest two interesting findings. First, in a blind tasting, 1 in 5 individuals does not appear to find the aroma of MA offensive, regardless of expertise. Second, among Californians, experts apparently begin to reject MA at lower concentrations than consumers, and high interest consumers may be more similar to the experts.

Figure 4-4: Rejection threshold for methyl anthranilate among California wine experts, and consumers divided into “high interest “ and “low interest” in wine.
4.5 Determining the Rejection Threshold for 2-aminoacetophenone in California Wine Consumers versus Wine Experts

4.5.1 Results and Discussion

There were 24 experts and 44 consumers who evaluated spiked wines to estimate rejection threshold for 2-aminoacetophenone in unoaked Chardonnay. Because 2AAP is typically found in lower concentrations in wine compared to MA, lower concentrations of 2AAP were used to maintain ecological validity. However, we would still expect to see significant effects of the added 2AAP, as the detection threshold for 2AAP is lower than that for MA (see chapter 3).

Contrary to expectations, neither group rejected the wine with added 2-aminoacetophenone. While the 4-parameter nonlinear regression resulted in higher $R^2$ values for fitting the consumer and expert data (0.7823 and 0.7551, respectively), these models plateau at 0.6 for consumers and 0.67 for experts (see Fig. 4-5), suggesting that the groups would never reach the established rejection criterion of 0.75. Alternatively, the linear regression analyses offer lower $R^2$ values (experts = 0.3796; consumers = 0.5430) but suggest that experts would reject wine containing 103 µg/L of 2AAP, compared to 240 µg/L for consumers. Considering that dose response curves are rarely linear in these situations, however, the former nonlinear regression analysis seems to be a more appropriate model. In fact, some participants spontaneously commented that in some trials, the wine appeared to smell “fruitier”, potentially offering a possible explanation for the slight preference for the sample with 0.630 µg/L of added 2AAP.

<table>
<thead>
<tr>
<th></th>
<th>Experts</th>
<th>Consumers</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-parameter regression fit</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>*2-parameter regression fit</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linear regression</td>
<td>103</td>
<td>240</td>
</tr>
</tbody>
</table>

*Hill equation with the bottom and top values constrained to 0.5 and 1.0, respectively.
This can be seen as the second data point in Figure 4-5, where the proportion for both groups appears shifted away from chance in preference to the sample wine with added 2AAP.

Untypical aging (UTA) is a known defect for old Rieslings, but the extent to which 2AAP contributes to the rejection of effected wines is not yet fully understood. Henick-Kling et al. (2008) suggested that rejection of effected wines, especially in the Finger Lakes wine-producing region in New York, might be more influenced by the perception and rejection of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN). It is therefore possible that a portion of the population does not mind low, detectable levels of 2AAP in wine. Only 58% of wine experts and 61% of consumers rejected the highest concentration of 2AAP added to the wine (24.4 µg/L). When these subsets of the data were analyzed, however, the data resembled typical rejection curves (see Fig. 4-6), with experts rejecting the compound at 2.27 µg/L and consumers rejecting 2AAP at 8.39 µg/L.

![Figure 4-5: Rejection threshold for 2-aminoacetophenone in wine among California wine experts and consumers. The 4-parameter nonlinear regression (“uncon”) fit to the data is shown along with the linear regression fit to model rejection threshold data.](image-url)
4.5.2 Conclusion

We failed to find a rejection threshold for 2-aminoacetophenone in unoaked Chardonnay, as evaluated by wine consumers and wine experts within the state of California. While linear regression analyses do provide an estimate of rejection thresholds, it is unknown how the shape of the dose-response curve may change at higher concentrations. Because these fits require extrapolating well beyond the concentration range tested in this study, we cannot state with certainty that rejection thresholds would be reached by consumers at 240 µg/L and by experts at 103 µg/L. Furthermore, the nonlinear regression analyses suggest that the response curves may flatten at higher concentrations and never reach minimum criterion for rejection (75%).
4.6 Determining the Rejection Threshold for Methyl Anthranilate in Pennsylvania Wine Consumers

4.6.1 Results and Discussion

A total of 47 individuals in Pennsylvania participated in a rejection threshold study to estimate the rejection threshold for methyl anthranilate in an unoaked Chardonnay. Group response proportions hovered around performance due to chance (P=0.5), failing to show any trend toward rejection of the added compound (see Fig. 4-7).

![Rejection threshold data for methyl anthranilate in unoaked chardonnay among Pennsylvania consumers.](image)

Figure 4-7: Rejection threshold data for methyl anthranilate in unoaked chardonnay among Pennsylvania consumers.

In addition to evaluating the wine samples, participants were also asked about their food preferences, wine interest level, as well as their knowledge of *vinifera* varieties. Participants were asked what type of jelly they preferred on toast and what type they preferred on a peanut butter
and jelly sandwich. Those participants who indicated grape for one or both of these questions were separated from those who indicated a non-grape flavor (e.g. strawberry, blueberry), and the rejection data were re-analyzed for each group. The results in Figure 4-8 suggest that there may be possible segmentation for rejection of methyl anthranilate in wine based on grape jelly liking/familiarity. While suggestive and intuitive, these data should be interpreted very cautiously, as the 95% confidence intervals for the slopes of the two regression models overlap, with a CI for grape jelly consumers of [-0.1298 to 0.06382] and [-0.05595 to 0.1231] for non-grape jelly consumers.

![RJT<sub>50</sub> for MA in PA Consumers](image)

Figure 4-8: Rejection threshold data for methyl anthranilate in unoaked chardonnay among Pennsylvania consumers, divided by jelly flavor preference.

The results achieved by segmenting the data based on jelly preference support the notion that methyl anthranilate may not be an undesirable odor to some Pennsylvania consumers and may, in fact, actually be an odor and taste that some wine consumers enjoy. When data from
participants who failed to reject the highest concentration (2440 µg/L) of MA in wine were removed from the data set, the rejection threshold evaluated by 53% of consumers rejecting MA resulted in a threshold value of 1160 µg/L (see Fig. 4-9).

Figure 4-9: Rejection threshold for methyl anthranilate in unoaked Chardonnay among wine consumers in Pennsylvania, excluding participants who failed to reject the sample with the highest concentration (2440 µg/L) of MA, compared to the detection threshold (DT).

Additionally, Pennsylvania consumers were also tested for their knowledge of *vinifera* varieties. Participants were provided with a list of 14 varieties and asked to check all that belonged to *Vitis vinifera*. Of the 14 varieties listed, 10 were *vinifera*, 2 were *labrusca*, and 2 were fictitious varieties. “Knowledge index” values were calculated by subtracting the number of incorrectly checked varieties from the number of correctly selected varieties. For example, if a participant correctly selected 4 *vinifera* varieties but also selected 1 *labrusca* variety, their knowledge index value was equal to 3. Participant data were then sorted into low and high
knowledge groups, based on a median split. With the exception of one outlier, there did not appear to be a significant split between the two groups segmented by *vinifera* knowledge, shown in Figure 4-10.

Participants also indicated their interest level in wine, with the same options available as provided in the California study. Two groups ("low" and "high" interest) were formed, as before. Unlike California where the majority (65%) of consumers tested at UC-Davis were categorized by high wine interest levels, only 23% of consumers tested at Penn State reported being highly interested in wine. Similar to the pattern seen in the California dataset, Figure 4-10 suggests that there may be a relationship between wine interest level and rejection of methyl anthranilate in wine. Unfortunately, due to small sample sizes after sorting the participants in the data set, the sample size is too small to generalize to the rest of the population in Pennsylvania.

Figure 4-10: Rejection threshold data for methyl anthranilate in unoaked chardonnay among Pennsylvania consumers, divided by *vinifera* knowledge (left) and self reported wine interest (right).
Comparing the rejection threshold data collected from California wine experts and consumers to that collected from Pennsylvania wine consumers (Fig. 4-11), there does appear to be some effect of location. Although consumers in California and Pennsylvania started at similar rejection proportions at the lowest concentration, the slopes of the two regression lines are statistically significantly different ($p < 0.05$), as the slope of CA consumers is much steeper ($m = 0.1102 ± 0.02358$) compared to PA consumers ($m = 0.01085 ± 0.03032$). The slopes of the regressions lines that fit the data of the California wine experts and the Pennsylvania consumers are more similar, but the group of CA wine experts start at a much higher rejection of the methyl anthranilate (proportion = 0.622) than the PA consumers (proportion = 0.447). Stated differently, the elevations of the lines for the CA experts and PA consumers are significantly different ($p < 0.01$)

Figure 4-11: Rejection threshold data for methyl anthranilate in unoaked chardonnay among California wine experts, California wine consumers, and Pennsylvania wine consumers relative to the detection threshold (DT) for MA, indicated by the shaded region, previously determined.
4.6.2 Conclusions

Consumers in Pennsylvania did not appear to favor the control (untreated) wine over the wine with added methyl anthranilate, even at higher concentrations. The highest concentration presented, 2.44 mg/L, which would never be representative of a *vinifera* wine, but could be found in a *labrusca* wine, was preferred by 46.8% of the participants and rejected by 53.2%. In a forced choice task, a participant that did not have a preference would still have to choose one of the two samples, so random chance would dictate that 50% would favor one and 50% would prefer the other. This suggests that Pennsylvania consumers simply do not reject *labrusca* odors, like methyl anthranilate, in wine.

This was in stark contrast to California non-expert consumers and wine experts, both groups of which demonstrated more rejection of methyl anthranilate in wine, although they still failed to reach complete rejection. Similar failures have even been seen for actual wine faults, such as cork taint (Prescott et al. 2005). While the methyl anthranilate rejection threshold for wine experts in California was estimated to be between 5.2 and 130 µg/L, the rejection threshold among wine consumers in California was estimated to be 1700 µg/L. However, this estimate, along with those determined for 2-aminoacetophenone in California experts (RjT<sub>50</sub> = 103 µg/L) and consumers (RjT<sub>50</sub> = 240 µg/L) are outside the concentration ranges tested in the experiments conducted in this study. It therefore is not clear if these extrapolations are accurate since the shape of the dose-response curve may change at higher concentrations and not follow the linear regression used to estimate the rejections thresholds in groups that failed to reach total rejection. 

Within the datasets for both CA and PA consumers, we observed evidence for an influential effect of wine interest on rejection thresholds for the two *labrusca* compounds. Additionally, within PA consumers, there was evidence of an inverse relationship between grape jelly consumption and rejection of methyl anthranilate. Additional work would be necessary to
clarify the effects and generalizability to the population at large. The work presented here assumed that the samples drawn from the Davis and State College populations were representative of California and Pennsylvania populations, respectively. As both towns attract demographically diverse individuals, we were not able to control for participants’ native regions. Even so, the segmented data suggest that liking or familiarity with other grape products might predispose consumers toward liking *labrusca* associated odors.
Chapter 5

Conclusions, Limitations and Future Studies

The two compounds used in this study were 2-aminacetophenone and methyl anthranilate, both of which are known to be character impact compounds for wines made from Vitis labrusca grapes. This study emphasized the importance of matrix consideration in detection threshold studies, as olfactory perception is presumably highly dependent on the concentration of aromatics available in the headspace being sampled. Detection thresholds for methyl anthranilate and 2-aminoacetophenone, determined orthonasally, were evaluated in three matrices: water, model wine, and white wine. As expected, the detection thresholds calculated in model wine and wine were higher than that in water for both of the volatile aroma molecules.

The threshold values found in this work are particularly relevant to the Pennsylvania wine industry, which produces wine made from both Vitis vinifera and Vitis labrusca grapes, as well as blends of the two. Characterization of hybrids made from labrusca varieties was not a focus of this work, but investigation of the levels of MA and 2AAP in hybrids of labrusca varieties would be an area to explore, as the concentration would be expected to decrease with an increasing deviation from labrusca parentage. Contamination of winery equipment used to process both species has been reported (Smith 2014). While Smith failed to find measurable amounts in 86% of the 35 V. vinifera wines collected across the commonwealth of PA, 5 of the wines had measurable quantities of MA, ranging from 0.03-76.1 µg/L, while only 1 of the wines had detectable levels of 2AAP, 2.28 µg/L (Smith et al. 2015). The results from the threshold experiments presented here suggest that the highest concentrations measured in these wines are detectable, but will not cause consumers in Pennsylvania to reject the wines.
Knowing the approximate concentrations of each aromatic compound necessary to elicit detection in a white wine, the objective that remained was the determination of the concentration (above detection threshold) that would cause samples to be rejected by participants. Differences in assessment of a food product by experts versus non-expert consumers are well documented for a variety of food matrices and beverages. To address these differences here, rejection thresholds for methyl anthranilate and 2-aminoacetophenone were separately determined by wine experts and non-expert wine consumers in California. Data were also collected from non-experts in both California and Pennsylvania to compare rejection of methyl anthranilate between participants who had little exposure to labrusca wines and Concord grape products (i.e. California), and those who had significant exposure to labrusca wines and Concord grape products (i.e. Pennsylvania). There was a difference in rejection thresholds for MA and 2AAP as assessed by experts versus consumers when judging wines with added methyl anthranilate; however, both groups failed to reject the wines with added 2-aminoacetophenone. While California wine experts did reach the minimum criterion for rejection threshold of methyl anthranilate, evaluations of the wines by Pennsylvania consumers fluctuated, thus demonstrating the predicted effects of region and exposure on rejection of compounds typically found in labrusca wines.

The negative correlation found between liking/familiarity of Concord grape products and rejection of MA lends itself to additional testing in populations that have not been exposed to labrusca grape products. Vineyards in a hot, arid climate like those in Australia, preferentially grow vinifera varieties (e.g. Shiraz, Cabernet Sauvignon) over labrusca varieties (e.g. Concord), therefore it is very likely that these grapes would be a novel item to Australian natives. Thus, to evaluate the possible correlation between familiarity and rejection threshold, evaluation of MA-spiked wines by Australian consumers would seem to be a natural extension of this work.
The consumer survey data described here indicated that, of the segment of the population polled, 47% of residents of Pennsylvania, New York, and New Jersey consume *labrusca* wines regularly. However, these data also suggest there is a dichotomy in situational appropriateness for consumption of *labrusca* and *vinifera* wines. While many native variety consumers reported drinking *labrusca* wines more frequently than *vinifera* wines, there remains a reported increase from everyday consumption of *vinifera* wines to consumption for entertaining or celebration purposes. This contrasts with a decrease in consumers who reported consuming *labrusca* when entertaining and/or celebrating a special occasion versus everyday drinking. This suggests that even among consumers who enjoy *labrusca* varieties, there is still an underlying belief that *vinifera* wines are somehow more appropriate for “special” occasions. This attitude was reinforced by data collected from wine experts, the majority of which reported rarely consuming *labrusca* wines and rarely gifting *labrusca* wines to their wine-enthusiast friends. There was some evidence in the rejection threshold data that consumers with high interest levels in wine were less accepting of higher concentrations of methyl anthranilate in their wine, suggesting some correlation between wine interest and rejection of *labrusca* associated odorants.

The solutions used to investigate evaluation of MA and 2AAP were simplified systems that may have underrepresented wine’s true composition. While similar work (Fontoin 2008) investigating odor thresholds for wine aromas have created model wine solutions using strictly ethanol, water, and tartaric acid; such a simplified approach may be an oversight for the compounds studied here as the derivatives of anthranilic acid may be affected by wine components such as glycerol, phenolics, and SO₂. Therefore, there are several additional studies that could be performed to make the data more representative and translatable to more complex wine systems. Using 4-methylcatechol as the system’s primary phenolic compound, the effects of pi stacking between the ring structures of 4-methylcatechol and MA, 2AAP could be investigated,
as well as the extent to which the pi stacking influences partitioning of the volatiles. SO₂, often present in wine, binds carbonyls, while glycerol can affect hydrophobic interactions, thus these compounds can also influence the partition coefficient of both MA and 2AAP.

The work described here evaluated only two of many known *labrusca* odorants. There are many other compounds, especially esters, known to be present in relatively high concentrations in volatiles of Concord grapes, such as ethyl acetate, ethyl crotonate, and ethyl anthranilate (Stern 1967). The aromas that these compounds contribute to *labrusca* wine were not investigated in this work, nor were possible synergistic effects between ethyl anthranilate, which has a similar odor as its methyl ester, and MA and 2AAP. The evaluation of MA and 2AAP, individually, in separate studies was useful for evaluating wine systems that would only have the presence of one of the compounds: for example, Rieslings with UTA problems would only need to consider 2AAP. Nonetheless, while it is unlikely that both MA and 2AAP would be found in measurable quantities in a *vinifera* wine, their interaction in a wine matrix was not studied here. Potential synergistic effects of MA and 2AAP at sub-threshold and barely detectable concentrations, shown by Atanasova (2004) and Berre et al. (2007) for woody and fruity aromatic volatiles, may exist in a *labrusca* wine. Berre et al. (2007), along with Moio et al. (1993) also showed that at concentrations well above threshold, fruity odors tended to decrease as the concentration of woody volatiles increased, therefore suggesting masking effects of the two volatiles at higher concentrations.

We observed differences for rejection thresholds between expert and consumer evaluators that assessed wines with added methyl anthranilate. Importantly, these rejection thresholds were based on *preference*, as participants were instructed to indicate which sample they *preferred*, as in a paired preference test. By extension, rejection thresholds could also be based on typicity, or how much a wine is representative of its grape variety. We would expect that experts, who are
more knowledgeable of variety nuances, to have a lower tolerance for wines that deviate from their standard descriptors due to added methyl anthranilate, compared to consumers, who have less experience with individual grape varieties and thus less of an established and remembered reference to which to compare the samples. Consistent with Port (2003), regional typicity may also be an influential variable to explain preference and rejection for methyl anthranilate. Port (2003) observed that some winemakers prefer their wines to be absolutely void of eucalyptol while others accepted it at moderate levels, considering it to be representative of regional character. Results from the work done by Saliba et al. (2009) reflect that which Port observed, with some consumers preferring moderate intensities of eucalyptol in wine compared to no eucalyptol. Perhaps, then, like eucalyptol, methyl anthranilate should not always be considered a wine taint as the rejection of MA data collected from Pennsylvania consumers illustrates that some consumers preferred the wines with moderate intensities of MA.

The detection threshold experiments relied solely on orthonasal olfaction for determination of the threshold values for methyl anthranilate and 2-aminoacetophenone in various matrices. Some prior work suggests there may be a difference in threshold values for orthonasal versus retronasal evaluation of wine (e.g. Pickering et al. 2007). Such speculated differences between orthonasal and retronasal evaluation may be able to explain how it is that the rejection threshold for MA that was observed for California experts falls below the detection threshold value for MA observed by Pennsylvania consumers. It is therefore uncertain how our simplified evaluation may translate to detection in practical applications for wine consumption. This is particularly relevant considering retronasal olfaction is critical for the perception of food and beverages when they are chewed or sipped before being swallowed.

While rejection thresholds were evaluated in the mouth, additional work is needed to investigate flavor release in the mouth, particularly for that of experts versus consumers, who
may taste wine very differently. Temperature in the oral cavity, as well as saliva, can influence volatile release within beverage matrices (Roberts & Acree 1995). Wine educator, Kevin Zraly, writes “When I taste wine I leave it in my mouth for three to five seconds before swallowing. The wine warms up, sending signals about the bouquet and aroma up through the nasal passage then on to the olfactory bulb…” (Zraly, pg. 20). Émile Peynaud, French oenologist and researcher, remarks that “there is a big difference between drinking and tasting. Good wines and great wines are not drinks which are simply swallowed; one savors them” (pg. 3). Wine experts, then, that hold wine in the oral cavity for a longer period of time may cause the wine to have a different physical and chemical presence in the headspace of the oral cavity as compared to wine sampled by consumers that may have a shorter oral residence time, which does not allow the wine to equilibrate in the oral cavity. This hypothesized difference in sensory experiences between experts and non-expert consumers could be tested by determining the concentration of volatile aromatics in a simulated oral cavity setup, as well as accompanying sensory data of threshold values among individuals instructed to sample the wines following two specified procedures imitating those that are likely to be performed by consumers versus wine judges. Knowing how tasting methodology affects the physical composition of the headspace in the oral cavity, and how that translates to potential differences in perception (as estimated by threshold values) would be a novel study that would increase our understanding of how wine is perceived among individuals with different levels of wine involvement.
References


Appendix A

Supplemental Survey Data

The data collected from wine professionals is provided in response to the questions asked in the survey. There were 61 respondents, but only data collected from individuals who identified as “wine experts” are provided beyond Table A-3 for n = 58. Self-taught wine experts were kept in the data set since each individual had an influential role in the sales of their business.

Table A-1: Demographic: Gender. “I identify my gender as:”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>72.1%</td>
<td>44</td>
</tr>
<tr>
<td>Female</td>
<td>27.9%</td>
<td>17</td>
</tr>
<tr>
<td>Other</td>
<td>0.0%</td>
<td>0</td>
</tr>
</tbody>
</table>

answered question 61

Table A-2: Demographic: Age. “Please indicate your age.”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 21</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>21-24</td>
<td>1.6%</td>
<td>1</td>
</tr>
<tr>
<td>25-34</td>
<td>21.3%</td>
<td>13</td>
</tr>
<tr>
<td>35-44</td>
<td>23.0%</td>
<td>14</td>
</tr>
<tr>
<td>45-54</td>
<td>11.5%</td>
<td>7</td>
</tr>
<tr>
<td>55-64</td>
<td>27.9%</td>
<td>17</td>
</tr>
<tr>
<td>65+</td>
<td>14.8%</td>
<td>9</td>
</tr>
</tbody>
</table>

answered question 61
Table A-3: Wine Knowledge. “How would you classify your wine knowledge?”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>I am a formally advocated wine expert; I have had formal training and/or hold a degree in viticulture and/or enology.</td>
<td>33.3%</td>
<td>20</td>
</tr>
<tr>
<td>I am a self-taught wine expert; I have not had formal training but I have been in the business long enough to know wine, and make financial decisions based on this knowledge.</td>
<td>36.7%</td>
<td>22</td>
</tr>
<tr>
<td>I am a self-taught wine enthusiast; I have not had formal training but I enjoy reading and learning about wine.</td>
<td>25.0%</td>
<td>15</td>
</tr>
<tr>
<td>*I don't know a lot about wine, but I enjoy trying new wines, and hearing about the differences between wine.</td>
<td>5.0%*</td>
<td>3*</td>
</tr>
<tr>
<td>I enjoy drinking wine, but I am not particularly curious about it.</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>I know nothing about wine.</td>
<td>0.0%</td>
<td>0</td>
</tr>
</tbody>
</table>

answered question 60

*The three participants who answered that they did not know a lot about wine were eliminated from subsequent data reported (n = 58).

Table A-4: Industry Involvement CATA. “How are you involved in the wine industry?”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>I am a professional winemaker.</td>
<td>53.6%</td>
<td>30</td>
</tr>
<tr>
<td>I am a vineyard manager.</td>
<td>25.0%</td>
<td>14</td>
</tr>
<tr>
<td>I am a tasting room manager or assistant.</td>
<td>16.1%</td>
<td>9</td>
</tr>
<tr>
<td>I am a cellar assistant.</td>
<td>3.6%</td>
<td>2</td>
</tr>
<tr>
<td>I am in sales.</td>
<td>14.3%</td>
<td>8</td>
</tr>
<tr>
<td>I am a winery owner.</td>
<td>37.5%</td>
<td>21</td>
</tr>
<tr>
<td>I am not involved in the wine industry on a profit basis, but I am a home</td>
<td>10.7%</td>
<td>6</td>
</tr>
<tr>
<td>I am a wine writer/journalist.</td>
<td>3.6%</td>
<td>2</td>
</tr>
<tr>
<td>I am a wine judge.</td>
<td>10.7%</td>
<td>6</td>
</tr>
<tr>
<td>I am a wine educator.</td>
<td>10.7%</td>
<td>6</td>
</tr>
<tr>
<td>Other (please specify)</td>
<td>14.3%</td>
<td>8</td>
</tr>
</tbody>
</table>

answered question 56
Table A-5:  V. vinifera Knowledge. “Please select which wines are made from V. vinifera grapes. (Note: if you are unsure, please do NOT consult an external reference to identify which varietals are vinifera.)”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albariño</td>
<td>71.9%</td>
<td>41</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>96.5%</td>
<td>55</td>
</tr>
<tr>
<td>Chardonnay</td>
<td>98.2%</td>
<td>56</td>
</tr>
<tr>
<td>Concord</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>Gewürztraminer</td>
<td>96.5%</td>
<td>55</td>
</tr>
<tr>
<td>Muller-Thurgau</td>
<td>49.1%</td>
<td>28</td>
</tr>
<tr>
<td>Petit Chableau</td>
<td>14.0%</td>
<td>8</td>
</tr>
<tr>
<td>Pinot Gris</td>
<td>94.7%</td>
<td>54</td>
</tr>
<tr>
<td>Riesling</td>
<td>96.5%</td>
<td>55</td>
</tr>
<tr>
<td>Rkatsiteli</td>
<td>42.1%</td>
<td>24</td>
</tr>
<tr>
<td>Seyval Blanc</td>
<td>8.8%</td>
<td>5</td>
</tr>
<tr>
<td>Seville Vioge</td>
<td>1.8%</td>
<td>1</td>
</tr>
<tr>
<td>Viognier</td>
<td>75.4%</td>
<td>43</td>
</tr>
</tbody>
</table>

answered question 57

Table A-6: Consumption Frequency: labrusca. “How often do you consume wine made from V. labrusca (Concord, Niagara, Delaware, Catawba, etc.) grapes?”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>32.7%</td>
<td>18</td>
</tr>
<tr>
<td>1-3 times a year</td>
<td>45.5%</td>
<td>25</td>
</tr>
<tr>
<td>Once a month</td>
<td>10.9%</td>
<td>6</td>
</tr>
<tr>
<td>Once every two weeks</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>1-2 times per week</td>
<td>9.1%</td>
<td>5</td>
</tr>
<tr>
<td>3-5 times per week</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>Every day</td>
<td>1.8%</td>
<td>1</td>
</tr>
</tbody>
</table>

answered question 55
Table A-7: Price Point: *labrusca*. “On average, how much are you willing to pay to purchase a standard bottle (750 mL) of table wine made from *labrusca* grapes?”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.00-$9.99</td>
<td>40.0%</td>
<td>22</td>
</tr>
<tr>
<td>$10.00-$14.99</td>
<td>49.1%</td>
<td>27</td>
</tr>
<tr>
<td>$15.00-$19.99</td>
<td>9.1%</td>
<td>5</td>
</tr>
<tr>
<td>$20.00-$24.99</td>
<td>1.8%</td>
<td>1</td>
</tr>
<tr>
<td>$25.00-$29.99</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>$30.00+</td>
<td>0.0%</td>
<td>0</td>
</tr>
</tbody>
</table>

answered question 55

Table A-8: Consumption Frequency: *vinifera*. “How often do you consume wines made from *V. vinifera* grapes (Cabernet Sauvignon, Cabernet Franc, Chardonnay, Riesling, Pinot Noir, Pinot Grigio/Gris, Syrah/Shiraz, Tempranillo, Merlot, Sangiovese, Zinfandel, etc.)?”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>1-3 times a year</td>
<td>3.6%</td>
<td>2</td>
</tr>
<tr>
<td>Once a month</td>
<td>7.3%</td>
<td>4</td>
</tr>
<tr>
<td>Once every two weeks</td>
<td>7.3%</td>
<td>4</td>
</tr>
<tr>
<td>1-2 times per week</td>
<td>21.8%</td>
<td>12</td>
</tr>
<tr>
<td>3-5 times per week</td>
<td>32.7%</td>
<td>18</td>
</tr>
<tr>
<td>Every day</td>
<td>27.3%</td>
<td>15</td>
</tr>
</tbody>
</table>

answered question 55
Table A-9: Price Point: *vinifera*. “On average, how much are you willing to pay to purchase a standard bottle (750 mL) of table wine made from *vinifera* grapes?”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.00-$9.99</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>$10.00-$14.99</td>
<td>16.4%</td>
<td>9</td>
</tr>
<tr>
<td>$15.00-$19.99</td>
<td>45.5%</td>
<td>25</td>
</tr>
<tr>
<td>$20.00-$24.99</td>
<td>18.2%</td>
<td>10</td>
</tr>
<tr>
<td>$25.00-$29.99</td>
<td>7.3%</td>
<td>4</td>
</tr>
<tr>
<td>$30.00+</td>
<td>12.7%</td>
<td>7</td>
</tr>
</tbody>
</table>

answered question 55

Table A-10: Wine Origin. “Most of the wine I purchase is made in:”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennsylvania</td>
<td>7.3%</td>
<td>4</td>
</tr>
<tr>
<td>New York</td>
<td>30.9%</td>
<td>17</td>
</tr>
<tr>
<td>California</td>
<td>23.6%</td>
<td>13</td>
</tr>
<tr>
<td>Oregon or Washington</td>
<td>5.5%</td>
<td>3</td>
</tr>
<tr>
<td>Italy</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>Spain</td>
<td>1.8%</td>
<td>1</td>
</tr>
<tr>
<td>Canada</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>1.8%</td>
<td>1</td>
</tr>
<tr>
<td>France</td>
<td>9.1%</td>
<td>5</td>
</tr>
<tr>
<td>Chile</td>
<td>3.6%</td>
<td>2</td>
</tr>
<tr>
<td>Argentina</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>Australia/New Zealand</td>
<td>3.6%</td>
<td>2</td>
</tr>
<tr>
<td>I don't know</td>
<td>1.8%</td>
<td>1</td>
</tr>
<tr>
<td>Other (please specify)</td>
<td>10.9%</td>
<td>6</td>
</tr>
</tbody>
</table>

answered question 55
Table A-11: “I like sweet table wines (residual sugar > 5%; excludes dessert wine).”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
<th>Rating Average</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>16</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>1.96</td>
<td>54</td>
</tr>
</tbody>
</table>

answered question 54

Table A-12: “Wines made from labrusca grapes are sweet.”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
<th>Rating Average</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>17</td>
<td>26</td>
<td>2</td>
<td>3.33</td>
<td>54</td>
</tr>
</tbody>
</table>

answered question 54

Table A-13: “When I purchase a bottle of wine to give to a wine enthusiast friend, I purchase vinifera wine.”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
<th>Rating Average</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>22</td>
<td>26</td>
<td>4.35</td>
<td>54</td>
</tr>
</tbody>
</table>

answered question 54

Table A-14: “When I open a bottle of wine for everyday drinking/a regular occasion; I open a wine made from labrusca grapes.”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
<th>Rating Average</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>17</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1.63</td>
<td>54</td>
</tr>
</tbody>
</table>

answered question 54

Table A-15: “When I open a bottle of wine to celebrate a special occasion, momentous event, or significant milestone; I open a wine made from labrusca grapes.”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
<th>Rating Average</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1.52</td>
<td>54</td>
</tr>
</tbody>
</table>

answered question 54
Table A-16: “Assuming the wines are clear of defects, a wine made from V. vinifera grapes is superior in quality to a wine made from V. labrusca grapes.”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
<th>Rating Average</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>10</td>
<td>24</td>
<td>11</td>
<td>3.63</td>
<td>54</td>
</tr>
</tbody>
</table>

answered question 54

Table A-17: “I like my wine to taste like Concord grapes.”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
<th>Rating Average</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32</td>
<td>13</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>1.61</td>
<td>54</td>
</tr>
</tbody>
</table>

answered question 54

Table A-18: “I believe quality wine can be made from labrusca grapes.”

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
<th>Rating Average</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>15</td>
<td>22</td>
<td>14</td>
<td>3.87</td>
<td>54</td>
</tr>
</tbody>
</table>

answered question 54
Appendix B

Supplemental Threshold Data

B.1 Group Best Estimate Thresholds; Comparison to Graphical Calculations

Group best estimate thresholds (BETs) were calculated for each experiment based on individual BETs. Individual BETs were calculated as the geometric mean of the highest concentration missed in the 3-AFC tasks and the next concentration in the ascending series that was chosen correctly and followed by at least three subsequent correct responses, or all remaining in the series.

The group BETs were based on individual BETs, which were determined from one trial. The experiments would need to be repeated to collect a standard deviation for individual BETs. The group BETs and 95% CI are combined with the detection thresholds determined graphically, which is to say the concentration of added compounds at which 50% of respondents perform greater than chance (67% correctly identify the “different” sample).
Table B-1: Group BETs (µg/L) and Graphically Determined Detection Thresholds (µg/L)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Water</th>
<th>Wine</th>
<th>Water</th>
<th>Wine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group BET</td>
<td>Graph</td>
<td>Group BET</td>
<td>Graph</td>
</tr>
<tr>
<td>12/10, 12/12/2014</td>
<td>3.924</td>
<td>7.514</td>
<td>20.29</td>
<td>44.98</td>
</tr>
<tr>
<td>95% CI</td>
<td>[2.339, 6.581]</td>
<td>[3.148, 17.93]</td>
<td>[12.46, 33.05]</td>
<td>[21.18, 95.54]</td>
</tr>
<tr>
<td>3/24, 3/26/2015</td>
<td>5.722</td>
<td>8.097</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>[3.184, 10.28]</td>
<td>[3.824, 17.15]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2AAP</td>
<td>0.5722</td>
<td>1.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>[0.3433, 0.9536]</td>
<td>[0.6852, 1.465]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/10/2015</td>
<td></td>
<td></td>
<td>[2.556, 7.680]</td>
<td>[3.791, 29.30]</td>
</tr>
<tr>
<td>2AAP</td>
<td></td>
<td></td>
<td>10.54</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td></td>
<td>[2.556, 7.680]</td>
<td>[3.791, 29.30]</td>
</tr>
<tr>
<td>9/3, 9/4/2015</td>
<td>7.633</td>
<td>7.572</td>
<td>50.32</td>
<td>89.36</td>
</tr>
<tr>
<td>95% CI</td>
<td>[4.140, 14.07]</td>
<td>[2.296, 24.97]</td>
<td>[28.82, 87.85]</td>
<td>[28.19, 283.3]</td>
</tr>
<tr>
<td>9/28, 9/30/2015</td>
<td>0.814</td>
<td>1.172</td>
<td>5.28</td>
<td>5.564</td>
</tr>
<tr>
<td>95% CI</td>
<td>[0.5230, 1.268]</td>
<td>[0.6137, 2.238]</td>
<td>[3.056, 9.124]</td>
<td>[2.942, 10.53]</td>
</tr>
</tbody>
</table>

95% CI: 95% Confidence Interval
Appendix C

GC-FID Data

To answer the question of whether the observed differences in detection threshold for methyl anthranilate and 2-aminoacetophenone in water, model wine and wine were due to partitioning differences or masking effects, static headspace analysis was performed using a gas chromatograph with a flame ionization detector (GC-FID). Samples of MA and 2AAP in each of the three matrices were prepared in concentrations of 0.5, 5, and 55.6 ppm (2AAP) and 0.5, 5, and 58.4 ppm (MA). 2 mL of each sample were pipetted into a 10 mL glass vial. The samples were then sealed with screw caps that had a Teflon/silicone septum. Samples were allowed to rest approximately one hour before headspace analysis.

Analysis was performed by a 6890 Agilent GC equipped with a FID, and coupled with a Gerstel multipurpose sampler (Gertel 1.0/2.5 mL syringe). A spitless inlet received the sample, maintained at 150°C. Helium, flowing at 1.4 mL/min, was used as the carrier gas. Compounds were separated on a HP-5MS column (30.0 m x 250 µm x 0.25 µm). The oven began at 35°C, held for 3 minutes, and then ramped to 250°C at 6°C/min, remaining at 250°C for a final 5 minutes. Compounds were detected by an FID held at 250°C with a hydrogen airflow of 40 mL/min and air flow of 400 mL/min, collecting data at a frequency of 20 Hz for a minimum peak width of 0.01 minutes.

The concentrations of MA and 2AAP in the headspace for even the highest concentrations were decidedly below the limit of detection of the instrument given the absence of any significant peaks diverging from the control samples.
Table C-1: GC-FID data collected for samples in water.

<table>
<thead>
<tr>
<th>Vial No.</th>
<th>Spiked Compound</th>
<th>Concentration (ppm)</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
<th>Peak Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2AAP</td>
<td>0.5</td>
<td>1.59</td>
<td>29.091</td>
<td>30.799</td>
</tr>
<tr>
<td>2</td>
<td>2AAP</td>
<td>5</td>
<td>1.451</td>
<td>35.788</td>
<td>35.788</td>
</tr>
<tr>
<td>3</td>
<td>2AAP</td>
<td>5</td>
<td>1.448</td>
<td>1.603</td>
<td>0.31</td>
</tr>
<tr>
<td>4</td>
<td>2AAP</td>
<td>5</td>
<td>1.442</td>
<td>1.603</td>
<td>0.31</td>
</tr>
<tr>
<td>5</td>
<td>2AAP</td>
<td>55.6</td>
<td>1.603</td>
<td>1.603</td>
<td>0.31</td>
</tr>
<tr>
<td>6</td>
<td>MA</td>
<td>0.5</td>
<td>1.601</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>MA</td>
<td>5</td>
<td>1.603</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>8</td>
<td>MA</td>
<td>5</td>
<td>1.601</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>9</td>
<td>MA</td>
<td>5</td>
<td>1.588</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>10</td>
<td>MA</td>
<td>58.4</td>
<td>1.603</td>
<td>2.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Table C-2: GC-FID data collected for samples in model wine.

<table>
<thead>
<tr>
<th>Vial No.</th>
<th>Spiked Compound</th>
<th>Concentration (ppm)</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
<th>Peak Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2AAP</td>
<td>0.5</td>
<td>1.601</td>
<td>2.1</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.819</td>
<td>6176.6</td>
<td>1194.4</td>
</tr>
<tr>
<td>12</td>
<td>2AAP</td>
<td>5</td>
<td>1.595</td>
<td>2.1</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.818</td>
<td>6053.2</td>
<td>1160.7</td>
</tr>
<tr>
<td>13</td>
<td>2AAP</td>
<td>5</td>
<td>1.598</td>
<td>2.3</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.818</td>
<td>5713.8</td>
<td>1119</td>
</tr>
<tr>
<td>14</td>
<td>2AAP</td>
<td>5</td>
<td>1.603</td>
<td>2.1</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.82</td>
<td>5925.7</td>
<td>1134.7</td>
</tr>
<tr>
<td>15</td>
<td>2AAP</td>
<td>55.6</td>
<td>1.603</td>
<td>2.2</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.819</td>
<td>5841.8</td>
<td>1119.1</td>
</tr>
<tr>
<td>16</td>
<td>MA</td>
<td>0.5</td>
<td>1.603</td>
<td>2.4</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.82</td>
<td>6191.1</td>
<td>1180</td>
</tr>
<tr>
<td>17</td>
<td>MA</td>
<td>5</td>
<td>1.601</td>
<td>2.3</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td></td>
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Table C-3: GC-FID data collected for 2-aminoacetophenone samples in wine.

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<th>Peak Height</th>
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Table C-4: GC-FID data collected for methyl anthranilate samples in wine.

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<th>Peak Height</th>
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Table C-5: GC-FID data collected for wine and air control samples (no chemical additions).

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<th>Vial No.</th>
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<th>Concentration (ppm)</th>
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Calculations

The formula used to calculate the number of participants needed for a specific test power, is provided by the equation:

**Equation D-1: Power Calculation**

\[ N = \left[ \frac{z_\alpha \sqrt{p_0 q_0} + z_\beta \sqrt{p_a q_a}}{p_0 - p_a} \right]^2 \]

N: number of participants needed

\( z_\alpha, z_\beta \): z scores chosen for given \( \alpha, \beta \) risk

\( p_0 \): chance probability

\( q_0 \): alternative probability (\( q = 1 - p_0 \))

\( p_0 - p_a \): level of performance desired

For an \( \alpha \) of 5%, \( z = 1.645 \); for a \( \beta \) of 5%, \( z = 1.645 \).

In a 3-alternative forced choice task with 3 samples presented: \( p_0 = 1/3 \) and \( q_0 = 2/3 \).

Detection threshold is, by definition, the concentration at which 50% (\( P = 1/2 \)) of participants identify the presence of the odorant, therefore:

\[ P_a = P_{\text{chance}} + P_d (1 - P_{\text{chance}}) \]

\[ P_a = (1/3) + (1/2)(1 - (1/3)) = 0.665 \]

Note that we do not use \( P_d = 0.67 \) (the Lawless corrected proportion) because we do not want to double compensate for chance. If, however, we did use a \( P_d \) value of 0.67, we would find that it results in \( N = 11 \).

Substituting values of \( p_a = 0.665; q_a = 0.335; z_\alpha, z_\beta = 1.645; p_0 = 0.33; q_0 = 0.67 \) into Equation D-1, we calculate a value of \( N = 22 \). Therefore, to reduce the risk for Type I and II error to 5%, we must use a minimum of 22 participants in each of the experiments.