

Characterization of Cold Soak on *Vitis vinifera* L. cv. Cabernet Sauvignon Grape and Wine Volatiles Using an Electronic Nose System

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ABSTRACT

The use of the electronic nose in the wine industry is an emerging technology. Although electronic nose systems have been used in other industries as tools to monitor fruit maturity or quality control, the use of these systems for wine analysis has been debated. Many electronic nose sensor types exist, but have often been accused of primarily discriminating varying ethanol concentrations of wines as opposed to aroma and flavor volatiles. This study evaluated the use of a commercial conducting polymer electronic nose to evaluate wines while minimizing ethanol interference. The first study presented evaluated the discrimination ability of an electronic nose with use of an ethanol baseline prior to wine evaluation. This experiment also determined the optimal wine temperature for electronic nose analysis. A second study reviewed the ability of the electronic nose to discriminate Cabernet Sauvignon grape and wine volatiles based on a pre-fermentation cold soak treatment. The electronic nose was used to monitor volatile changes throughout a five day cold soak, and to discriminate differences in control versus cold soak wines. These results were compared with juice and wine analytical data, GC-MS evaluation of individual volatiles, and sensory analysis of the finished wines. It was found that discrimination of wines improved from 33% to 60% when using an ethanol baseline. Influence of the baseline was indicated by a drop in sensor response prior to wine evaluation. A 30°C sample temperature was chosen for wine analysis based on sensor response and adequate discrimination in canonical distributions. The electronic nose was found to discriminate grape volatiles in cold soak musts as shown in canonical distributions at a 95% significance level. PCA distributions of electronic nose data, chemistry data, and GC-MS data showed varying degrees of discrimination based on analysis. Electronic nose data often showed 100% of variation of samples accounted by PC1. Despite differences in treatment by ENose and analytical data, sensory results did not find a difference in control and cold soak wines.

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TABLE OF CONTENTS

Abstract	
Dedication	iii
Acknowledgements	iv
List of Tables and Figures	vii
Introduction	1
 Chapter I: Review of the Literature	 3
A. Aroma and Flavor Compounds of Grapes and Wine	3
Non-Volatile Compounds	3
Volatile Compounds	6
B. Electronic Nose	9
System and Sensors	9
Sensitivity, Selectivity, and Wine Analysis Using an Electronic Nose	12
Precision and Accuracy of the Electronic Nose	13
C. Cold Soak Process	16
References	20
 Chapter II: Optimizing Red and White Wine Analysis using the Conducting Polymer Cyranose® 320 Electronic Nose	 28
Abstract	28
Introduction	29
Materials and Methods	30
Results and Discussion	31
Conclusion	32
References	42
 Chapter III: Electronic Nose Analysis on the Effect of Pre-fermentation Cold Soak <i>Vitis vinifera</i> L. cv. Cabernet Sauvignon Grape and Wine Volatiles	 43
Abstract	43
Introduction	44
Materials and Methods	46
Results and Discussion	49
Conclusion	54
References	75
 Summary	 81

Appendix	83
A.1: Regression Data for 2007 Cabernet Sauvignon Juice During Cold Soak	84
A.2: Regression Data for 2008 Cabernet Sauvignon Juice During Cold Soak	86
A.3: IRB Approval Documentation	88
A.4: IRB Sensory Consent Form	89
A.5: Copyright Permission Letter from Smith's Detection	91

LIST OF TABLES AND FIGURES

Chapter I

Table 1: The use of an electronic nose in wine/grape studies	13
---	-----------

Chapter II

Figure 1: Representation of change in resistance due to sensor swelling during volatile adsorption in the Cyranose® 320 (Used with permission courtesy of Smith's Detection© 2009).....	34
--	-----------

Table 1: Cyranose® 320 methods developed for (A) grape (Athamneh et al. 2008) and (B) wine evaluation.....	35
---	-----------

Figure 2: Electronic nose set up during wine evaluation (Photograph by Denise M. Gardner, December 2007).....	36
--	-----------

Figure 3: Sensor response screen from PC Nose software showing 32 sensors with influence of ethanol baseline (circled in blue). Ethanol causes initial dip in sensor response.....	37
---	-----------

Figure 4: Cross validation (confusion charts) from PC Nose software of Traminette and Cabernet Franc wines (A) without and (B) with the use of an ethanol baseline during wine evaluation.....	38
---	-----------

Figure 5: Canonical distribution of Traminette and Cabernet Franc wines (A) without and (B) with an ethanol baseline during wine evaluation.....	39
---	-----------

Figure 6: Canonical distribution from PC Nose software of Traminette (numbers “6” through “10”) and Cabernet Franc (numbers “1” through “5”) wine samples at four sampling temperatures (7°C, 15°C, 20°C, 30°C).....	40
---	-----------

Figure 7: Sensor response with sample temperature at (A) 7°C, (B) 15°C, (C) 20°C, and (D) 30°C. First curve is from the initial purge cycle, second from the sample draw, and the final curve is from the volatile purge cycle and removing ENose from sample.....	41
---	-----------

Chapter III

Table 1: Cabernet Sauvignon must additions (per volume) to adjust TA, Brix, and fermentable nitrogen content prior to fermentation.....	56
--	-----------

Table 2: Cyranose 320® electronic nose wine evaluation parameters. The last four settings describe statistical analysis details.....	57
---	-----------

Figure 1: Canonical distribution of differences detected by ENose analysis during five day cold soak of (A) 2007 and (B) 2008 Cabernet Sauvignon must (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).....	58
Figure 2: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon cold soak must volatiles detected by ENose during cold soak.....	59
Table 3: Juice (A) pH, titratable acidity (TA), Brix, phenol-free glycosyl-glucose (PFGG), and total glycosyl glucose (TGG) and (B) absorbance (280 nm, 420 nm, 520 nm) and color (hue and intensity) throughout five day cold soak in 2007 Cabernet Sauvignon must (n = 3).....	60
Table 4: Juice (A) pH, titratable acidity (TA), Brix, phenol-free glycosyl-glucose (PFGG), and total glycosyl glucose (TGG) and (B) absorbance (280 nm, 420 nm, 520 nm) and color (hue and intensity) throughout five day cold soak in 2008 Cabernet Sauvignon must (n = 3).....	61
Table 5: Regression slopes for juice chemistry attributes of Cabernet Sauvignon must throughout five day cold soak [*Indicates slope is significantly different than zero (0.0)].	62
Figure 3: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon cold soak must chemistries determined by pH, TA, Brix, PFGG, TGG, absorbance (280 nm, 420 nm, 520 nm) and color (hue and intensity) during cold soak.....	63
Figure 4: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon control and cold soak wine volatiles detected by the ENose.....	64
Figure 5: Canonical distribution of 2007 Cabernet Sauvignon wines (A) post-fermentation and (B) six months post-fermentation (significant differences at $\alpha = 0.05$ level indicated by non-intersecting points).....	65
Figure 6: Canonical distribution of 2008 Cabernet Sauvignon wines (A) post-fermentation and (B) six months post-fermentation (significant differences at $\alpha = 0.05$ level indicated by non-intersecting points).....	66
Table 6: Volatile compounds analyzed by SPME GC-MS, odor thresholds, and reported concentrations for control and cold soak Cabernet Sauvignon wines.....	67
Figure 7: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon control and cold soak wine volatiles detected by GC-MS.....	69
Figure 8: Canonical distribution of wines (A) without and (B) with the use of an ethanol baseline using a conducting polymer electronic nose system.....	70

Table 7: Wine (A) pH, titratable acidity (TA), ethanol concentration, volatile acidity (VA), phenol-free glycosyl-glucose (PFGG), and total glycosyl-glucose (TGG) and (B) absorbance (280 nm, 420 nm, 520 nm) and color (hue and intensity) of 2007 and 2008 Cabernet Sauvignon.....	71
Figure 9: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon control and cold soak wine chemistries determined by pH, TA, ethanol concentration, volatile acidity, PFGG, TGG, absorbance (280 nm, 320 nm, 420 nm, 520 nm) and color (hue and intensity).....	72
Table 8: Cabernet Sauvignon wine aroma (sample temperature 19°C) triangle difference sensory results (n = 54) of control vs. cold soak treatments per vintage year ($\alpha = 0.05$, $\beta = 0.10$, $\rho_{\max} = 30\%$, 25 correct responses needed for significant difference).....	73
Table 8: Aroma units of select volatile compounds in 2007 and 2008 Cabernet Sauvignon control and cold soak wines.....	74

INTRODUCTION

Wine quality is prominently determined by varietal aroma and flavor composition. Each grape variety has a specific varietal aroma and flavor profile that can be evaluated by winemakers, industry professionals, and consumers. The manipulation of aroma and flavor of wine occurs through a number of steps from the vineyard to pre-fermentation processes (i.e. crushing, cold soaking, extended maceration, etc.) to post-fermentation processes (i.e. yeast contact, pressing, barrel aging, bottle aging, etc.). Cold soak is a pre-fermentation maceration process applied at cold temperatures and is believed to increase color stability of reds wines. Several studies have evaluated the cold soak process for effects on color stability and also noted changes in aroma and flavor development of those wines. However, little research has focused on identifying and quantifying changes caused by cold soak using volatile analytical techniques and sensory evaluation.

The electronic nose (ENose) is an analytical tool composed of a sensor array system that analyzes volatile compounds, some of which may contribute to aroma. Depending on sensor type and polymer coatings, the ENose can detect a broad variety of volatile compounds by static headspace analysis. A “smell print” is used to identify a given juice or wine sample. The “smell print” represents a unique pattern recognizable only to that set of samples, and can be used to show relationships among samples by classifying similarities among wine varieties or treatments.

Ethanol interference with sensors has been a noted problem in electronic nose research. Ethanol variation has been accused of contributing some, if not most, of the variation among wines during electronic nose analysis. The use of hot operating temperature ENose systems have been used most frequently in wine analysis. However, the use of a conducting polymer electronic nose has potential to minimize the influence of ethanol in sensor responses, indicating

a possibility of using such a system for wine analysis. Little research has used a conducting polymer ENose system to measure volatile differences in wines under different processing treatments and couple results with more traditional analytical and sensory methods.

This research was conducted to monitor juice and analyze wine volatiles by electronic nose analysis, and compare electronic nose results with wine analytical data, GC-MS, and sensory analysis. The null hypothesis is that there is no difference in the volatile component between control and cold soak treatments, while the alternative hypothesis is that there is a difference.

CHAPTER I: REVIEW OF THE LITERATURE

Aroma and Flavor Compounds of Grapes and Wine

Non-Volatile Compounds

Aroma and flavor of wine, consisting of free volatiles and non-volatile precursors, greatly affects quality. The non-volatile component of grape and wine aroma precursors consists of conjugated compounds in which a sugar moiety is bound to an aglycone unit. The aglycone unit can include anthocyanins, phenolic compounds, or aroma and flavor compounds (Dimitriadis and Williams 1984). An aroma and flavor aglycone has potential to contribute to varietal aroma characteristics of wine (Abbott et al. 1993). Grape varieties low in free volatile concentrations are typically high in glycosylated compounds, and as wines age, increase in free volatile compounds (Abbott et al. 1989, Sefton et al. 1993).

Glycosides can exist as mono- and disaccharide glycosidic bound units; some of the most common including 6-O- α -L-arabinofuranosyl- β -D-glucopyranoside, 6-O- α -L-rhamnopyranosyl- β -D-glucopyranoside, and 6-O- β -D-glucopyranosyl- β -D-glucopyranoside (Strauss et al. 1986b, Williams et al. 1993, Williams and Allen 1996). Upon hydrolysis, glycosides may liberate volatile compounds such as monoterpenes, aliphatic compounds, C₁₃-norisoprenoids, and shikimic acid metabolites. In aromatic wine varieties (such as Muscat, Riesling, and Gewürztraminer), the aglycone typically consists of a monoterpene, whereas in non-aromatic varieties, the aglycone is typically a norisoprenoid, but liberation of aglycones is non-specific and a function of hydrolysis type (Sefton et al. 1993, Straus et al. 1986a, Williams et al. 1993).

Glycosides are odorless, but provide an indication of potential wine aroma due to the possible release of free volatiles from the sugar moiety. Liberation of the aglycone upon enzymatic and/or acidic hydrolysis may occur during processing and aging, contributing to

changes in the volatile component (Strauss et al. 1986b, Williams et al. 1993). The basis of analytical testing for glycosides involves the quantification of the released glucose unit after the glycosidic bond is broken. Theoretically, a one-to-one molar ratio exists between the sugar moiety, glucose, and the aglycone (Whiton and Zoecklein 2002). Analysis involves extraction of glycosides from berries, juice, or wine, liberation of the aglycone unit, and quantification of D-glucose (referred to as glycosyl-glucose) previously bound to aglycones (Illand et al. 1986, Abbott et al. 1993, Williams et al. 1995). Alterations to the analysis have been made including skin extraction techniques and development of eliminating glycosidically bound anthocyanins (Illand et al. 1996) and phenolic compounds (Zoecklein et al. 2000).

Enzymatic hydrolysis cleaves the 1→6 glycosidic bond to potentially increase hydrolysis of mono- and disaccharide precursors (Zoecklein et al. 1997). Glucosidase enzymes (α -glucosidase, α -arabinosidase, β -glucosidase, and β -galactosidase) are unspecific to the aglycone moiety and have potential to release undesirable compounds such as 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and sulfur-containing compounds (Aryan et al. 1987, Williams and Allen 1996). Enzyme hydrolysis particularly from endogenous grape glucosidases, although possible, can be inhibited by increased glucose concentrations, as well as changes in ethanol concentrations, pH, temperature, and phenolic content (Aryan et al. 1987, Mansfield et al. 2002).

Acid hydrolysis of glycosides can occur at pHs from 3.0 to 3.5 (Williams et al. 1989). Acid hydrolysis splits the aglycone unit from the 1→6 glycosidic bond by protonation to form a hydroxyl group onto the sugar (Timell 1964). For analysis, pH is dropped to about 3.2 for extraction and concentration of glycosidic precursors (Williams et al. 1989). Application of acid hydrolysis in glycosyl-glucose analysis improved accuracy of quantifying glycosidic compounds (Williams and Francis 1996, Williams et al. 1996, Zoecklein et al. 2000).

Research in viticulture and enology has focused on ways to increase the aromatic volatile potential through vineyard practices and enhance them through grape and wine processing. Vineyard practices, including leaf removal, have been noted to increase the concentration of glycosides in the berries (Zoecklein et al. 1998a). Other vineyard practices that influence the concentration of glycosidically bound and free secondary metabolites include bunch thinning, berry weight changes, water supply regulation, leaf area, and canopy management, especially increased sun exposure (Illand et al. 1986, Zoecklein et al. 1998b). In wine processing, decreased concentrations of glycosides have been found post-fermentation with thermal processing indicating the possible release of volatile moieties (Zoecklein et al. 1997). Yeast and bacterial selection have been of particular importance due to their varying glycosidase properties (Lambrechts and Pretorius 2000, Swiegers et al. 2005). Non-*Saccharomyces* yeasts species have been used to enhance wine aroma (Mendes Ferreira et al. 2001). Due to non-specificity of the aglycone moiety, however, these species also have the potential to enhance non-desirable aroma and flavor attributes of wine (Mendes Ferreira et al. 2001). *Brettanomyces bruxellensis*, a wild yeast strain, and *Oenococcus oeni*, malolactic fermentation (MLF) bacteria strain, have displayed glucosidase activity, which have potential to alter the aromatic profile of wines put through MLF (Mansfield et al 2002). Other research has not shown an increase in aglycone concentration after glycoside hydrolysis from malolactic bacteria, which was speculated as a function of aglycone stabilization (Boido et al. 2002).

Additional odorless non-volatile components in wine include sulfur-cysteine-conjugated compounds. Hydrolysis of these compounds occurs primarily during fermentation and elicits volatile thiol compounds (Tominaga et al. 2000). Volatile thiols, including 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexanol (3MH), 4-mercapto-4-methylpentan-2-ol

(4MMPOH), and 3-mercapto-3-methylbutan-1-ol (3MMB), are important to wine aroma, associated with black currant, broom tree, and cat's urine, grapefruit and passion fruit, citrus zest, and leek aromas respectively (Tominaga et al. 2000, Howell et al. 2005). 4MMP and 3MH are mostly associated with S-cysteine conjugation (Tominaga et al. 2000).

Volatile Compounds

Over 1300 volatile compounds have been identified in alcoholic beverages (Ebeler 2001). Although of greatest importance, these compounds are found in the smallest concentration (<1%) compared to water and ethanol (Etievant 1991, Miranda-Lopez et al. 1992). Major volatile components of wine include monoterpenoides (i.e. free monoterpenes), C₁₃-norisoprenoids, esters, higher (fusel) alcohols, carbonyl compounds (i.e. acetaldehyde), and shikimic acid metabolites (Etievant 1991, Swiegers et al. 2005). Monoterpenes are free volatile terpene subunits that contribute to floral and fruity aroma characteristics. Of the varietal aroma compounds discussed, monoterpenes are the most potent and have been isolated in highly aromatic varieties including Riesling, Muscat, and Gewürztraminer at concentrations as low as one to two mg/L in the berries of these aromatic varieties (Williams et al 1989). Other wine grape varieties such as Chardonnay, Sauvignon Blanc, and Cabernet Sauvignon lack high concentrations of free monoterpenes, and aroma is contributed by other volatile classes (Sefton 1998). Some of the most common monoterpenes found in grapes are geraniol, linalool, nerol, citronellol, terpineol and furan (Strauss et al. 1986b, Sefton 1998) which have associated rose-like, vegetative, geranium, and lilac aroma descriptors (Ebeler 2001, Swiegers et al. 2005).

C₁₃-norisoprenoids consist of thirteen-carbon backbone cyclohexene units and are believed to be derived from carotenoid degradation (Williams et al. 1993). These compounds have been found to have a predominant affect on wine aroma of Cabernet Sauvignon wines and

other non-terpene varieties (Sefton 1998). Generally, norisoprenoids accumulate in grape berries as glycosides and are released during fermentation and processing (Swiegers et al. 2005). In non-aromatic varieties, such as Chardonnay, norisoprenoids were found to maintain 70% of the aroma volatiles (Williams et al. 1993). Of particular importance to the varietal character of Cabernet Sauvignon is the norisoprenoid, β -damascenone, which has a low odor threshold of 0.05 to 1.6 mg/L (Sefton et al. 1989, Etievant 1991). Additional norisoprenoid compounds isolated and identified in Cabernet Sauvignon include actinidol, vitispirane, and 2-(3-hydroxybut-1-enyl)-2,6,6-trimethylcyclohex-3-en-1-one (Sefton 1998). Using sensory analysis comparison, such compounds have been linked to higher quality aged red wines (Gunata et al. 1986, Strauss et al. 1986, Williams et al. 1996, Ebeler 2001).

Esters are constituents of wine aroma and flavor that arise from organic acids, acetates (acetic acid), and fatty acids (Etievant 1991). Such compounds typically provide fruity, pleasant smelling aromas that are naturally produced in the grape and extracted during fermentation (Etievant 1991, Lambrechts and Pretorius 2000, Swiegers et al. 2005). Even at low odor threshold levels, esters have been noted to interact synergistically and enhance fruity aromas of wines (Etievant 1991). Through aging, esters are degraded, which reduces the fruity characters of most aged red wines (Etievant 1991). The most important ester in wine is ethyl acetate, which at concentrations of 150 to 200 mg/L implements a nail polish character which may be confused with acetic acid aromas at such high concentrations (Etievant 1991, Lambrechts and Pretorius 2000). There are many factors that affect the ester concentration in wine, including grape variety, grape maturity, sugar content, yeast strain, fermentation temperature, fermentation methods, skin contact time, pH, and sulfur dioxide levels (Gerbaux et al. 2002, Lambrechts and Pretorius 2000, Swiegers et al. 2005).

Higher alcohols are long-chained alcohol units (i.e. 2-phenylethyl alcohol, tyrosol) that contribute to many undesired aromas that have been described as fusel-like, alcoholic, pungent, and green (Etievant 1991, Sweigers et al. 2005). They are produced through fermentation primarily by catabolism of amino acids and higher concentrations associated with high-rate fermentations (Etievant 1991, Rapp and Versini 1996). Concentrations above 300 mg/L degrade wine quality (Etievant 1991).

Carbonyl compounds contain a carbon atom double bonded to an oxygen atom. The most common carbonyl compound found in wine is acetaldehyde with a sensory threshold at 100 mg/L (Swiegers et al. 2005). Typically, acetaldehyde is an indication of wine oxidation, but is present in all wines to some degree (Etievant 1991). Diacetyl (2,3-butanedione), a compound implemented into wine during oak fermentation or aging is also a prevalent carbonyl compound that provides a buttery aroma and flavor (Swiegers et al. 2005). Additional compounds in this class include aliphatic compounds, which do not contain an aromatic ring structure, and are typically four to ten carbon chains attached to various functional groups. Examples of aliphatic alcohols found in wine include propanol, isobutyl alcohol, amyl alcohol, and isoamyl alcohol (Lambrechts and Pretorius 2000).

Shikimic acid is a component of chlorogenic acid that is used in the development of phenolic and tannin compounds (Amerine et al. 1972). These components are found in the highest concentration of young wines, especially whites (Amerine et al. 1972). Some volatiles are formed through the shikimic metabolic pathway and contribute to aromatic amino acids, benzoic acids, cinnamic acids, and lignins (Sefton et al. 1993). Concentrations of shikimic acid metabolites have been noted to decrease during MLF (Boulton et al. 1996).

In a grape berry, free volatile development occurs during final stages of ripening after sugar accumulation has reached a maximum concentration (Conde et al. 2007). During the final maturation stages of grape berries, other aromatic and flavor components such as methoxypyrazines that contribute to earthy, vegetative aromas, begin to decrease in concentration and aromatic intensity (Conde et al. 2007). The sudden increase in grape varietal free volatile compounds and simultaneous decrease in methoxypyrazines is the best indication of grape maturity and quality. Varietal aromatic component accumulation during late stage grape ripening has been coined *engustment* (Coombe and McCarthy 1997). Coombe and McCarthy (1997) have found that the increase in free volatiles, as well as their glycosidic forms, is one of the last stages of grape maturation. This stage is of greatest importance because it establishes varietal character (Coombe and McCarthy 1997). Grapes with extended hang time are likely to develop more varietal aroma and flavor compounds. Accumulation of aroma and flavor compounds indicates a greater potential for high quality wine.

Electronic Nose

The electronic nose (ENose) is an analytical tool composed of a sensor array system that analyzes volatile compounds, some of which may contribute to aroma. This is an objective tool that evaluates volatile headspace comparable to the human olfactory system (Craven et al. 1996). Several ENose sensor classes exist including quartz-microbalanced sensors (QMS), conducting polymer sensors, metal oxide sensors (MOS), and surface acoustic wave (SAW)-based sensors (Pearce et al. 2003, Mallikarjunan 2005). Selectivity, sensitivity, and detection of volatile compounds differ according to sensor type.

Systems and Sensors

Quartz-microbalanced sensors measure the change in frequency of oscillation for a given volatile compound (Mallikarjunan 2005). QMS sensors contain a thin polymer coating over a quartz base. Selectivity of volatile compounds is determined by the functional groups that make up each polymer surrounding the quartz crystal. Volatile compounds pass through the polymer coatings causing a change in mass of the sensor. Quartz, at a defined thickness (less than one μm), oscillates at a constant frequency when a conductive current is applied (Applied Sensors 2001). As volatiles interact with the polymer coating, a change in mass occurs and causes a change in frequency of oscillation (Schaller et al. 1998). This phenomenon is known as the piezoelectric effect (Pearce et al. 2003, Mallikarjunan 2005). As little as a one nanogram mass change can cause a change in frequency of the sensor oscillation (Nanto et al. 1995).

Conducting polymer sensors are among of the most selective and sensitive sensors used in ENose systems (Mallikarjunan 2005). Sensors are composed of a carbon- or silicone-base polymer substrate attached to a conductive polymer and an electrode bridge (Mallikarjunan 2005). Common polymer coatings that are selective for polar volatiles are polypyrrole, polyaniline, polythiophene, polyindole, and polyacetylene (Janata and Josowicz 2003, Pearce et al. 2003). Adsorption of volatiles causes the sensors to swell, which results in a change in electrical resistance. The maximum change in resistance between the baseline (sensor steady-state) and sample cycles is recorded for each coated sensor (Dickinson et al. 1998).

MOS sensors are composed of metal oxide films covering a ceramic base. Adsorption of volatiles causes a change in conductance due to the interaction of gases on the surface of the sensor (Pearce et al. 2003). Reducing compounds cause an increase in conductance while oxidizing compounds cause a decrease in conductance (Pearce et al. 2003). MOS-based sensors are commonly referred to as “hot sensors” because they require a high operational temperature

(200-600°C) (Pearce et al. 2003, Mallikarjunan 2005). Compared to other ENose systems, high operating temperatures make MOS-based sensors the least sensitive to water vapor concentrations and reduce needed sensor recovery time (Dickinson et al. 1998). The use of MOS sensors has been applied to a number of wine studies including vineyard denomination discrimination (Di Natale et al. 1996), oak barrel toasting level discrimination (Chatonnet and Dubourdieu 1999), wine volatile sensor sensitivity (Santos et al. 2004), and wine style classification or discrimination (Lozano et al. 2005, McKellar et al. 2005, Garcia et al. 2006).

Surface acoustic wave (SAW)-based sensors detect volatile compounds on the surface of a crystal causing a change in crystal oscillation (Dickinson et al. 1998). The commercial zNose™ that utilizes SAW-based sensors operates as a miniature fast-reading gas chromatography (GC) unit. Samples are drawn into a sampling port and sent through a column by use of helium carrier gas. Volatiles interact with the column's given polarity similar to what occurs in a GC (Electronic Sensor Technology 2001). The zNose™ serves two functions: identification of volatile compounds through a chromatogram in relation to peak retention time in the column and quantification of those compounds due to a change in oscillation of the quartz crystal (Lammertyn et al. 2004). The zNose™ differs from other ENose systems in that it can isolate, identify, and quantify individual compounds that are detected through the column. Quartz crystals are not coated and oscillate at a constant frequency (500 Hz). A change in frequency relates to the concentration of an individual volatile compound as it elutes off the column. The commercial 7300 zNose™ can detect five Hz differences in frequency, which can occur with detection limits as low as picogram mass changes (Electronic Sensor Technology 2001). The column is most sensitive to volatile organic compounds in up to µg/L concentrations (Electronic Sensor Technology 2001).

Sensitivity, Selectivity, and Wine Analysis Using an Electronic Nose

Generally, sensitivity to volatiles varies according to sensor type and polymer coatings. Polymers are selected according to chemical properties such as polarity, molecular size, chilarity, and specific functional groups (Applied Sensors 2001). QMS machines are most selective for organic compounds larger than two carbons in length (Applied Sensors 2001). MOS systems are most selective for alcohols and/or non-nitrogen and sulfur containing volatiles (Mallikarjunan 2005). Depending on the type of compound analyzed and sensor coating, sensitivity for MOS systems can be in the mg/L or $\mu\text{g/L}$ concentration levels (Chatonnet and Dubourdieu 1999, Mallikarjunan 2005). Conducting polymer sensors are sensitive to a wide range of compounds, dependent on polymer coatings, at concentrations less than 20 mg/L at ambient temperatures (Pearce et al. 2003). The zNose™ reportedly has greater sensitivity than other ENose machines (Watkins and Wijesundera 2006). A study has shown sensitivity at concentrations of $\mu\text{g/L}$ for volatile compounds and pg/L (picogram/L, parts per trillion) for semi-volatile compounds (Staples 2000).

Wine aroma consists of water vapor, alcohols (predominately ethanol), and other volatile compounds in concentrations of 900 g/L, 100 g/L, and about 1 g/L respectfully (Etievant 1991). Wine variety and quality is differentiated by aroma and flavor compound differences, but contribute such a small concentration compared to water and ethanol. Wine is composed of a various mixture of aroma and flavor compounds including esters, terpenes, higher (fusel) alcohols, lactones, organic acids, aldehydes, ketones, acetals, phenolic compounds, sulfur-containing compounds, and nitrogen-containing compounds (Etievant 1991).

An ENose analyzes a complex collection of volatiles and identifies differences through sensor response changes of samples without quantifying individual components (Pearce et al.

2003), creating a specific “smell print” for a given wine. A “smell print” is a bar graph with sensor adsorption changes in relation to the sensor steady-state condition (Dickinson et al. 1998).

Table 1 lists some EN research to date on grape and wine volatile compounds.

Table 1: The use of an electronic nose in wine/grape studies

Author	Year	Type of EN Used	Study Focus
DiNatale et al.	1996	MOS	Wines of different locations
DiNatale et al.	1997	QMS	Changes in wine aroma after opening bottle
Chatonnet & Dubourdieu	1999	MOS	Oak toasting levels
Guadarrama et al.	2001	Conducting Polymer	Spanish wine identification
McKellar et al.	2005	MOS	Wine varieties
Lozano et al.	2005	MOS (tin oxide array)	White wine classification
Garcia et al.	2006	MOS (tin oxide)	Wine discrimination
Athamneh et al.	2008	Conducting Polymer (Cyranose)	Grape maturity
Martin et al.	2008	QMS	Wine discrimination

Precision and Accuracy of the Electronic Nose

There are a number of factors that can alter the precision, accuracy, and sensitivity of EN systems, including sensor drift, temperature alterations, humidity, incorrect storage, and ethanol/water vapor concentrations. Small changes in sensor responses may be caused by carry-over of volatiles from one evaluation to another. However, sensor drift occurs when volatile compounds remain in the sensor absorbance phase and cause a shift in sensor performance (Pearce et al. 2003). This may occur if a strong interaction exists between sample volatiles and sensor coatings (Pearce et al. 2003). Water vapor is one type of volatile compound that can increase sensor drift (Ragazzo-Sanchez et al. 2005).

Operating and sample temperatures alter the measuring capabilities of these machines (Dickinson et al. 1998). At higher operating temperatures, which differ according to the ENose model, sensitivity of the sensors decreases (Pearce et al. 2003, Rice, 2007, personal communication). For optimal results using the QMS, SAW, and conducting polymer systems, operating temperatures should be as low as possible, but high enough to obtain a response (Cyran Sciences 2000, Sunshine, 2007, personal communication).

In static headspace sampling, equilibrium between the liquid and vapor phase is reached over a specific time period (Pearce et al. 1993). Equilibrium between the vapor and liquid phases is temperature and pressure dependent (Mestres et al. 2000, Meilgaard et al. 2007). The most volatile components diffuse into the headspace first (van Ruth and Roozen 2002). However some volatile aroma and flavor compounds are more immiscible in alcohol solutions (van Ruth and Roozen 2002). Compared to liquid-liquid extraction, solid phase microextraction (SPME), and dynamic headspace analysis, static headspace analysis is diluted in volatile compounds (Jennings et al. 1972, Mestres et al. 2000, Ebeler 2001, Ortega-Heras et al. 2002). The dilution of volatiles often results in low sensitivity (Ortega-Heras et al. 2002). Regardless, static headspace is believed to best represent the sample volatile composition (Jennings et al. 1972). Wine volatiles that have been identified using static headspace techniques include esters, alcohols, acids, monoterpenes, and norisoprenoids (Ebeler 2001).

Higher sampling temperatures can alter the volatile composition and concentration (Robbins et al. 1993). It is important to note that sample temperatures should be about 10°C cooler than operating sensor temperatures for optimal results using most commercial systems (Cyran Sciences 2000, Electronic Sensor Technology 2001, Rice, 2007, personal communication). Higher sample temperatures also increases volatile concentrations as volatiles

escape from the liquid phase into the headspace (Robbins et al. 1993). The increased concentration of some volatiles may lead to sensor saturation. It is also true that increasing the sampling temperature may alter the true volatile profile. To avoid variability among samples, sample temperature should remain constant throughout analysis.

Ethanol can interfere with sensors, especially when using a conducting polymer unit. Ethanol can saturate sensors and mask changes in other contributing volatiles (Ragazzo-Sanchez et al. 2005). This is of considerable importance analyzing wines because ethanol is present at much greater concentrations than aroma and flavor volatiles (Etievant 1991). It has been suggested that ENose discrimination amongst wine samples may reflect changes in ethanol content as opposed to varietal aroma and flavor changes (Ragazzo-Sanchez et al. 2005). Various methods have been used to dilute the concentration of ethanol during sampling. Some of these methods include purge-and-trap, direct dilution, non-polar resins, and thermal alterations including distillation (Ragazzo-Sanchez et al. 2005). Purge-and-trap systems are typically useful at diluting ethanol concentration (Jennings et al. 1972). Several techniques of these systems have been offered including trap-and-purge methods (Porapak traps, cryogenic traps) and altering headspace sampling techniques (static, dynamic, direct injection, etc.) (Ragazzo-Sanchez et al. 2005). The use of cryogenic traps has been noted to increase water vapor content, hence leading to sensor drift issues when applied continuously (Francis and Cynkar 2000, Ragazzo-Sanchez et al. 2005). In addition, these techniques affect the efficacy of using the ENose to analyze and discriminate among wine samples quickly and efficiently. Therefore, training a commercial ENose system prior to sampling is most often recommended (Cyrano Sciences 2000, Applied Sensors 2001, Electronic Sensor Technology 2001).

Currently, ENose systems have been used to evaluate fruit maturity for blueberries, strawberries, bananas, grapes, apples, pears, peaches and nectarines (Simon et al. 1996, Llobet et al. 1999, Brezmes et al. 2005, Marrazzo et al. 2005, Buratti et al. 2006, Pathange et al. 2006, Athamneh et al. 2008). Many studies have indicated that an ENose can predict maturity levels of these fruits (blueberries, apples, pears, nectarines) or appropriate quality levels dependent on ripeness (Simon et al. 1996, Brezmes et al. 2005, Pathange et al. 2006). For blueberries, an ENose was found to differentiate levels of quality and maturity as berries neared ripeness better than when berries were immature (Simon et al. 1996). In addition, most of these studies have compared ENose responses with standard maturity indices including GC/MS, firmness, pH, TA, color, and soluble solids content.

Although the use of the ENose in enology studies is limited, advancements in sensor technology and standardization of ethanol-based samples have allowed for further investigation. To analyze wines, most studies have focused on the use of MOS and QMS based ENose systems, as well as a number of prototypes. Buratti et. al. (2006) claimed that MOS ENose systems had potential to analyze wine aroma objectively and quickly, but further research was needed to enhance the systems' predictive power in relation to wine aroma composition. Current research shows use of conductive polymer or SAW systems to analyze wine volatiles is innovative and shows potential for aroma discrimination.

Cold Soak Process

Cold soak [also termed “cold maceration” or “Accard process” (Heatherbell et al. 1996)] is a pre-fermentation process that is used to develop color stability and enhance water soluble tannin and anthocyanin extraction of red grape skins. Most research has focused on red varieties including Pinot Noir which has difficulty retaining color (Heatherbell et al. 1996), Cabernet

Sauvignon (McMahon et al. 1999, Mansfield and Zoecklein 2003, Baki 2004), Pinotage (Marais 2003a), Sangiovese (Parenti et al. 2004), Shiraz (Reynolds et al. 2001), and Monastrell (Alvarez et al. 2006). While these studies show consistent changes in cold soak wines, variation among varieties appears to occur. Some debate remains on the quantity of time that should be devoted to this process. In some winemaking regions (Chile), red wines can be cold soaked up to 20 days (Baki 2004). However, commercial cold soak typically lasts for about 24 hours to five days (Baki 2004). Cold soak temperatures typically range from 10 to 15°C (Heatherbell et al. 1996, McMahon 1999, Marais 2003b, Sacchi et al. 2005), but process temperature does vary: 0 to 2°C (Alvarez et al. 2006), 4°C (Heatherbell et al. 1996), 7°C (Mansfield and Zoecklein 2003, Baki 2004, Alvarez et al. 2006). Temperatures below 0°C have been used, but involve the use of cryogens (Parenti 2004).

During cold soak, natural yeast populations are inhibited through cooler temperatures and often additions of antimicrobials such as sulfur dioxide (McMahon et al. 1999). Cold soak can increase extraction of grape components including water soluble tannins, anthocyanins, and glycosides (Heatherbell et al. 1996, McMahon et al. 1999). Cold soak application has been linked to decreasing extraction of harsher, ethanol-soluble tannins during primary fermentation (Alvarez et al. 2006) as well as increasing polymeric pigment formation without the presence of alcohol (Williams et al. 1982, McMahon et al. 1999). Polymeric pigments are associated compounds of anthocyanins and tannins, particularly catechins and epicatechin (Timberlake and Bridle 1976). Enhanced extraction of anthocyanins and polymeric pigment formation pre-fermentation improves color stability during bottle aging (Alvarez et al. 2006).

Increased concentrations of post-fermentation glycosides were found in cold soaked Cabernet Sauvignon wines (McMahon et al. 1999, Mansfield and Zoecklein 2003). Glycosides

are volatile precursors extracted from grape skins and pulp that act as potential carriers for aromatic compounds in wine (Mansfield and Zoecklein 2003). Longer skin contact time in an aqueous environment and a higher degree of berry breakage may allow greater extraction of glycosides from grape skins (Williams et al. 1982) although this concept is debated (Sacchi et al. 2005). An increase in glycoside extraction during cold soak enhances the possibility of aroma alteration as wines age (Alvarez et al. 2006).

There is a lack of consistent information on cold soaked wines. The use and effects of sulfur dioxide during cold soaking is not clearly understood. Extractability of tannins and anthocyanins from Pinot Noir grapes during cold soak was found slightly dependent on sulfur dioxide concentrations in grape juice (Heatherbell et al. 1996). Heatherbell et al. (1996) reported no change in pigments during cold soaking without sulfur dioxide treatment. Increased anthocyanin extraction was correlated with an increase in sulfur dioxide. However, other studies have shown negative effects of polymeric pigment formation with use of higher sulfur dioxide levels (Boulton et al. 2001), limiting the effects of a cold soak period. Another study (Alvarez et al. 2006) found that the use of cold soak increased ethanol content in finished wines, but this is not consistent with the report by Heatherbell et al. (1996).

A majority of cold soak research focuses on color extraction and color stability. Few studies have also noted aroma and sensory differences among cold soaked wines. Heatherbell et al. (1996) reported that Pinot Noir wines held at 10°C for six days had higher woody, tobacco, and berry aromas, as well as greater mouthfeel, suggesting a change in flavor and textural attributes of cold soaked wines. Aroma and flavor intensity differences were also found among Shiraz wines cold soaked at 2°C for 10 days (Reynolds et al. 2001). An increase in berry and plum aroma and taste intensities in cold soaked Pinotage wines were found to be of higher

sensory quality (Marais 2003a). Over four growing seasons, highest quality was associated with wines cold soaked for two and four days at 10°C (Marais 2003a).

Despite noted sensory changes, little research has focused on analyzing wine aroma analytically in cold soaked wines. A study by Alvarez et al. (2006) used GC analysis to quantify wine volatiles three months after cold soak treated wines were bottled. An increase in acetates and esters were found after juice had been fermented and stored for three months at 14°C in cold soak treated wines (Alvarez et al. 2006). Cold soaked wines also contained higher concentrations of ethyl acetate compared to controls (Alvarez et al. 2006). Regardless of these results, a lack of understanding aroma or volatile changes in cold soaked wines remains, and can be of considerable interest to regions that practice this processing technique.

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CHAPTER II: OPTIMIZING RED AND WHITE WINE ANALYSIS USING THE CONDUCTING POLYMER CYRANOSE® 320 ELECTRONIC NOSE

Abstract

Volatile aroma and flavor compounds make up less than one percent of wine headspace. The majority of wine headspace consists of water vapor and ethanol. However, aroma and flavor compounds create varietal character. Various methods have been established to evaluate volatile aroma and flavor compounds, including electronic nose evaluation. The following study was conducted to minimize the influence of ethanol and monitor its interaction with conducting polymer sensors during wine evaluation. A standard ethanol/water solution was created to match ethanol concentrations of three wine varieties. Varying sample temperatures (7°C, 15°C, 20°C, 30°C) of Cabernet Franc and Traminette wines were also evaluated to establish an appropriate sample temperature. Use of an ethanol standard was shown to minimize ethanol influence in selected sensors by a drop in sensor response during the ENose purge cycle. An increase of 33% to 60% correctly classified wine samples in the cross validation was found when using an ethanol baseline. Samples analyzed with an ethanol baseline showed better canonical discrimination at the 95% significance level. A sample temperature of 30°C was chosen for wine evaluation based on clustering in the canonical distribution. This temperature also showed greatest sensor stability and cleanest sensor response during wine evaluation, as indicated by the Cyranose® 320 scrolling sensor chart.

Introduction

Wine aroma consists of water vapor, alcohols (predominately ethanol), and other volatile compounds in concentrations of 900 g/L, 100 g/L, and about 1 g/L respectfully (Etievant 1991). Wine variety and quality is differentiated by aroma and flavor compound differences, but contribute such a small percentage of headspace compared to water and ethanol. Wine is composed of a mixture of various aroma and flavor compounds including esters, monoterpenes, higher (fusel) alcohols, lactones, organic acids, aldehydes, ketones, acetals, phenolic compounds, sulfur-containing compounds, and nitrogen-containing compounds (Etievant 1991).

The usefulness of electronic nose (ENose) systems in wine aroma and flavor analysis is debatable. Previous studies have reported wine discrimination may be due to varying ethanol concentrations as opposed to aroma and flavor changes (Nanto et al. 1995, Pinheiro et al. 2002, Ragazzo-Sanchez et al. 2005, 2006). Ethanol can interfere with sensors, causing saturation and may mask effects of other volatiles (Pinheiro et al. 2002, Ragazzo-Sanchez et al. 2005). This is of considerable importance when analyzing wines because ethanol is present at much greater concentrations than aroma and flavor volatiles (Etievant 1991). For this reason, the majority of ENose wine research has been conducted using metal oxide sensor (MOS) systems, which operate at much higher temperatures (200 - 400°C) and minimize interference of ethanol and water vapor (Pearce et al. 2003, Mallikarjunan 2005). However, operation at such high temperatures can potentially alter the volatile composition (Mallikarjunan 2005).

Various methods have been used to dilute the ethanol concentration during wine analysis. Several mechanisms have been proposed, purge-and-trap systems being most popular, in efforts to remove ethanol from an alcoholized food system (Ragazzo-Sanchez et al. 2005). Purge-and-trap systems (Porapak traps, cryogenic traps) are typically useful at diluting ethanol concentration, but not removing it completely (Jennings et al. 1972, Ragazzo-Sanchez et al. 2005). Cryogenic traps have been noted to increase water vapor content, hence leading to sensor drift issues when applied continuously (Francis 2000, Ragazzo-Sanchez et al. 2005). In addition, these techniques affect the efficacy of using an ENose to analyze and discriminate among wine samples quickly and efficiently.

Conducting polymer sensors are among of the most selective and sensitive sensors used in ENose systems (Mallikarjunan 2005), but have not been used in many wine studies. Sensors are composed of a carbon- or silicone-base polymer substrate attached to a conductive polymer

and an electrode bridge (Mallikarjunan 2005). Primary polymers used in conductive polymer systems are polypyrrole, polyaniline, polythiophene, polyindole, and polyacetylene, which are doped into a standard oxidative or reductive state (Janata and Josowicz 2003, Pearce et al. 2003). Adsorption of volatiles causes sensor swelling, which results in an electrical conductance change from steady-state conditions and is recorded as a change in resistance (Figure 1) (Janata and Josowicz 2003). The maximum change in resistance between the steady-state and sample cycles is recorded for each coated sensor (Dickinson et al. 1998). The commercial Cyranose® 320 is a conducting polymer ENose that can be trained to minimize ethanol fluctuations among samples and monitor changes in sensor resistance. The objective of this study was to determine an appropriate method for minimizing the ethanol response in ENose sensors, and establish the optimal sample temperature of red and white wines using the conducting polymer Cyranose® 320.

Materials and Methods

The Cyranose® 320 (Smiths Detection®, Pasadena, California) had been previously optimized for use as an efficient system to analyze wine grape volatiles (Athamneh et al. 2008), and was used as a base for wine volatile analysis (Table 1). Purge, sample draw, and gas purge times were altered, according to the manual, to account for the change in headspace volume during wine analysis.

Three wine varieties, Traminette, Cabernet Franc clone 1, and Cabernet Franc clone 313 were evaluated with the Cyranose® 320, using the wine method, without and with an ethanol baseline. Wine samples were drawn from 2 bottles of the same variety and vintage year. Samples evaluated without an ethanol baseline were equilibrated at 30°C for 20 minutes before evaluation. A standard solution of ethanol was prepared using food grade ethanol and distilled water to match the ethanol concentration (v/v) of the three wine samples. Wine samples were equilibrated, as described above. The ethanol solution headspace was drawn into the electronic nose during the 30 second inlet purge cycle prior to analyzing each wine sample.

A 2006 Traminette and Cabernet Franc clone 313 were used as representative wine varieties for optimization of the Cyranose® 320. Both were evaluated by FTIR for alcohol concentration, and a standard ethanol solution was created to act as a baseline during wine analysis.

Twenty replicate samples of Traminette and Cabernet Franc clone 313 wines were prepared for analysis. Twenty mL of wines were volumetrically pipetted into 40 mL glass vials and sealed with a septa. Five samples of each variety were placed at one of four sampling temperatures (7°C, 15°C, 20°C, or 30°C) for 20 minutes for sample equilibrium. After 20 minutes, samples were analyzed by the Cyranose® 320 using the wine method. The Cyranose® 320 contained four internal classification files, one for each sample temperature.

Before analysis, the standard ethanol solution was connected to the Cyranose® 320 via a purge inlet port on the side of the machine (Figure 2). During the Baseline Purge cycle, the machine would draw up the volatile component of the standard solution. Volatiles would interact with sensors prior to the Sample Draw cycle, which could be recorded on a scrolling monitor graph of the sensors. The screen was recorded for one sample of each red and white wine at four sample temperatures.

Data was statistically analyzed using canonical distributions in PC Nose software (Smiths Detection©, Pasadena, California) and JMP version 7.0 (SAS Institute, New Jersey). Additionally, response screens were evaluated on resistance readings of each sensor during evaluation. If the response screen did not show sensor stability during the sample draw cycle (determined via the manufacturer's guidance), the sample temperature was considered un-optimal.

Results and Discussion

A review of how the ENose sensors record changes in volatile concentrations and the method used for wine evaluation are shown in Figures 1 and 2, and Table 1 respectively. The monitoring scroll chart displays the difference between sensor responses with an ethanol baseline during the inlet purge cycle prior to sample evaluation (Figure 3). Sensors that interact with ethanol volatiles dip lower prior to sample evaluation in the steady state, baseline analysis. This initial change in sensor response equilibrates the sensors at the appropriate ethanol concentration. For example, if a thirteen percent ethanol standard is purged into the machine prior to sample evaluation, when a wine of thirteen percent ethanol concentration is evaluated, an increase in sensor response due to ethanol will return back to the initial baseline. Therefore, the ethanol content is not included in resistance change and ethanol fluctuations among wine samples are minimized (Sunshine, 2007, personal communication). This allows for better detection and

discrimination, as well as improved pattern recognition among samples, of potential aroma volatiles (Gardner and Bartlett 1996).

Cross validation results with and without an ethanol baseline are also reported (Figure 4). An increase of 33% to 60% of correctly identified samples in the cross validation results was due to the use of an ethanol baseline. For example, without an ethanol baseline one of the five replicates of Traminette was correctly identified as Traminette by the ENose. However, with the ethanol baseline three of the five Traminette replicates were correctly identified.

Canonical distributions showed improved discrimination of wines when using a standard ethanol/water solution (Figure 5). These results indicate changes in sensor responses to minimize sensitivity of ethanol and water vapor during analysis while maintaining an operating temperature closer to the ambient environment.

In terms of wine sampling temperatures of Traminette and Cabernet Franc clone 1, the greatest degree of canonical distribution clustering was found at 30°C (Figure 6). This result is conducive with manufacturer recommendations. Samples should be at least 7°C to 10°C below the sensor temperature of 40°C for enhanced repeatability during evaluation.

The scrolling charts of wines at each sample temperature are shown in Figure 7. Stabilization of Cyranose® 320 sensors is classified by a specific chart, with a dominate curve after the initial baseline purge cycle. This chart is best represented in Figure 7D with a sampling temperature of 30°C. Based on the canonical discrimination and sensor response scrolling charts, 30°C was chosen for the standard sampling temperature of red and white wines for evaluation by the Cyranose® 320.

Conclusion

This study evaluated the use of a conducting polymer ENose to analyze wine volatiles. The results from this study indicate the potential use of the Cyranose® 320 for wine evaluation with minimized influence from ethanol during evaluation. Therefore, the focus on wine discrimination is potentially based on aroma and flavor volatile variation. Additionally, discrimination of wines at a temperature closer to ambient temperatures was found to adequately differentiate wine volatiles. Compared other ENose analytical methods that require a high degree of heating samples, this is an improvement to evaluation which better represents wine

headspace. These settings optimize discrimination of wines based on actual aroma and flavor volatile differences.

Figure 1: Representation of change in resistance due to sensor swelling during volatile absorption in Cyranose® 320 (Used with permission courtesy of Smith's Detection© 2009)

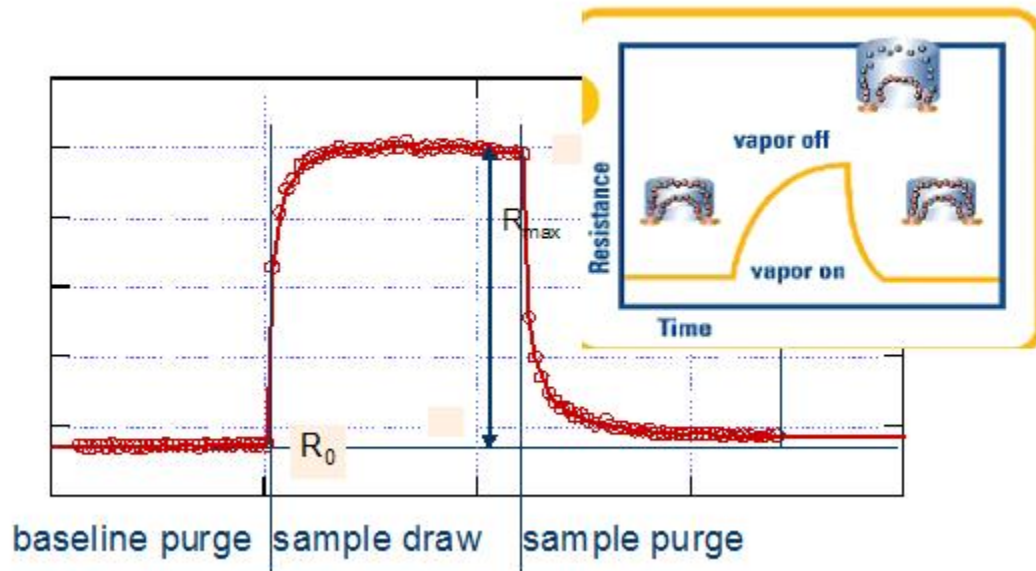


Table 1: Cyranose® 320 methods developed for (A) grape (Athamneh et al. 2008) and (B) wine evaluation

(A) Grape Method			(B) Wine Method		
<i>Setting Name</i>	<i>Setting 1</i>	<i>Setting 2</i>	<i>Setting Name</i>	<i>Setting 1</i>	<i>Setting 2</i>
Baseline Purge	30 sec	Med.	Baseline Purge	30 sec	Med.
Sample Draw 1	40 sec	Med.	Sample Draw 1	20 sec	Med.
Sample Draw 2	0 sec	Med.	Sample Draw 2	0 sec	Med.
Snout Removal	0 sec	Low	Snout Removal	0 sec	Low
1 st Sample Gas Purge	0 sec.	High	1 st Sample Gas Purge	0 sec.	High
1 st Air Intake Purge	10 sec.	High	1 st Air Intake Purge	10 sec.	High
2 nd Sample Gas Purge	90 sec.	High	2 nd Sample Gas Purge	60 sec.	High
2 nd Air Intake Purge	0 sec.	High	2 nd Air Intake Purge	0 sec.	High
Digital Filtering	On		Digital Filtering	On	
Substrate Heater	On		Substrate Heater	On	
Training Repeat Count	1		Training Repeat Count	1	
Identify Repeat Count	1		Identify Repeat Count	1	
Algorithm	Canonical		Algorithm	Canonical	
Preprocessing	Auto-Scaling		Preprocessing	Auto-Scaling	
Normalization	Normal 1		Normalization	Normal 1	
Identification Quality	Medium		Identification Quality	Medium	

Figure 2: Electronic nose set up during wine evaluation (Photograph taken by Denise M. Gardner, December 2007)

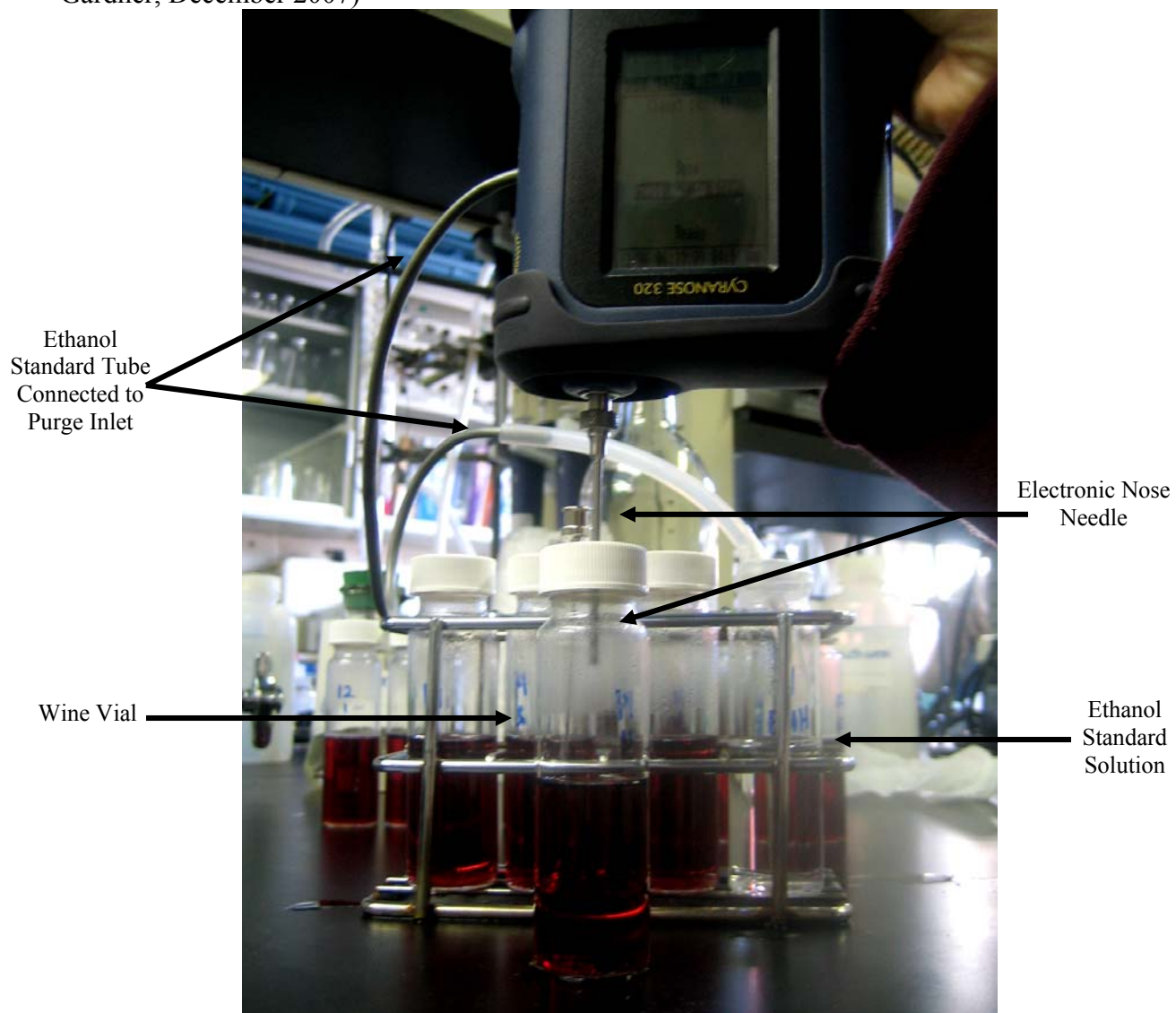


Figure 3: Sensor response screen from PC Nose software showing 32 sensors with influence of ethanol baseline (circled in blue). Ethanol causes the initial dip in sensor response.

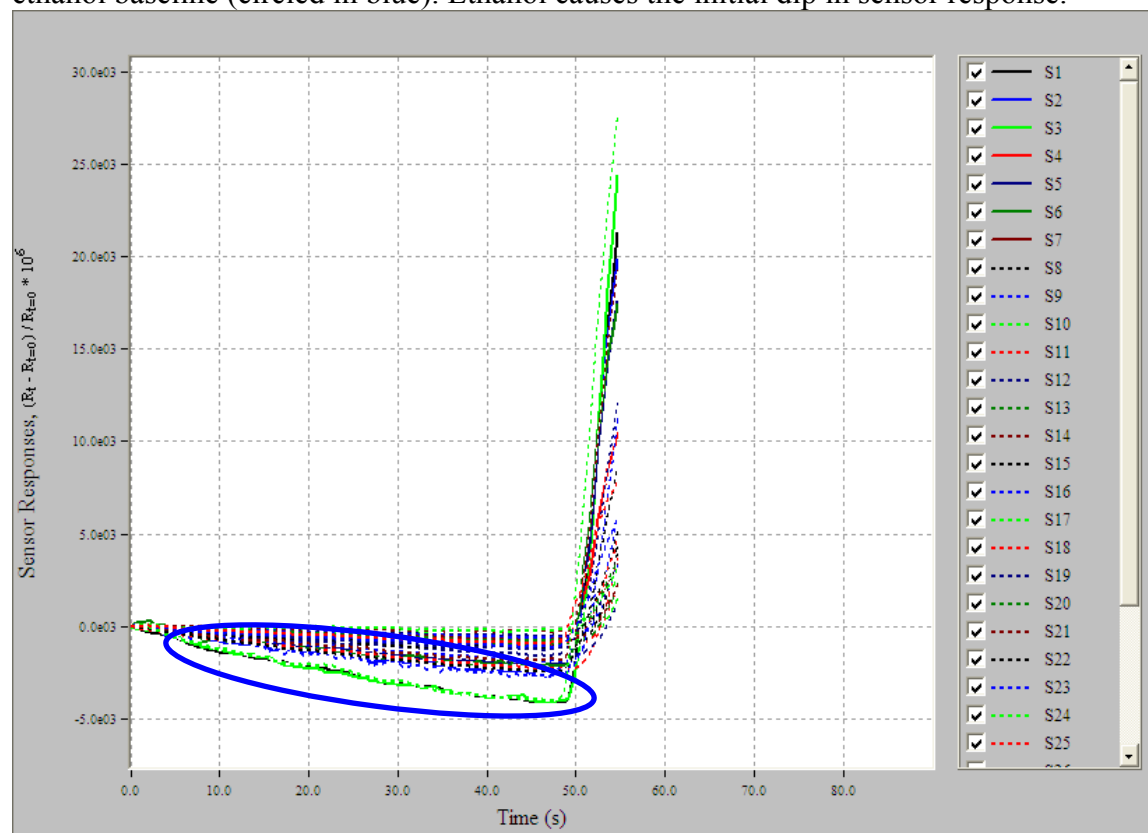


Figure 4: Cross validation (confusion charts) from PC Nose software of Traminette and Cabernet Franc wines (A) without and (B) with the use of an ethanol baseline during wine evaluation

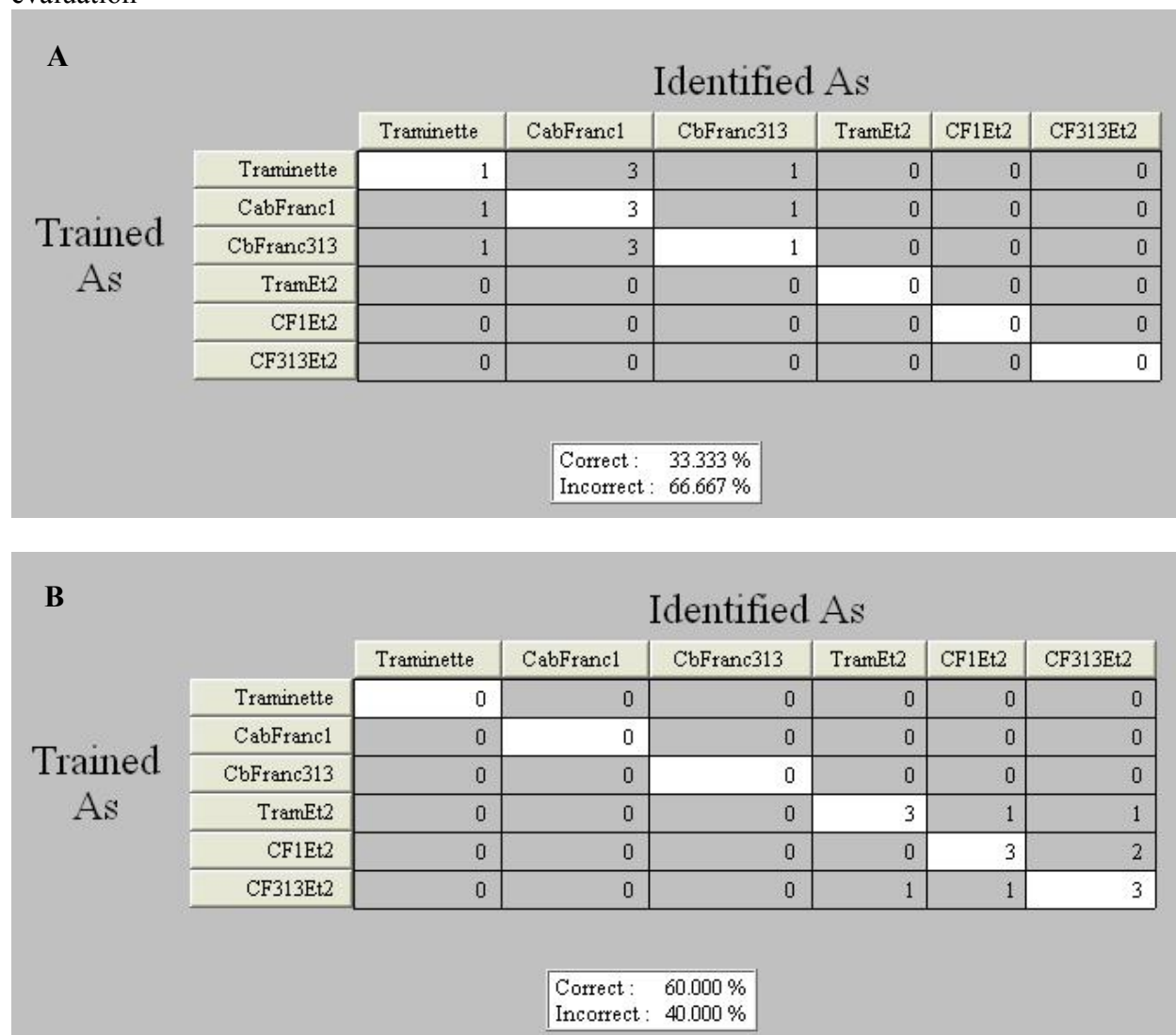


Figure 5: Canonical distribution of Traminette and Cabernet Franc wines (A) without and (B) with an ethanol baseline during wine evaluation

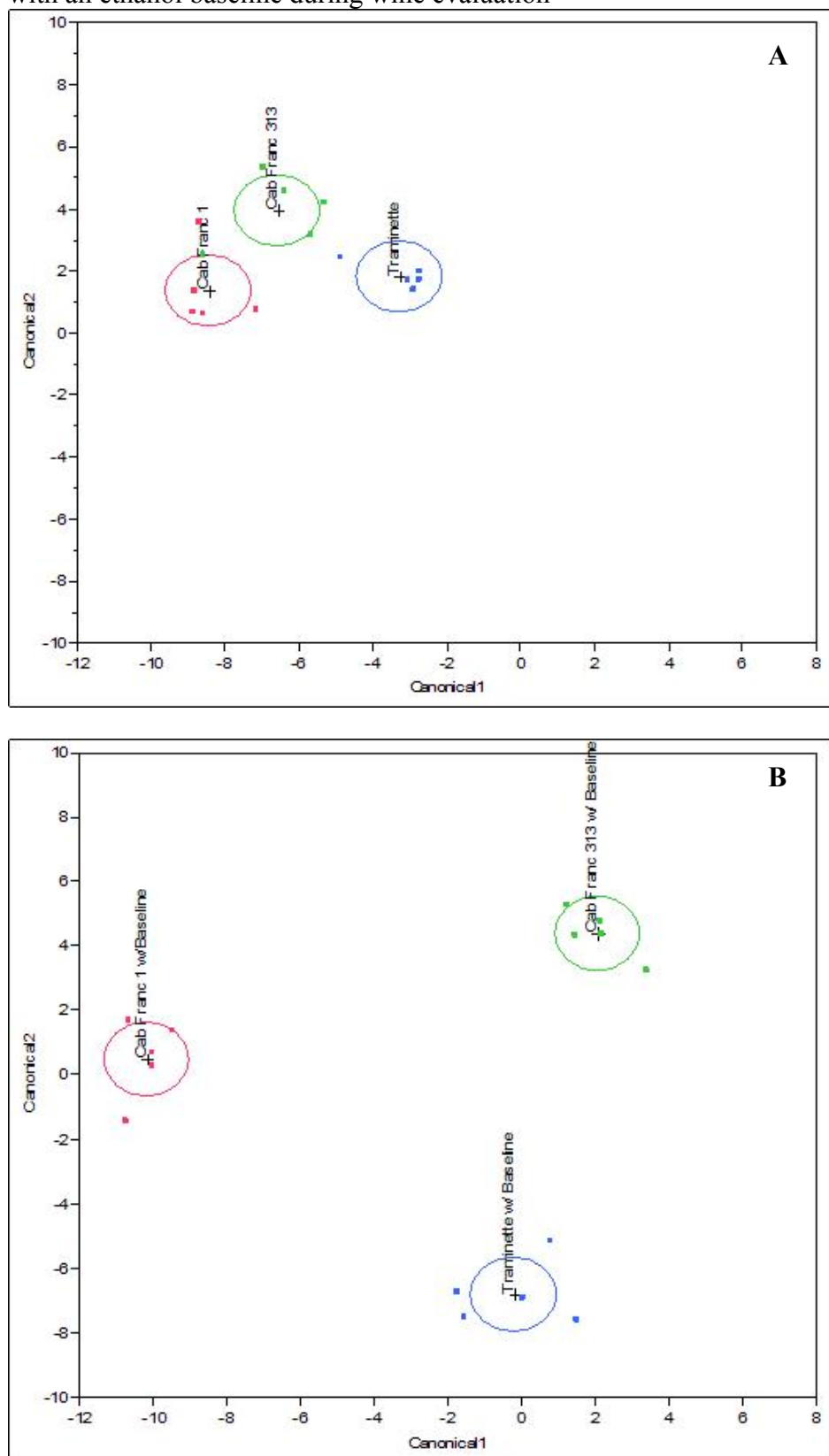


Figure 6: Canonical distribution from PC Nose software of Traminette (numbers “6” through “10”) and Cabernet Franc (numbers “1” through “5”) wine samples at four sampling temperatures (7°C, 15°C, 20°C, 30°C). Non-intersecting numbers are considered significantly different. Black circles indicate clustering of wine varieties. Note that greatest clustering occurs in samples that were heated to 30°C prior to ENose evaluation.

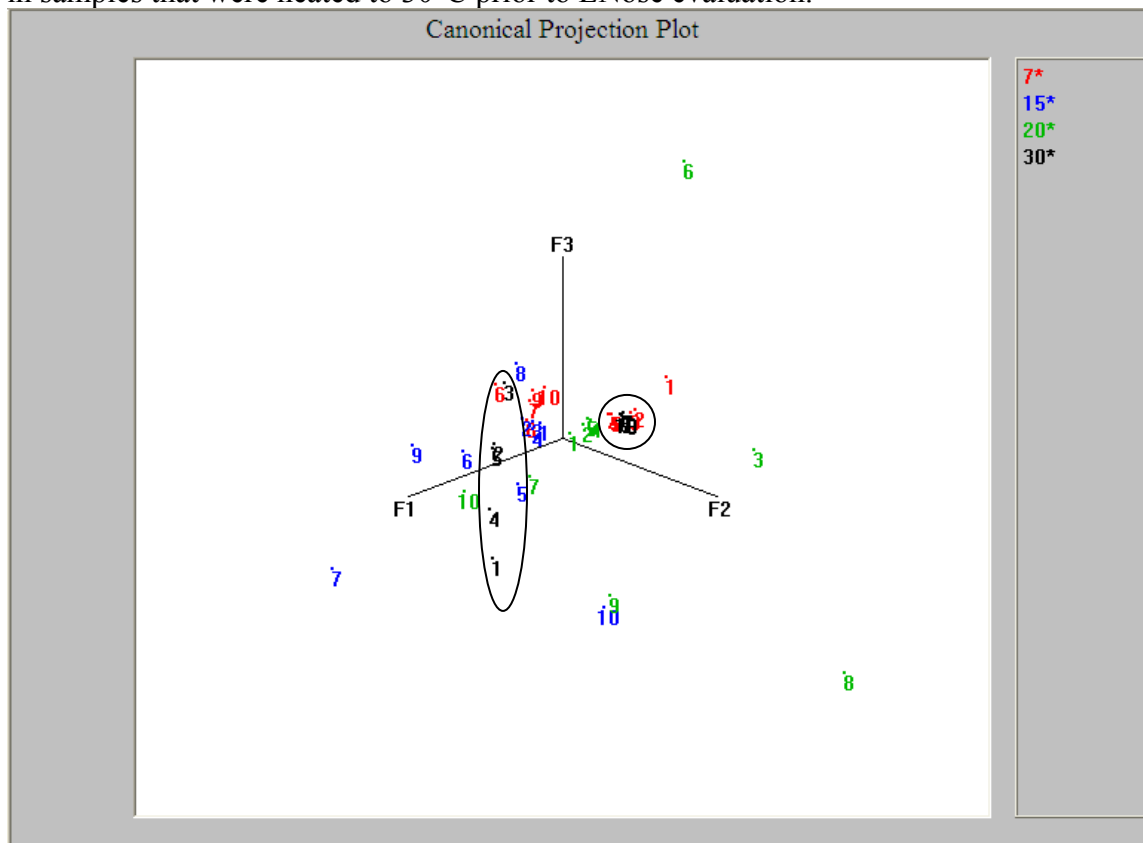
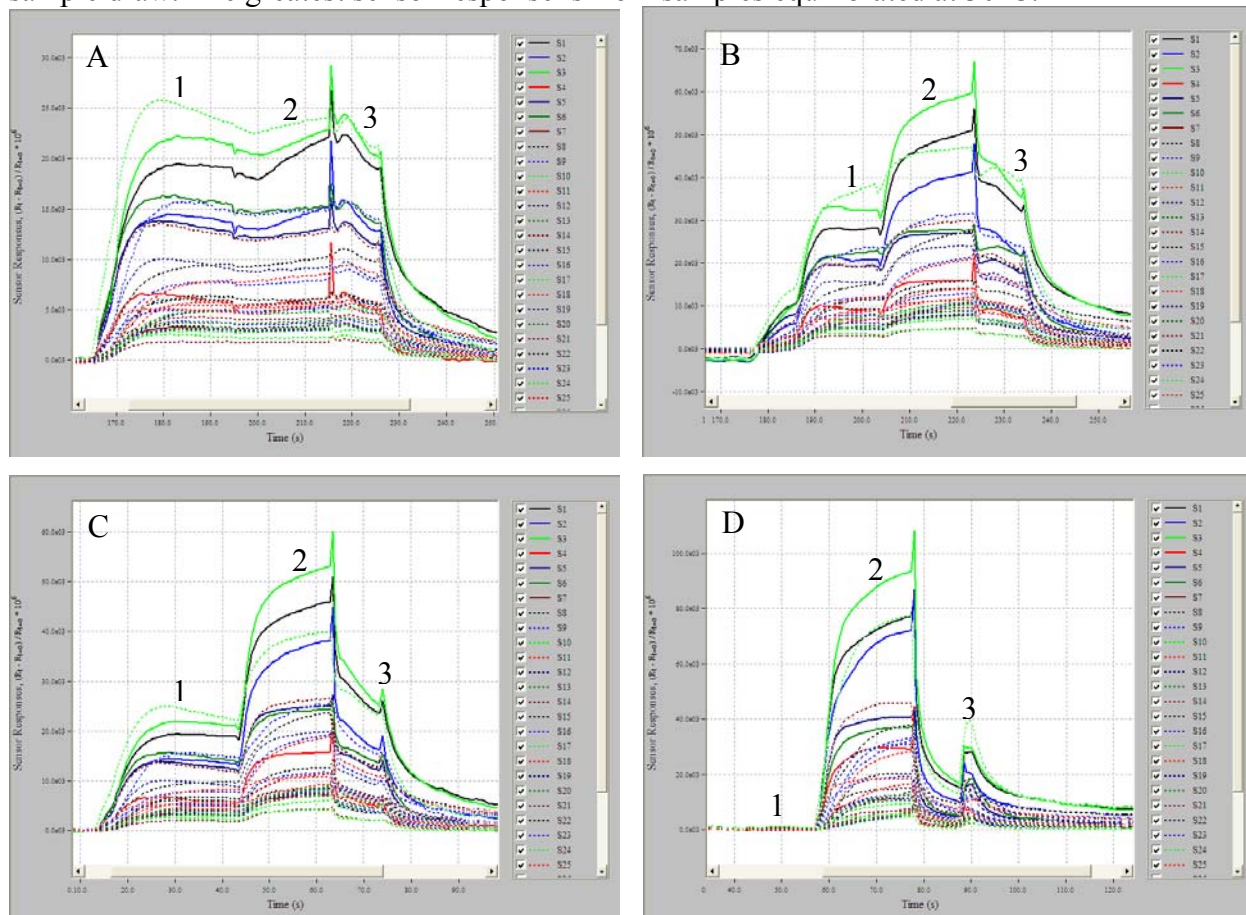


Figure 7: Sensor response with sample temperature at (A) 7°C, (B) 15°C, (C) 20°C, and (D) 30°C. First curve (1) is from the initial purge cycle or baseline, second curve (2) from the sample draw, and the final curve (3) is from the volatile purge cycle and removing ENose from sample. Graph (D) does not display first curve due to the degree of sensor response during sample draw. The greatest sensor response is from samples equilibrated at 30°C.



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CHAPTER III: CHARACTERIZATION OF COLD SOAK ON VITIS VINIFERA L. CV. CABERNET SAUVIGNON GRAPE AND WINE VOLATILES USING AN ELECTRONIC NOSE SYSTEM

Abstract

Cold soak is a pre-fermentation maceration process at cold temperatures used traditionally to enhance red wine color. This study monitored changes in volatiles during a five day cold soak and post-fermentation volatiles of *Vitis vinifera* L. cv. Cabernet Sauvignon in the 2007 and 2008 growing seasons using a commercial conducting polymer electronic nose (ENose). Juice and control and cold soak wine chemistries, GC-MS analysis, and triangle difference sensory evaluation were conducted. PCA distribution of ENose data obtained during cold soak showed PC1 accounted for 92.7% of the variation in 2007 grape musts and 95.7% of the variation in 2008. In comparison, PCA distribution of must chemistries (pH, TA, Brix, absorbance, color hue and intensity, and glycoside content) showed 78.7% of the variation accounted by PC1 in 2007, and 52.4% in 2008. Volatile variations detected by the ENose were displayed in canonical distributions, which were significantly different by treatment. PCA distributions of wine volatiles detected by the ENose had a PC1 which accounted for 100% of the variation. Volatile concentration differences by GC-MS were found based on treatment, although specific compounds varied by vintage year. In 2007, the PCA of volatile compounds 94.1% of the variation accounted by PC1. In 2008, PC1 accounted for 97.1%. Both years showed control wine volatiles associated with ethyl esters, while cold soak wine volatiles were associated with phenethyl acetate and benzaldehyde. Although analytical differences were also shown, sensory evaluation did not demonstrate significant differences between control and cold soak wines of either season.

Introduction

Wine sensory attributes can be influenced by pre-fermentation processing and fermentation techniques, including temperature (McMahon et al. 1999, Reynolds et al. 2001), yeast species and strains (Ramey and Ough 1980), malolactic fermentation (MLF) species and strains (Swiegers et al. 2005), and maceration (Ramey et al. 1986, Sims and Bates 1994, Baldwin 1996). Research has shown cap management (including pre-fermentation maceration) can also influence wine composition (McMahon et al. 1999). Although studies have explored how production methods affect wine composition, few have investigated the cold soak process. Cold soak, or pre-fermentation maceration at temperatures usually less than 10°C, is primarily used for red varieties which have relatively poor wine color stability. Cultivar variation in color stability is related to presence of anthocyanin types (basic anthocyanins vs. acylated derivatives) and concentrations of such forms (Adams 2006). Anthocyanins exist as free (anthocyanidins) and monoglycoside complexes, the most prevalent being malvidin 3-glucoside or its derivatives (Brouillard and Dangles 1994). Stabilization of anthocyanins occurs by complexing with colorless tannins, primarily flavan-3-ol polymers and their monomers (catechin and epicatechin) (Brouillard and Dangles 1994, Adams 2006). Polymerization of anthocyanidin-tannin complexes in grape must is dictated through hydrophobic interactions, and to a lesser extent covalent bonding, by condensation reactions (Timberlake and Bridle 1976, Brouillard and Dangles 1994). It has been proposed that acetaldehyde may mediate these reactions in wines, but polymerization rates may decrease in the presence of alcohol (Timberlake and Bridle 1976, Brouillard and Dangles 1994, Remy et al. 2000). Regardless, cap contact time has been related to enhanced extraction and accumulation of water-soluble tannins, some of which may contribute to anthocyanidin-tannin complexes (Berg and Akiyoshi 1956, Boulton et al. 1996, Heatherbell et al. 1996, Mazza et al. 1999). Such research has influenced the use of cold soak in red varieties.

While Cabernet Sauvignon generally produces wines of high color intensity and stability, and therefore, may not be cold soaked, there is interest in increasing varietal aroma and flavor. Aroma is contributed by volatile compounds that are sensed orthonasally, while flavor is a retronasal sensation of tastes (sweet, sour, bitter, salty, umami) and aromas (Lambrechts and Pretorius 2000, Meilgaard et al. 2007). Several chemical classes of aroma and flavor compounds are found in grapes and wine including monoterpenes, C13-norisoprenoids, fusel (higher molecular weight) alcohols, esters, shikimic acid metabolites, and carbonyl compounds (Etievant

1991, Swiegers et al. 2005). Some of these compounds exist as free volatiles or as non-odorous glycosidically-bound sugar conjugates. Many glycosides, commonly found in higher concentrations than free volatiles in fruit, are in part, aroma and flavor precursors (Abbott et al. 1993, Mansfield and Zoecklein 2003). It has been shown that cold soak can increase glycoside concentrations (Heatherbell et al. 1997, McMahon et al. 1999). Liberation of glycosidically-bound aroma and flavor precursors occurs via acid or enzymatic hydrolysis (Williams et al. 1989, Sefton et al. 1993, Williams et al. 1993, Williams et al. 1996). Acid hydrolysis of glycosides can occur at a pH range from 3.0 to 3.5, splitting the aglycone from the 1→6 glycosidic bond by protonation to form a hydroxyl group onto the sugar (Timell 1964, Williams et al. 1989). Enzymatic hydrolysis cleaves the 1→6 linkage liberating the aglycone from the mono- or disaccharide (Zoecklein et al. 1997). Liberation of aroma and flavor compounds may be exemplified further during aging (Zoecklein et al. 1997).

Aglycones and sugar moieties may vary among grape varieties contributing, in part, to aroma volatile variations (Strauss et al. 1986, Sefton et al. 1993, Williams et al. 1993). These differences may be exemplified through alterations in processing, such as the use of cold soak. Heatherbell et al. (1996) reported Pinot Noir held at 10°C for six days produced wines with higher intensities of woody, tobacco, and berry aromas, as well as greater mouthfeel. Reynolds et al. (2001) showed black current and flavor intensity differences in cold soaked Shiraz wines. Marais (2003) reported cold soaked Pinotage wines had increased berry and plum aroma and flavor intensities, which contributed to improved sensory quality six months post-fermentation.

Sensory evaluation is an important research tool, is subjective, and may require extensive preparation and panel training (Lesschaeve 2007). In addition, it may be difficult to relate individual aroma and flavor compounds to a sensory response or attribute in a complex matrix such as wine (Genovese et al. 2005). Volatile analytical methods are time consuming, require extensive sample preparation, and require training (Mallikarjunan 2005). Therefore, there is interest for an alternative simple and time efficient method for analyzing volatile aroma and flavor.

Use of electronic nose (ENose) systems is an emerging technology using multi-sensor array systems that measure headspace volatiles. ENose variations exist primarily in sensor type, which denotes their selectivity and sensitivity to volatile compounds, and includes metal oxide sensors (MOS), surface acoustic wave (SAW), quartz micro-balance sensors (QMS), and

conducting polymers (CP) (Pearce et al. 2003, Mallikarjunan 2005). ENose systems are analogous to the human nose, in which qualitative and quantitative variations in volatiles may be found usually without identifying or quantifying individual compounds (Craven et al. 1996). ENose systems represent an objective method to estimate volatile composition rapidly (Mallikarjunan 2005).

This study used a conducting polymer ENose consisting of 32 carbon- or silicone-based sensors. As volatiles are adsorbed onto the surface of polymer coatings sensors swell which causes a change in their electrical resistance (Mallikarjunan 2005). A graphical representation of resistant change is interpreted as a “smell print” for a given sample, and can be analyzed using multivariate canonical distribution. The objective of this study was to monitor Cabernet Sauvignon cold soak musts and wine volatiles by ENose analysis, and to compare ENose results with GC-MS and sensory analysis.

Materials and Methods

Harvest and Fermentation

Cabernet Sauvignon (*Vitis vinifera* L.) clone 8 grapes were grown on an open lyre training system at Winchester, Virginia. Approximately 136 kg and 227 kg of grapes were hand harvested on 29 October 2007 and 13 to 15 October 2008, respectively. Grapes harvested in 2007 were frozen at -20°C and thawed to ambient temperature prior to processing. Grapes harvested in 2008 were stored at 7°C and processed within 24 hours of harvesting. Grapes were crushed and destemmed using a Wottle (Anton, Poysdorf, Austria) destemmer crusher with 50% berry breakage, estimated visually. Must and berries were evenly distributed into six open-top 60 L Nalgene fermenting bins of equal height and volume. Treatment consisted of three control and three cold soak replicates randomly selected. Each replicate was treated with 250 mg/L Velcorin® (Scott Laboratories, California) dimethyl-dicarbonate (DMDC) and chilled for 24 hours at 7°C .

Prior to inoculation, TA, pH, and fermentable nitrogen adjustments were made using tartaric acid, sucrose, and FermAid K (Lallemand, Rexdale, Ontario) and diamonium phosphate (DAP) one third of the way through fermentation (Table 1). Adjustments to nitrogen were made for an optimum of 150 mg/L fermentable nitrogen availability. The three cold soak replicates for each season were stored at 7°C for an additional five days. During cold soak, caps were punched

once a day, and sampled daily. At the end of the cold soak period, musts were inoculated, fermented, and stored as described below. Musts were inoculated with *Saccharomyces cerevisiae* ICV-D21® (Lallemand, Rexdale, Ontario) at a rate of 25 g/hL following hydration as per supplier recommendations with additions of 30 g/hL GoFerm (Lallemand, Rexdale, Ontario). Caps were punched three times daily. Fermentation was monitored by hydrometry and proceeded at 23°C ± 2°C. At dryness (<1% residual sugar), wine was pressed using a basket press. Free and press fractions were kept separate and stored at 7°C for 24 hours prior to racking into 3.80 L and 1.90 L glass bottles and stored at 7°C.

Berry, Juice, and Wine Chemistry

For 2007 fruit, six randomly selected clusters were weighed and crushed in Premium Red Line 6" X 8" plastic sampling bags (Minigrip®, Texas) while in 2008, 600 berries were selected, weighed, and crushed for estimated fruit chemistry. Crushed fruit was filtered through 0.45 µm syringe filters (Whatman®, New Jersey) and analyzed for pH, TA, and Brix using standard methods as described by Zoecklein et al. (1999). Malic acid content was determined using an L-Malic Acid enzyme analysis kit (R-Biopharm AG, Germany). Color intensity ($A_{420} + A_{520}$) and color hue (A_{420}/A_{520}) were estimated using a Genesys™ 5 spectrophotometer (Spectronic®, UK). Fermentable nitrogen was measured enzymatically (Megazyme®, Ireland).

Juice samples, taken at crush and throughout cold soak, and wines were analyzed for pH, TA, Brix, malic acid content, and color (intensity and hue) as described above. Juice results were used for any possible must adjustments. Phenol-free glycosyl-glucose (PFGG) and total glycosyl-glucose (TGG) were determined as described by Williams (1995) and modified by Zoecklein et al. (2000). Wine residual sugar concentration was estimated by Clinitest (Bayer, Elkhart, Indiana) and ethanol percentage (v/v) was evaluated using FTIR (Foss WineScan™ FT 120, Eden Prairie, Minnesota).

Wine samples for GC-MS analysis were prepared using 4 mL sample with salt (1.0 g) in 10 mL clear glass vials sealed with a septa (MicroLiter® Analytical Supplies, Inc., Georgia). Vials had a pre-incubation time of 30 seconds at 30°C with agitation at 250 rpm. A CAR/DVB/PDMS Grey SPME Fiber (Supelco Sigma-Aldrich, St. Louis, Missouri) was used to penetrate vials to a 32 mm depth. A GC-MS (Agilent Technologies, 6890N Network GC System, 5975B inert MSD) with injector temperature of 250°C, DB-Wax column (30x25x25), and helium carrier gas with a flow rate of 1 mL/min were used. Oven temperature was 40°C

with a ramp rate of 6°C per minute to 230°C. Volatile quantification was done using 42 standard volatiles reported in Cabernet Sauvignon wines (Shimoda et al. 1993, Genovese et al. 2005, Gurbuz et al. 2006). Forty-two standard compounds and wine samples were manually integrated and quantified. In wine samples 54 compounds were identified via ion matching.

Juice and wines were analyzed using a conducting polymer, 32 sensor, Cyranose 320™ unit (Smiths Detection®, Pasadena, California). Instrument settings were altered using a previously optimized evaluation method (Athamneh et al. 2008) and from manufacturer suggestions (Table 2). Ethanol standard solutions were used to create a baseline to minimize differences in alcohol concentrations among wine samples. For ENose evaluation of samples, three aliquots (20 mL) from each replicate throughout cold soak and post-fermentation were pipetted into 40 mL clear glass vials sealed with Teflon/Silicone 0.100" septa top (MicroLiter® Analytical Supplies, Inc., Suwanee, Georgia) and incubated in a 30°C water bath for 20 minutes. A venting needle (22 gauge) was injected into the septa followed by injection of the Cyranose 320™ needle for headspace evaluation.

Sensory Analysis

Sensory testing was conducted in the Sensory Evaluation Laboratory at Virginia Tech using standard conditions as described by Meilgaard et al. (2007). Untrained panelists were seated at individual light controlled booths for evaluation. The majority of panelists were students between the ages of 21 to 25, who consumed wine regularly (more than once a week), as recorded by a demographic survey given to each panelist prior to wine evaluation. Participation in sensory evaluation was a class requirement for most students. Triangle difference sensory tests for wine aroma were conducted at two intervals: six months post-fermentation in 2007 wines and five months post-fermentation in the 2008. A triangle test to compare 2007 control and 2008 control comparison was also conducted at the second sensory session during the 2008 evaluation. Treatment replications were pooled prior to evaluation following a sensory screening for sulfur-like off odors.

A balanced order of presentation for all combinations (n=6) was used. Wine samples were each identified with a randomly selected three digit code, poured into standard ISO glasses, and filled with 20 mL of wine. Each glass was topped with plastic Petri dishes and presented at 19°C ± 2°C under red lights. A total of 54 panelists were used ($\alpha = 0.05$, $\beta = 0.10$, $\rho_{\max} = 30\%$, 25 correct responses needed for significant difference) for evaluations (Meilgaard et al. 2007).

Statistical Analysis

ENose data was analyzed by linear canonical distribution and principal component analysis (PCA) using JMP Version 7 (SAS Institute, New Jersey). Chemistries and GC-MS data was also analyzed by PCA. Juice and wine chemistry data, and GC-MS volatile concentrations were statistically analyzed using one-way ANOVA and Student's t-test for least significant differences in JMP Version 7 (SAS Institute, New Jersey). Chemistry data was additionally analyzed using linear regression in JMP Version 7 (SAS Institute, New Jersey).

Results and Discussion

Must adjustments and ENose operational parameters are listed in Tables 1 and 2, respectively. In 2007 and 2008, canonical distribution showed 100% separation of the volatile component, detected by the ENose, every day during cold soak (Figure 1). Three-dimensional canonical distributions cluster ENose data based on the greatest degree of variation using three canonical scores. Each circle surrounding data clusters represents the multivariate mean for that group at a 95% significance level. Circles that do not intersect or overlap, which is not a cause of axis depth, represent significant differences. Principal component analysis (PCA) distribution of 2007 and 2008 juice is shown in Figures 2A and 2B, respectively. In 2007, PC1, which is the three-dimensional axis not labeled on the graph, accounted for 92.7% of the variation, PC2 as 6.4%, and PC3 as 0.6%. Days 1 and 2 are below the biplot ray plane, but day 3 was associated primarily with sensors 7 and 27, day 4 with sensors 6 and 17, and day 5 with sensors 21 and 28. Cross validation, also known as a confusion chart and used to determine electronic nose system performance (Santos et al. 2004a), reported a 69% of samples correctly classified (data not shown). This indicates the percentage of samples that were matched correctly with their appropriate class designation. In 2008, PC1 accounted for 95.7% of the variation, PC 2 as 3.7%, and PC3 as 0.4%. Based on the biplot ray position, days 1, 2, and 3 are below the biplot ray plane, but day 4 was mostly associated with sensors 24, 30, and 31 while day 5 with sensor 5. Cross validation indicated 82% correctly classified samples using the commercial ENose. These results indicate the ability of the ENose to discriminate volatile differences over time due to processing alterations, which has also been applied in previous studies on beer (Pearce et al. 1993), yogurt (Cimander et al. 2002), tea (Dutta et al. 2003), and ginseng (Lee et al. 2005).

Must chemistries are listed in Tables 3 and 4. Total glycosides (TGG) did not differ either year during cold soak. Phenolic-free glycosides (PFGG) decreased throughout cold soak. Greater concentrations of TGG and PFGG were estimated in 2008 than in 2007, possibly as a function of seasonal variation (Lee and Jaworski 1989) and/or extraction variation during cold soak. Williams et al. (1996) has contributed concentration decreases of GG to glycoside hydrolysis, which may be a function of native enzymatic hydrolysis. Aryan et al. (1987) observed that β -glucosidase activity increased as berry ripeness increased. The presence of sugar can reduce enzyme activity of β -glucosidase, but native grape enzymes can retain 50% of the maximum activity at 100 mM glucose concentrations (Aryan et al. 1987).

An increase in pH during cold soak occurred in 2007 and 2008 musts, which is supported by Ough (1969) and Heatherbell et al. (1996), and may have resulted from increased extraction (Boulton 1980). Seasonal variation may have affected the slighter increase of pH in 2008.

A decrease in Brix during cold soak was found in 2007 and 2008. However, Brix values were not significantly different at inoculation of control or cold soak treatment and not a function of fermentation. Therefore, it was assumed that differences in Brix may have been contributed by possible analytical error.

In 2007, cold soak musts increased in hue and decreased in intensity. In 2008, hue decreased and intensity increased. An increase in absorption at 420 nm, as was found in 2008, has been recorded with increased skin contact (Singleton et al. 1980, Reynolds et al. 2001). Small changes in absorbance at 520 nm may be due to slow extraction of pigmented compounds at cold soak temperatures (Sacchi et al. 2005). In 2007, the increase in hue was due to a decrease in absorption at 520 nm while in 2008, absorption at this wavelength increased. Increased absorption at 520 nm may have been indicative of greater extraction of anthocyanic glycosides during cold soak. Variation between the two years was contributed to seasonal variation and possible extraction differences during cold soak.

Regression analysis indicated differences in juice chemistry attributes over the five day cold soak (Table 5, raw data shown in Appendix). In 2007, pH, TA, absorbance at 420 nm and 520 nm, color intensity, and PFGG had trends associated with the cold soak time period. In 2008, attributes that differed over time included pH, TA, Brix, absorbance at 280 nm and 520 nm, color hue and intensity, and PFGG. The increased absorbance at 280 nm estimates increased extraction of phenolic compounds. Increased concentrations of non-volatile phenolic

compounds have been shown to decrease the volatility of some volatile aroma and flavor compounds (Aronson and Ebeler 2004). Similar trends found in both years included an increase in pH, decrease in TA, and a decrease in PFGG throughout cold soak. Such trends may be due to the cold soak process.

A PCA distribution of 2007 chemistries resulted in 78.7% variation from PC1, 10.2% from PC2 and 8.1% from PC3 (Figure 3A). Based on the biplot ray positions, day 1 was mostly associated with absorbance at 520nm and color intensity, day 2 with PFGG, day 3 with PFGG and pH, day 4 with pH and color hue, and day 5 with absorbance at 320. In 2008, PC1 accounted for 52.4% of the variation, PC2 with 32.0%, and PC3 with 10.5% (Figure 3B). Day 1 was associated with color hue, day 2 with Brix, day 3 with absorbance at 280 nm and 320 nm, day 4 with absorbance at 520 nm, and day 5 with color intensity.

In the PCA distribution of 2007 and 2008 volatiles analyzed by the ENose, PC1 accounted for 100% of the variation (Figure 4). In 2007, control volatiles were associated with sensors 10 and 25 while the cold soak volatiles had no particular association. In 2008, control volatiles were associated with sensors 6, 13, 24, 26, and 32 while cold soak volatiles were directly opposite of the control placement below the biplot ray plane. ENose measurements were also analyzed by a canonical distribution in which volatile differences based on treatment immediately post-fermentation (Figures 5A and 6A) and six months post-fermentation (Figures 5B and 6B). Cross validation indicated 59% correct classification for 2007 wines immediately post-fermentation, and 95% for 2008 wines (data not shown). This indicates better discrimination in 2008 wines than in 2007, possibly due to greater differences in the volatile composition based on treatment. Differences detected between treatments demonstrate the potential use of a conducting polymer ENose to analyze wine volatiles.

These results were conducive with GC-MS data, which showed concentration variation of individual volatile compounds based on treatment (Table 6). Significance in volatile concentrations varied between years, which were expected due to inherent seasonal variation and possible freezing effects of 2007 grapes. However, several general trends were found. Ethyl esters were found in higher concentrations in control wines, which may be a factor of skin contact duration (Falque and Fernandez 1996, Lambrechts and Pretorius 2000). In the 2007 vintage, most ethyl esters were found significantly higher in control wines with exception to ethyl palmitate. However, ethyl palmitate was significantly higher in 2008 control wines. Ethyl

acetate was found at a higher concentration in the cold soak wines, significantly in 2007. It has been linked to increased skin contact time (Falque and Fernandez 1996). Other compounds that were found in greater concentrations of cold soak wines include benzaldehyde in 2007 and diethyl succinate in 2008.

PCA distributions of GC-MS data for 2007 and 2008 wines are shown in Figure 7. In 2007, 94.1% of the variation was from PC1. Control wines were related with ethyl palmitate, ethyl myristate, ethyl dodecanoate, ethyl decanoate, octanoic acid, phenethyl alcohol, methyl salicylate, and citronellol as determined by biplot ray positions. Cold soak wines were associated with phenethyl alcohol, benzyl alcohol, benzaldehyde, cis-3-hexenol, hexyl acetate, and ethyl acetate. In 2008, PC1 accounted for 97.1% of the variation. Control wines were associated with many of the same volatiles as 2007 control wines with exception to octanoic acid, phenethyl alcohol, methyl salicylate, and citronellol, but additionally with 2-ethyl-1-hexanol. In general, ethyl esters were primarily coupled with control wines in PCA distributions, which are consistent with concentration results from GC-MS data. Cold soak wines were related to phenethyl acetate, hexanoic acid, benzaldehyde, n-hexanol, n-butanol, gamma-nonalactone, and 1-octanol.

Each sensor in the conductive polymer ENose is selective towards a broad range of compounds (Mallikarjunan 2005). Polymers that are primarily used include polypyrrole, polyaniline, polythiophene, polyindole, and polyacetylene at different oxidative or reductive states providing selectivity (Mallikarjunan 2005, Pearce et al. 2003). One of the primary problems with conductive polymer sensors includes the influence of ethanol (Pinheiro et al. 2002, Ragazzo-Sanchez et al. 2006) and water vapor (Reddy and Payne 1999, Janata and Josowicz 2003). To avoid this interference, MOS systems have been the ENose units of choice for wine analysis (Di Natale et al. 1996, Chatonnet and Dubourdieu 1999, Santos et al. 2004b). However, MOS systems heat samples to 400 to 600°C thereby potentially changing volatile composition (Mallikarjunan 2005).

The conductive polymer system used in this study minimized the effects of both ethanol and water vapor interferences. An increase of 33% to 60% of correctly identified samples in the cross validation results was due to the use of an ethanol baseline (data not shown). Canonical distributions from a previous evaluation of three wines have shown improved discrimination of samples when using a standard ethanol/water solution (Figure 8). These results indicate

minimized sensitivity of ethanol and water vapor during analysis while maintaining an operating temperature closer to the ambient environment. It also implies that treatment discrimination based more prominently on possible wine aroma and flavor volatiles.

Wine chemistries are described in Table 7. No differences were found in TGG or PFGG of either vintage, but an increase in concentration of glycosides occurred from the end of cold soak to the end of fermentation in both years. Rates of extraction and/or hydrolysis may have differed contributing to lack of difference in PFGG between treatments as suggested by Williams et al. (1996). A reduction in PFGG may have contributed to possible liberation of volatiles, detected by GC-MS and ENose analysis. A possible influence to glycoside hydrolysis is wine pH, which was higher in cold soak wines in 2007 and lower in 2008. Studies on glycoside hydrolysis have shown that at pH 3.2, liberation of various aroma compounds, including linalool, α -terpineol, β -damascenone, benzaldehyde, and benzyl alcohol, which were found in Cabernet Sauvignon wines of this study, at concentrations from 0.1 to 500 $\mu\text{g/L}$ (Williams et al. 1989, Francis et al. 1996, Francis et al. 1998).

Wine chemistries were analyzed in PCA distributions for both years (Figure 9). In 2007, PC1 accounted for 92.7% of the variation. Control wines were related to TGG while cold soak wines were associated with color hue. In 2008, PC1 accounted for 100% of the variation in which control wines were associated with TGG and PFGG, while cold soak wines were related to absorbance at 420 nm and color intensity. Results indicate discrimination of control wines based on glycoside content while cold soak wines were predominated affected by color components.

No significant differences based on treatment were found during sensory evaluation (Table 8). Aroma unit (AU) values for select compounds are found in Table 9. Compounds with an AU greater than 1.0 are considered to have a contribution to the aroma of that wine sample. Ethyl acetate and phenethyl alcohol were the only two compounds found to have a greater contribution in cold soak wines. Based on AU values ethyl heptanoate, ethyl hexanoate, ethyl octanoate, ethyl palmitate, and isoamyl acetate contributed more to the control wines, which is consistent with the GC-MS data. Other compounds that were found to have AU values greater than one, but exhibited no treatment pattern between the two years included isovaleric acid, β -damascenone, phenethyl acetate, and 3-methyl butanol.

Past studies that have evaluated cold soaked wines have consistently found variation in aromas (Heatherbell et al. 1996, Reynolds et al. 2001, Marais 2003). However, these studies all evaluated aroma differences using an expert or trained sensory panel. Although differences may have been detected in this study if a trained panel was used, there is a large difference between the purpose of trained panels and consumer acceptance. However, judge variability is often one of the greatest variables in consumer evaluation (Meilgaard et al. 2007, Cortell et al. 2008).

Ethanol concentration was higher in cold soak wines. Variation in ethanol concentrations between treatments may have affected panelists' degree of perception as suggested by Williams and Rosser (1981). Changes in ethanol (0.5 to 0.75%) can alter the perception of some volatile compounds (Williams and Rosser 1981). Ethanol concentrations in cold soak wines varied from controls by about 1.5 to 1.6%, indicating that ethanol may have influenced sensory panelists. This fluctuation in ethanol is minimized during ENose evaluation due to the ethanol baseline during wine analysis, which may provide some insight into why differences are detectable by ENose and not sensory.

The ENose is one of the only analytical tools to closely resemble the human sensory system (Mallikarjunan 2005). However, differences between volatile detection by ENose and aroma discrimination during sensory evaluation do exist. ENose evaluation is based on sensor interaction with volatile compounds of a particular molecular weight, chemical class, and concentration. In aroma sensory evaluation, an aroma is detected by receptor proteins in the olfactory epithelium in which a threshold concentration must be met in order for the brain to recognize what that aroma is or variations in intensity. Aroma volatiles act antagonistically or synergistically towards one another, often in which some volatiles minimize or mask the perception of others in a wine matrix (Etievant 1991). One primary example of this is the presence of ethyl acetate, detected in this study, which masks the perception of other volatiles at lower concentrations (Etievant 1991). Such interactions may have affected sensory evaluation during this study.

Conclusion

This study evaluated the effects of cold soak on changes in volatile composition. The results from this study demonstrate use of a conducting polymer ENose to monitor grape volatiles over time and when possible volatile changes occur during cold soak. In regards to

wine analysis, it shows the potential of using a conducting polymer ENose system while minimizing influences of ethanol and water vapor. It is also one of the first studies to compare ENose results with standard wine chemistries, GC-MS, and sensory analysis which provide insight into what may be detectable by or impact results of the ENose. In this study, although consumers did not detect aroma differences during sensory evaluation that were detected by ENose and GC-MS, use of the ENose exhibited results that may reflect the possibility of using it as a screening tool. The ENose can be used for a rapid analysis and provides quick results. ENose data would provide researchers with insight on allocating resources and time into a sensory panel.

The use of ENose systems in the wine industry has become of greater interest in the past years. Many question its usefulness in the winery. This study is one of the first to show the ENose and its practical potential as a quality assurance instrument to monitor changes in wine volatiles. Although this study focused on the discrimination and training of an electronic nose, industry professionals may potentially use the machine to “match” wines to a previously trained system of a desired quality and enhance uniformity of production wines. Such practices may ultimately improve wine quality for a commercial winery.

Table 1: Cabernet Sauvignon must additions (per volume) to adjust TA, Brix, and fermentable nitrogen content prior to fermentation

Vintage	Tartaric Acid (g)	Sucrose (g)	FermAid K (g)	DAP (g)
2007	44.9	343.67	1.40	0.90
2008	NA	NA	6.10	6.1

NA – Indicates that no additions were added to that vintage year

Table 2: Cyranose® 320 electronic nose wine evaluation parameters. The last four settings describe statistical analysis details.

Method Setting	Setting Parameters
Baseline Purge	30s
Sample Draw	20s
Air Intake Purge	10s
Sample Gas Purge	60s
Digital Filtering	On
Substrate Heater	On: 40°C
Training Repeat Count	1
Identifying Repeat Count	1
Algorithm	Canonical
Preprocessing	Auto-scaling
Normalization	Normal 1
Identification quantity	Medium

Figure 1: Canonical distribution of differences detected by ENose analysis during five day cold soak of (A) 2007 and (B) 2008 Cabernet Sauvignon must (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles)

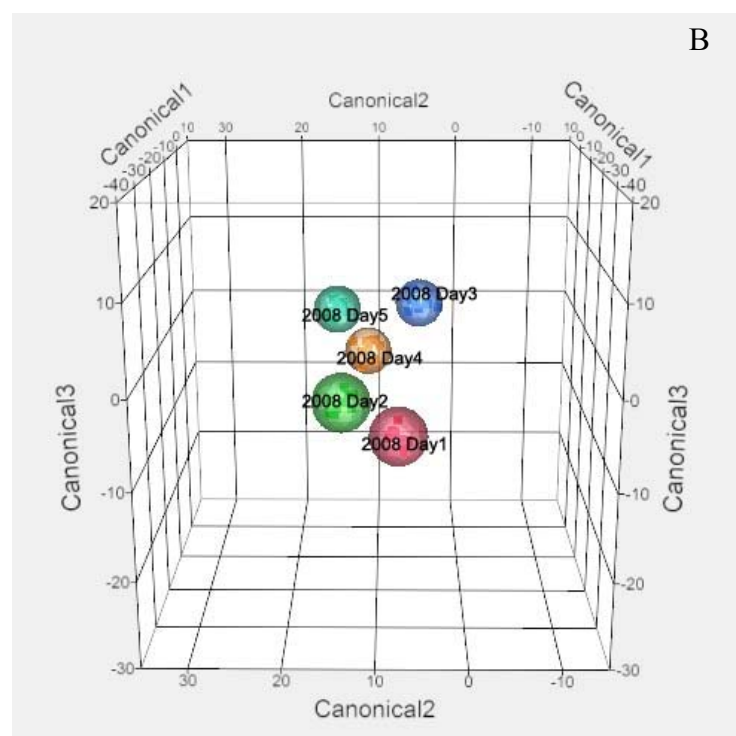
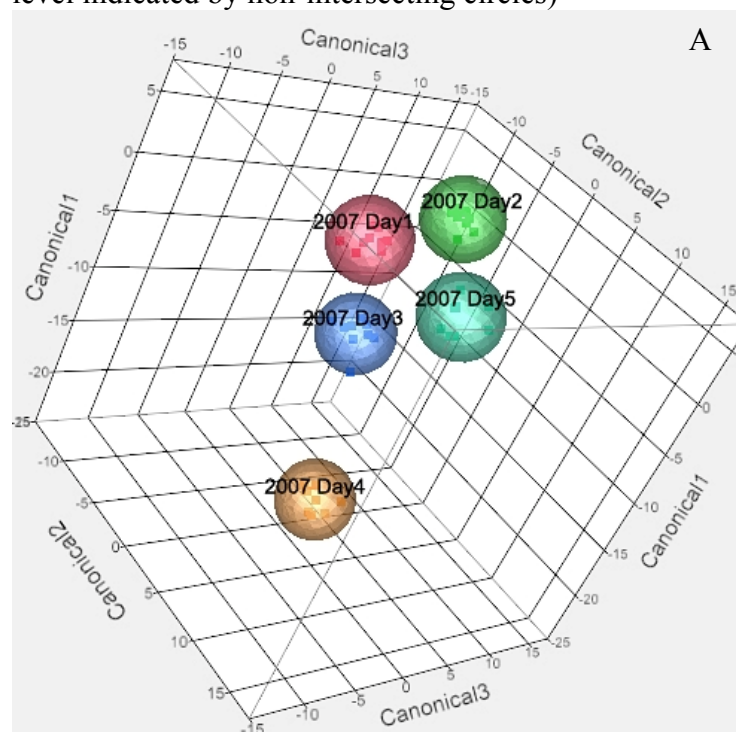


Figure 2: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon cold soak must volatile detected by ENose during cold soak

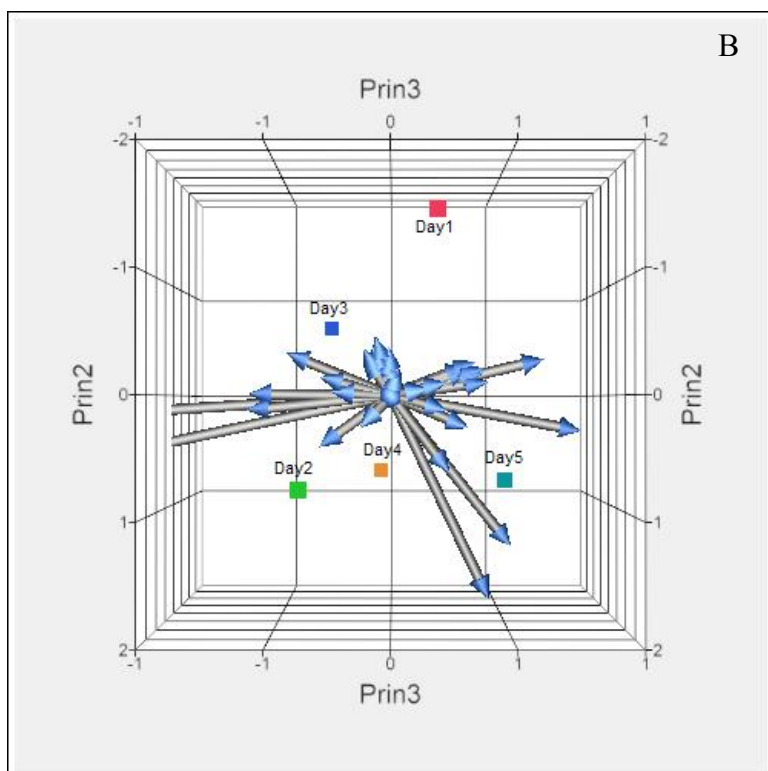
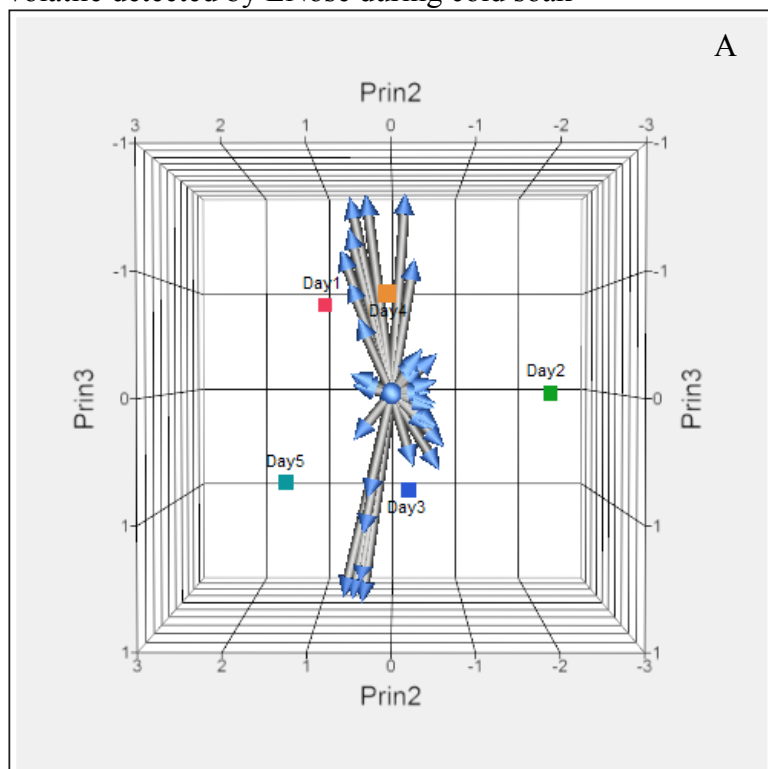


Table 3: Juice (A) pH, titratable acidity (TA), Brix, phenol-free glycosyl-glucose (PFGG) and total glycosyl-glucose (TGG), and (B) absorbance (280 nm, 420 nm, 520 nm) and color (hue and intensity) throughout five day cold soak in 2007 Cabernet Sauvignon musts (n=3)

A. Cold Soak Day	pH	TA (g/L tartaric)	Brix	PFGG (μM)	TGG (μM)
Day 0	3.28±0.03 ^d	7.16±0.20 ^a	22.2±0.1 ^b	NA	NA
Day 1	3.21±0.03 ^d	7.12±0.20 ^a	23.0±0.1 ^a	431±91 ^{ab}	875±113 ^a
Day 2	3.45±0.03 ^c	6.31±0.20 ^b	22.9±0.1 ^a	568±91 ^a	830±113 ^a
Day 3	3.58±0.03 ^b	5.80±0.20 ^{bc}	22.2±0.1 ^b	465±91 ^{ab}	833±113 ^a
Day 4	3.68±0.03 ^a	5.50±0.20 ^c	22.3±0.1 ^b	225±91 ^b	765±139 ^a
Day 5	3.65±0.03 ^{ab}	5.30±0.20 ^c	22.2±0.1 ^b	260±91 ^b	805±113 ^a

NA – Indicates that no data points are available for those samples

B. Cold Soak Day	A_{280nm}	A_{420nm}	A_{520nm}	Color Hue	Color Intensity
Day 0	1.11±0.02 ^a	0.14±0.02 ^a	0.22±0.01 ^a	0.63±0.03 ^{ab}	0.35±0.03 ^a
Day 1	1.11±0.02 ^a	0.10±0.02 ^{ab}	0.18±0.01 ^{ab}	0.55±0.03 ^b	0.28±0.03 ^{ab}
Day 2	1.12±0.02 ^a	0.09±0.02 ^{ab}	0.16±0.01 ^{bc}	0.59±0.03 ^{ab}	0.25±0.03 ^b
Day 3	1.12±0.02 ^a	0.09±0.02 ^b	0.15±0.01 ^{bc}	0.63±0.03 ^{ab}	0.24±0.03 ^b
Day 4	1.12±0.02 ^a	0.09±0.02 ^b	0.14±0.01 ^c	0.66±0.03 ^a	0.23±0.03 ^b
Day 5	1.13±0.02 ^a	0.09±0.02 ^{ab}	0.14±0.01 ^c	0.66±0.03 ^a	0.23±0.03 ^b

Values with different letters within a column indicate significant differences ($\alpha = 0.05$)

Table 4: Juice (A) pH, titratable acidity (TA), Brix, , phenol-free glycosyl-glucose (PFGG), and total glycosyl-glucose (TGG), and (B) absorbance (280 nm, 420 nm, 520 nm) and color (hue and intensity) throughout five day cold soak in 2008 Cabernet Sauvignon must (n=3)

A. Cold Soak Day	pH	TA (g/L tartaric)	Brix	PFGG (μM)	TGG (μM)
Day 0	3.62±0.01 ^d	7.23±0.09 ^a	23.4±0.1 ^{bc}	680±78 ^a	681±70 ^b
Day 1	3.80±0.01 ^a	4.98±0.05 ^b	23.8±0.1 ^a	642±96 ^a	965±70 ^a
Day 2	3.73±0.01 ^c	4.95±0.05 ^b	23.8±0.1 ^a	400±78 ^b	917±70 ^a
Day 3	3.81±0.01 ^a	4.71±0.05 ^c	23.7±0.1 ^{ab}	431±78 ^{ab}	839±70 ^{ab}
Day 4	3.75±0.01 ^b	4.71±0.05 ^c	23.2±0.1 ^c	294±78 ^b	841±70 ^{ab}
Day 5	3.82±0.01 ^a	3.82±0.05 ^d	23.1±0.1 ^c	330±78 ^b	902±70 ^a

B. Cold Soak Day	A_{280nm}	A_{420nm}	A_{520nm}	Color Hue	Color Intensity
Day 0	0.94±0.04 ^c	0.06±0.01 ^a	0.07±0.01 ^{ab}	0.80±0.17 ^b	0.12±0.02 ^{ab}
Day 1	1.25±0.03 ^b	0.06±0.01 ^a	0.05±0.01 ^b	1.30±0.10 ^a	0.12±0.01 ^b
Day 2	1.25±0.03 ^b	0.05±0.01 ^a	0.06±0.01 ^c	0.88±0.10 ^b	0.11±0.01 ^b
Day 3	1.35±0.03 ^a	0.06±0.01 ^a	0.08±0.01 ^{ab}	0.83±0.10 ^b	0.14±0.01 ^{ab}
Day 4	1.35±0.03 ^a	0.06±0.01 ^a	0.08±0.01 ^{ab}	0.83±0.10 ^b	0.14±0.01 ^{ab}
Day 5	1.32±0.03 ^{ab}	0.07±0.01 ^a	0.10±0.01 ^a	0.67±0.10 ^b	0.16±0.01 ^a

Values with different letters within a column indicate significant differences ($\alpha = 0.05$)

Table 5: Regression slopes for juice chemistry attributes of Cabernet Sauvignon must throughout five day cold soak [* Indicates slope is significantly different than zero (0.0)]

Wine Attribute	2007	2008
pH	0.10*	0.02*
TA	-0.42*	-0.27*
Brix	-0.07	-0.10*
A280	0.00	0.05*
A420	-0.01*	0.00
A520	-0.02*	0.01*
Color Hue	0.02	-0.09*
Color Intensity	0.02	0.01*
PFGG	-68.38*	-68.17*
TGG	-19.10	22.79

Figure 3: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon cold soak must chemistry differences determined by pH, TA, Brix, PFGG, TGG, absorbance (280 nm, 420 nm, 520 nm) and color (hue and Intensity) during cold soak

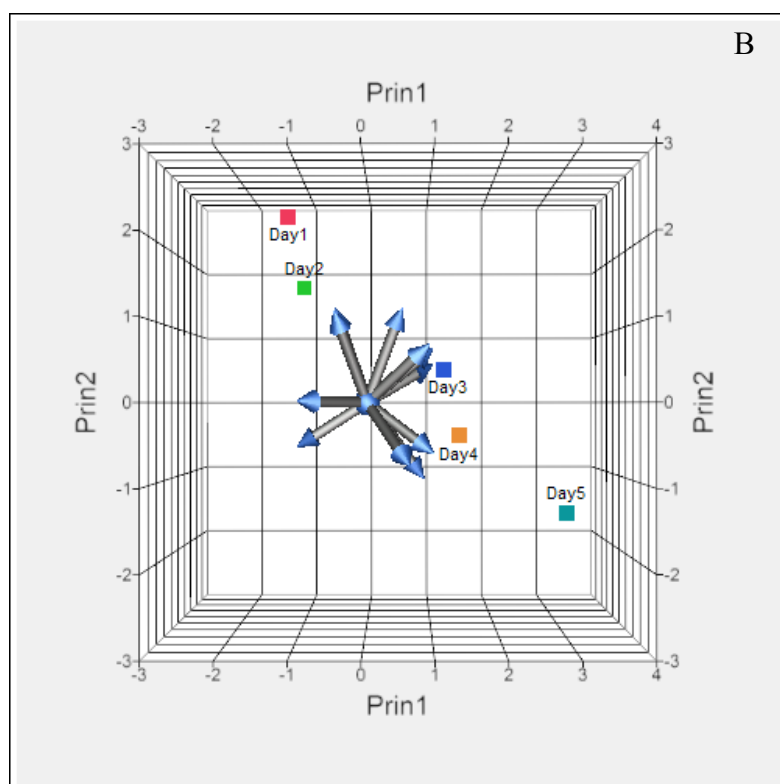
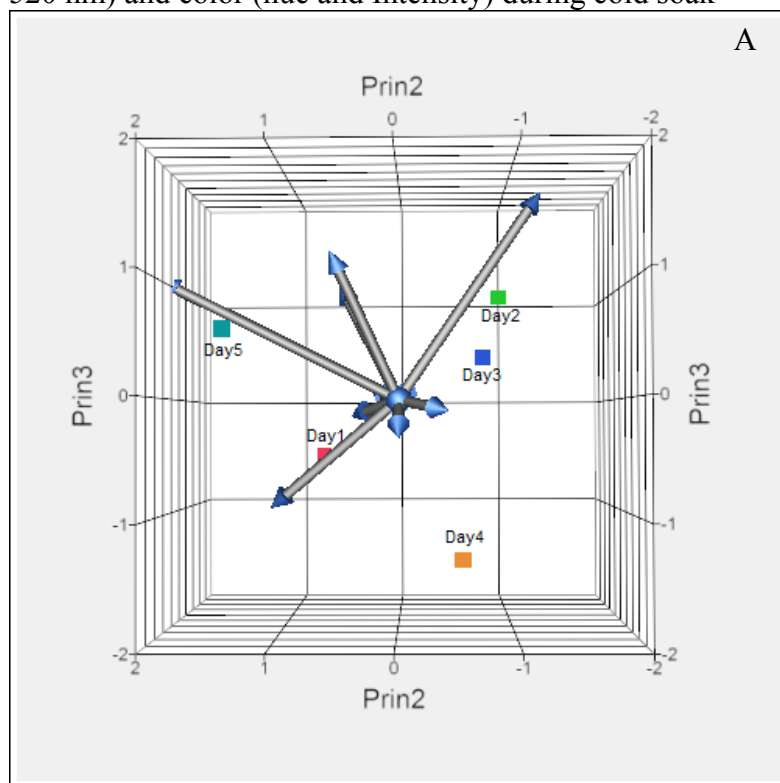


Figure 4: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon control and cold soak wine volatiles detected by the ENose

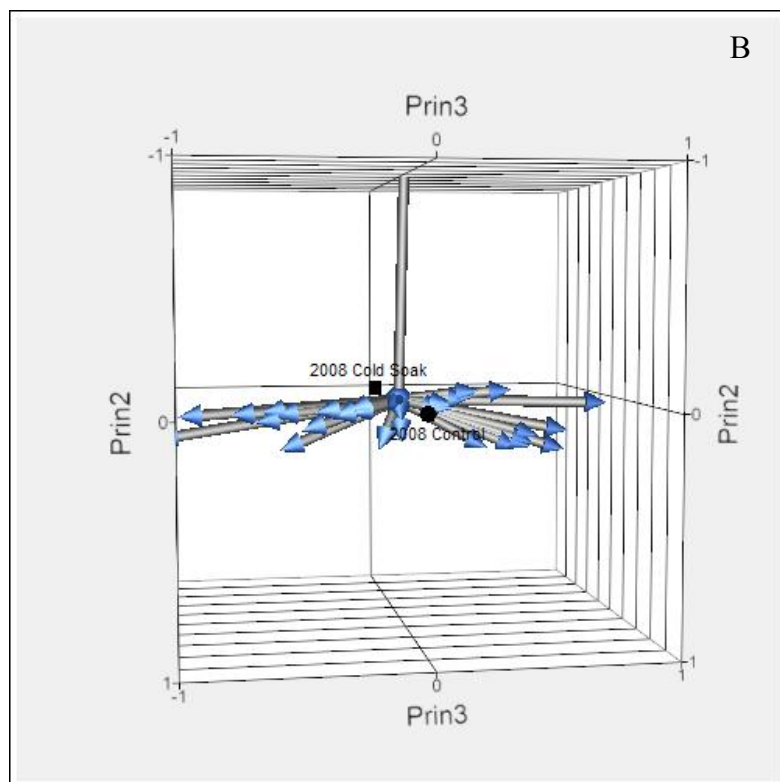
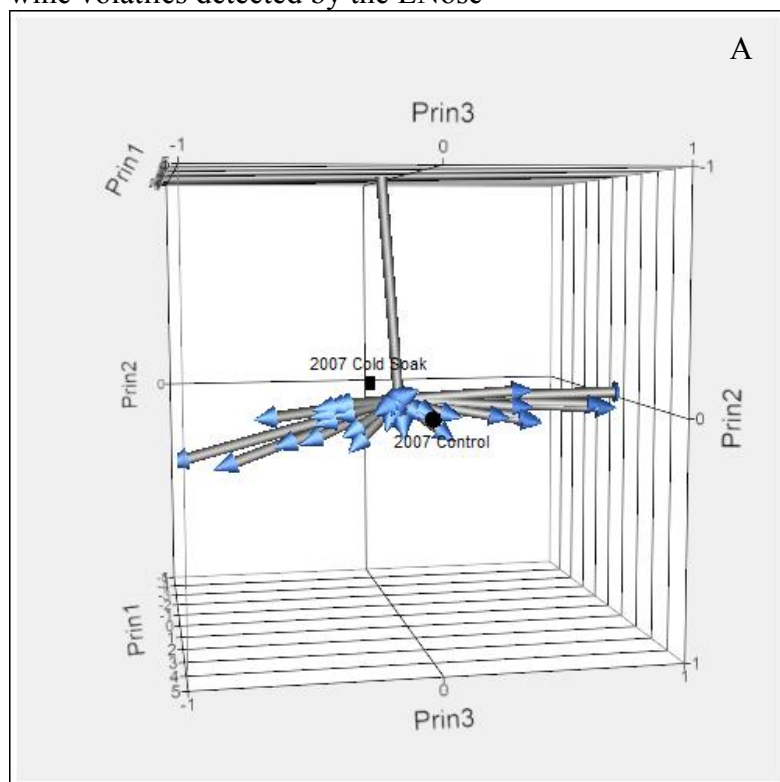


Figure 5: Canonical distribution of 2007 Cabernet Sauvignon wines (A) post-fermentation and (B) six months post fermentation (significant differences at $\alpha = 0.05$ level indicated by non-intersecting points)

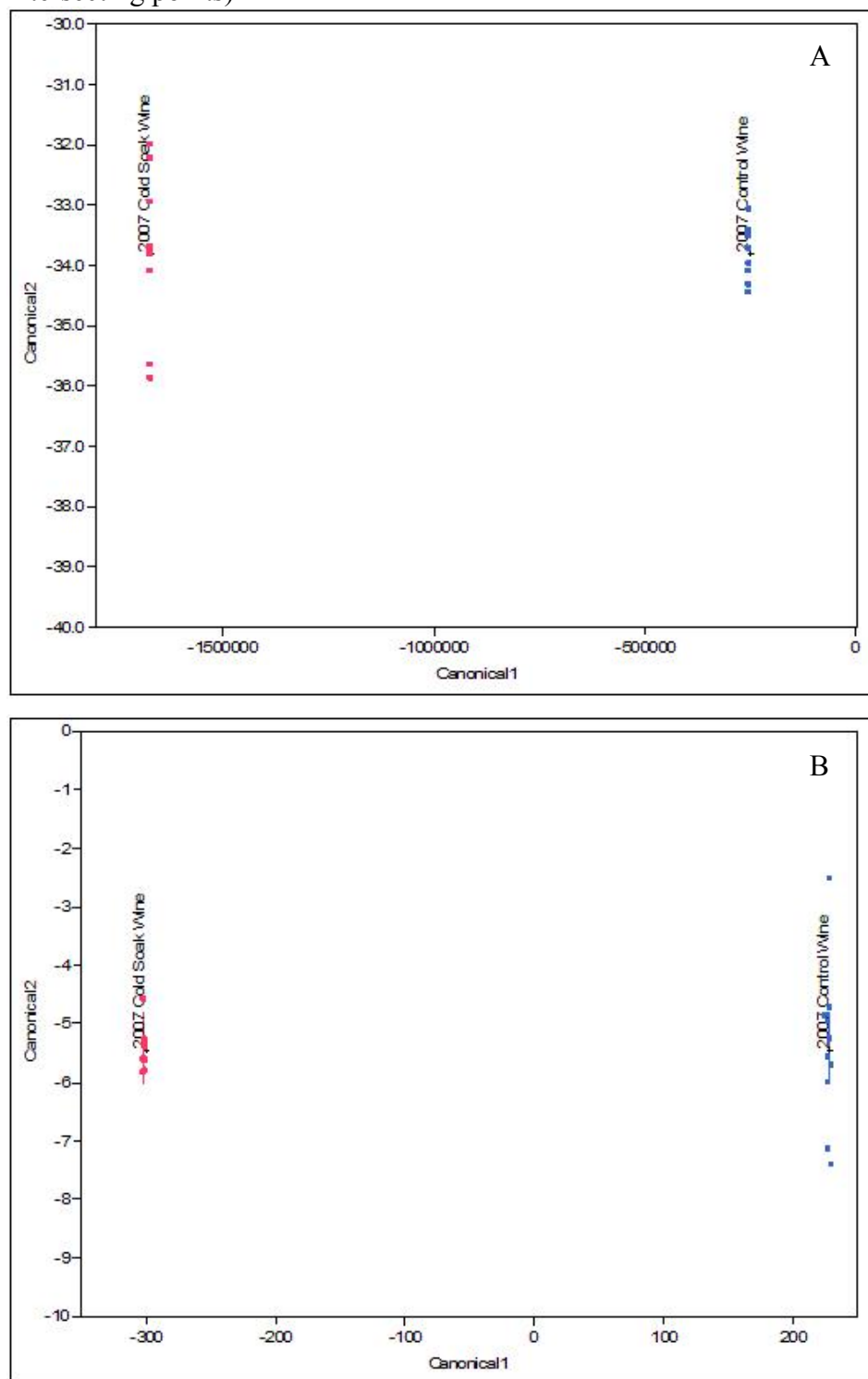


Figure 6: Canonical distribution of 2008 Cabernet Sauvignon wines (A) post-fermentation and (B) six months post fermentation (significant differences at $\alpha = 0.05$ level indicated by non-intersecting points)

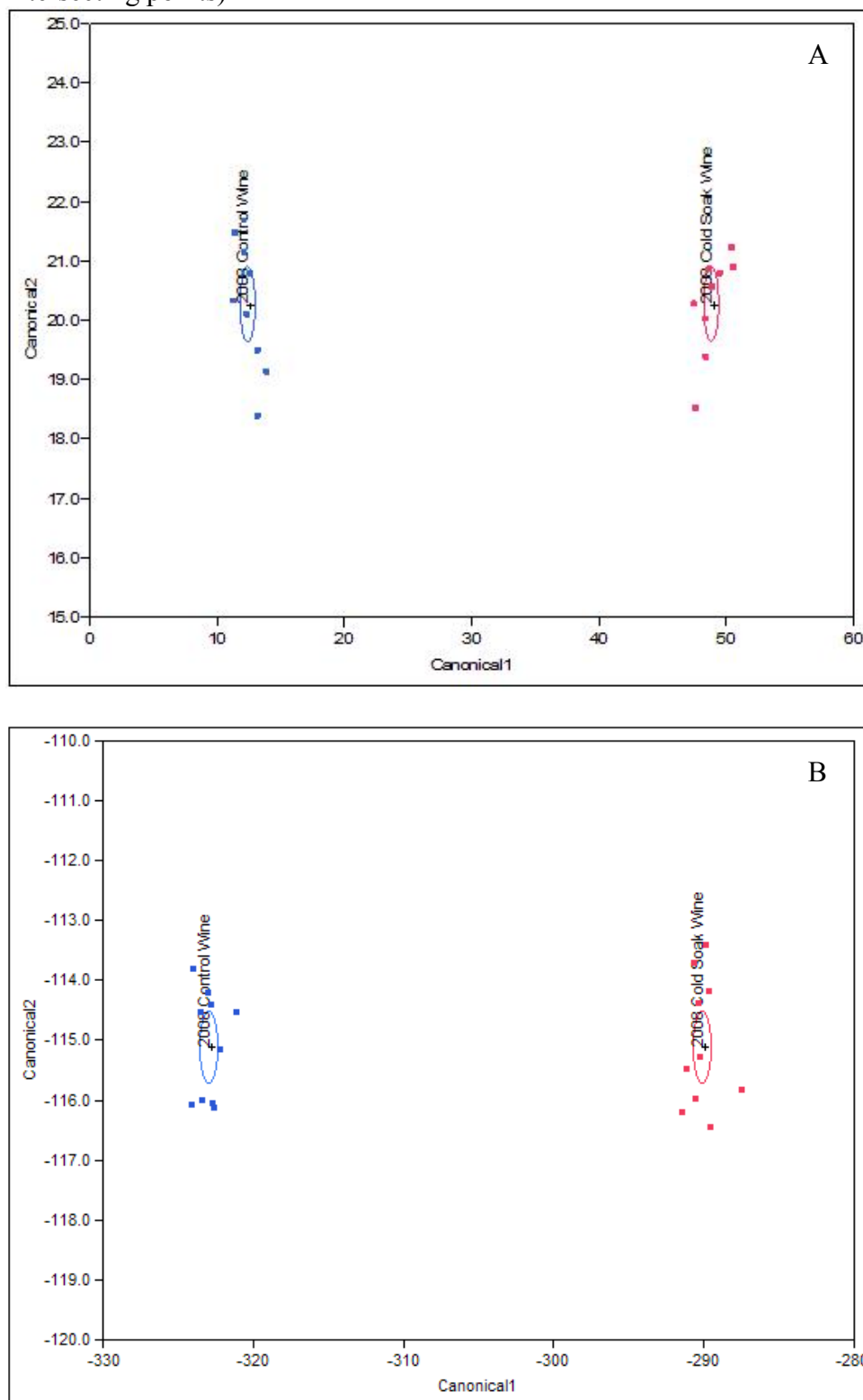


Table 6: Volatile compounds analyzed by SPME GC-MS, odor thresholds, and reported concentrations for control and cold soak Cabernet Sauvignon wines

Compound	Odor Threshold ($\mu\text{g/L}$)	Concentration ($\mu\text{g/L}$)			
		2007 Control	2007 Cold Soak	2008 Control	2008 Cold Soak
2,3-butanediol	2.3 – 6.5 ^w	+	+	ND	ND
1-octanol	110 – 130 ^w	32.7 $\pm$ 2.5 ^a	25.3 $\pm$ 2.5 ^a	51.2 $\pm$ 4.1 ^a	56.2 $\pm$ 4.1 ^a
1-propanol	9000 ^w	+	+	+	+
2,3-benzofuran	NA	+	+	+	+
2-dodecanone	270,000 ^w	0.55 $\pm$ 0.1 ^a	0.24 $\pm$ 0.1 ^b	ND	ND
2-ethyl-1-hexanol	NA	3.72 $\pm$ 0.3 ^a	3.27 $\pm$ 0.3 ^a	3.18 $\pm$ 0.13 ^a	2.75 $\pm$ 0.13 ^a
2-ethylhexanoic acid	NA	33096.7 $\pm$ 1536.7 ^a	33086.7 $\pm$ 1536.7 ^a	22073.3 $\pm$ 2969.1 ^a	29390.0 $\pm$ 2969.1 ^a
2-methyl benzofuran	NA	+	+	ND	ND
2-methyl propanol	40,000 ^z	+	+	+	+
3-methyl butanol	30,000 ^z	100997 $\pm$ 1465.3 ^a	93730 $\pm$ 1465.3 ^b	89136.7 $\pm$ 6037.7 ^a	91876.7 $\pm$ 6037.7 ^a
3-methyl-1-pentanol	NA	+	+	+	+
6-methyl-5-heptene-2-one	2000 ^w	+	+	+	+
acetic acid	200,000 ^z	+	+	+	+
α -terpineol	350 ^y	+	+	+	+
benzaldehyde	350 – 3500 ^w	8.51 $\pm$ 2.0 ^a	27.7 $\pm$ 2.0 ^b	10.9 $\pm$ 2.63 ^a	20.2 $\pm$ 2.63 ^a
benzyl alcohol	10,000 ^w	508.2 $\pm$ 21.9 ^a	563.0 $\pm$ 21.9 ^a	478.2 $\pm$ 21.5 ^a	560.8 $\pm$ 21.5 ^a
β -damascenone	0.05 ^z	2.90 $\pm$ 0.1 ^a	2.89 $\pm$ 0.1 ^a	3.22 $\pm$ 1.00 ^a	4.53 $\pm$ 1.00 ^a
cis-3-hexenol	70 ^w	1.63 $\pm$ 0.3 ^a	2.10 $\pm$ 0.3 ^a	17.8 $\pm$ 2.0 ^a	19.8 $\pm$ 2.0 ^a
citronellol	100 ^z	44.7 $\pm$ 4.3 ^a	36.4 $\pm$ 4.3 ^a	24.3 $\pm$ 2.8 ^a	33.3 $\pm$ 2.8 ^a
decanal	0.1 – 2 ^w	NP	NP	+	+
decanoic acid	15,000 ^z	+	+	+	+
diethyl succinate	NA	91.9 $\pm$ 4.4 ^a	43.4 $\pm$ 4.4 ^b	90.8 $\pm$ 9.3 ^a	133.3 $\pm$ 9.3 ^b
dodecanoic acid	10,000 ^w	+	+	+	+
estragole	NA	+	+	ND	ND
ethyl acetate	7500 ^z	63720.0 $\pm$ 3111.4 ^a	77293.3 $\pm$ 3111.4 ^b	56770.0 $\pm$ 3539.3 ^a	60200.0 $\pm$ 3539.3 ^a
ethyl decanoate	NA	84.8 $\pm$ 1.0 ^a	56.3 $\pm$ 1.0 ^b	85.4 $\pm$ 10.4 ^a	74.8 $\pm$ 10.4 ^a
ethyl dodecanoate	NA	22.1 $\pm$ 3.1 ^a	17.9 $\pm$ 3.1 ^a	26.9 $\pm$ 4.3 ^a	18.7 $\pm$ 4.3 ^a
ethyl heptanoate	2.2 ^w	3.58 $\pm$ 0.2 ^a	2.12 $\pm$ 0.2 ^b	1.23 $\pm$ 0.21 ^a	1.07 $\pm$ 0.21 ^a
ethyl hexanoate	5 ^z	221.6 $\pm$ 16.7 ^a	126.0 $\pm$ 16.7 ^b	261.7 $\pm$ 52.6 ^a	209.0 $\pm$ 52.6 ^a
ethyl myristate	NA	29.3 $\pm$ 0.2 ^a	28.1 $\pm$ 0.2 ^b	27.9 $\pm$ 0.1 ^a	27.6 $\pm$ 0.1 ^a
ethyl nonanoate	NA	22.1 $\pm$ 0.1 ^a	20.1 $\pm$ 0.1 ^b	19.8 $\pm$ 0.4 ^a	19.6 $\pm$ 0.4 ^a
ethyl octanoate	2 ^z	1894.8 $\pm$ 45.1 ^a	1056.1 $\pm$ 45.1 ^b	1662.9 $\pm$ 183.8 ^a	1509.3 $\pm$ 183.8 ^a
ethyl palmitate	>2000 ^w	98.0 $\pm$ 6.9 ^a	75.1 $\pm$ 6.9 ^a	63.6 $\pm$ 2.6 ^a	52.4 $\pm$ 2.6 ^b
γ -butyrolactone	NA	19420.0 $\pm$ 1089.5 ^a	18663.3 $\pm$ 1089.5 ^a	22970.0 $\pm$ 735.7 ^a	23070.0 $\pm$ 735.7 ^a
γ -nonalactone	NA	40.0 $\pm$ 2.4 ^a	36.7 $\pm$ 2.4 ^a	13.3 $\pm$ 2.4 ^a	20.0 $\pm$ 2.4 ^a
hexanoic acid	3000 ^z	1586.7 $\pm$ 117.9 ^a	1393.3 $\pm$ 117.9 ^a	2090.0 $\pm$ 212.2 ^a	2666.7 $\pm$ 212.2 ^a
hexyl acetate	2 ^w	1.36 $\pm$ 0.08 ^a	1.39 $\pm$ 0.08 ^a	12.7 $\pm$ 2.14 ^a	9.04 $\pm$ 2.14 ^a
isoamyl acetate	30 ^z	4934.9 $\pm$ 822.0 ^a	4001.5 $\pm$ 822.0 ^a	7460.7 $\pm$ 1969.4 ^a	6815.5 $\pm$ 1969.4 ^a
isoamyl octanoate	NA	+	+	+	+
isovaleric acid	120 – 700 ^w	2976.7 $\pm$ 220.6 ^a	1983.3 $\pm$ 220.6 ^b	1256.7 $\pm$ 310.0 ^a	1726.7 $\pm$ 310.0 ^a
linalool	15 ^z	+	+	+	+
methionol	NA	3876.7 $\pm$ 183.8 ^a	3696.7 $\pm$ 183.8 ^a	5320.0 $\pm$ 191.7 ^a	5686.7 $\pm$ 191.7 ^a
methyl salicylate	40 ^w	13.7 $\pm$ 0.9 ^a	10.5 $\pm$ 0.9 ^a	9.45 $\pm$ 0.02 ^a	9.38 $\pm$ 0.02 ^a
n-butanol	500 ^w	5746.7 $\pm$ 86.6 ^a	5220.0 $\pm$ 86.6 ^b	6680.0 $\pm$ 384.4 ^a	6980.0 $\pm$ 384.4 ^a
nerol	300 ^w	+	+	ND	ND
n-hexanol	8000 ^z	220.0 $\pm$ 26.3 ^a	210.0 $\pm$ 32.2 ^a	2550.0 $\pm$ 373.1 ^a	3056.7 $\pm$ 373.1 ^a
nonanoic acid	3000 ^w	+	+	+	+
nonanol	50 ^w	+	+	+	+
nonyl aldehyde (nonanal)	1 ^w	+	+	+	+
octanoic acid	3000 ^w	903.3 $\pm$ 109.8 ^a	823.3 $\pm$ 109.8 ^a	1726.7 $\pm$ 303.9 ^a	2090.0 $\pm$ 303.9 ^a

phenethyl acetate	250 ^z	1212.5 ± 36.1 ^a	1162.0 ± 36.1 ^a	724.9 ± 61.5 ^a	749.9 ± 61.5 ^a
phenethyl alcohol	750 ^y	35340.0 ± 1220.5 ^a	36280.0 ± 1220.5 ^a	39686.7 ± 884.3 ^a	41920.0 ± 884.3 ^a
propyl benzoate	NA	+	+	+	+
terpinene-4-ol	NA	1.45 ± 0.04 ^a	1.13 ± 0.04 ^b	8.02 ± 0.70 ^a	9.37 ± 0.70 ^a

^wOdor detection threshold determined in water from Leffingwell & Associates Database (<http://www.leffingwell.com/odorthre.htm>); ^yOdor recognition threshold determined in water (Ohloff 1978); ^zOdor recognition threshold determined in 90% water and 10% ethanol (Guth 1997); ^{a,b}Designates significant differences in a row for a given vintage ($\alpha = 0.05$); NA indicates threshold level is not available; ND indicates that the compound was not detected during analysis; + indicates that the compound was not quantified, but present in the chromatogram as matched with appropriate MS ions

Figure 7: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon control and cold soak wine volatiles detected by GC-MS

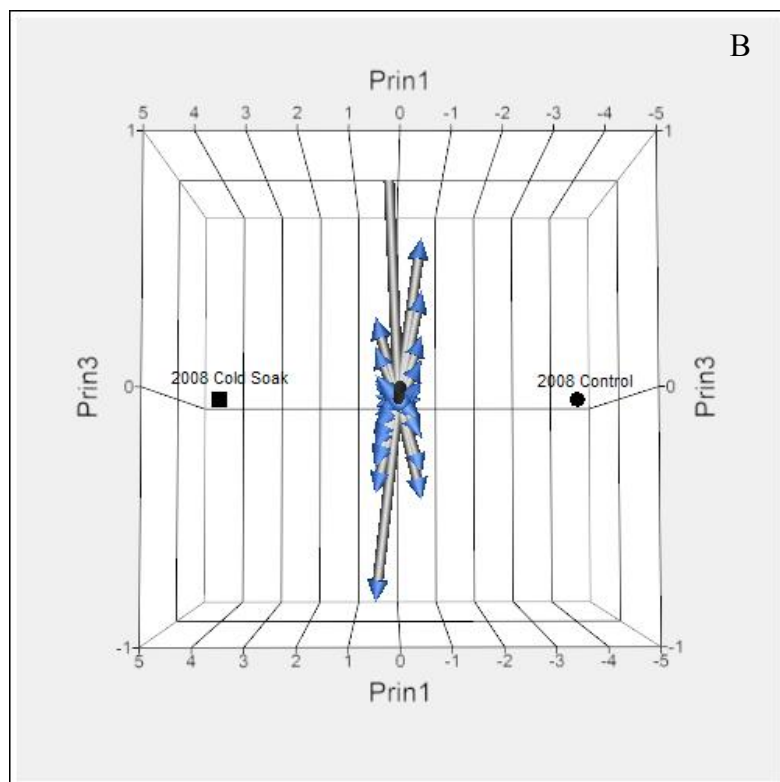
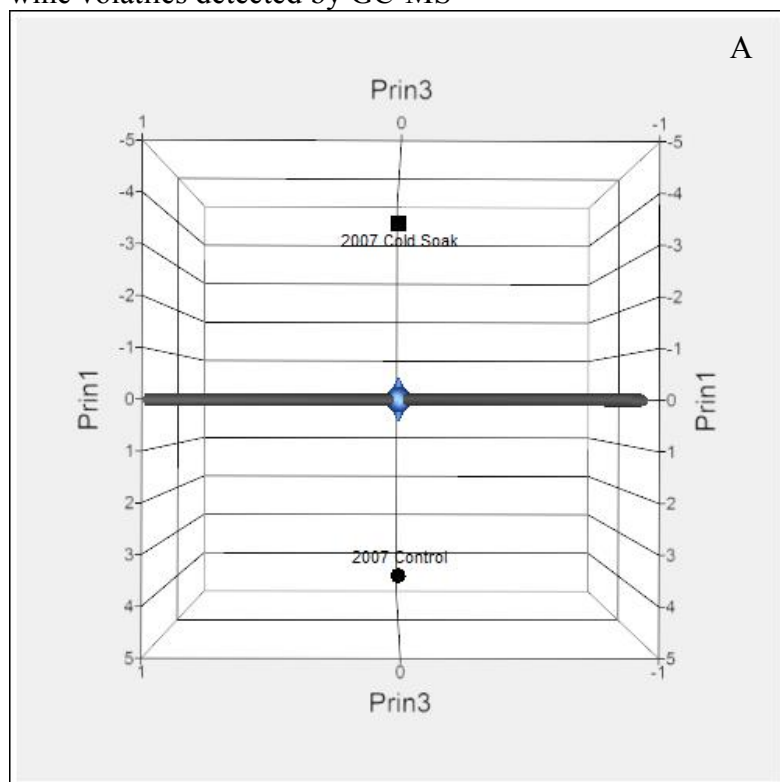


Figure 8: Canonical distributions of wines (A) without and (B) with the use of an ethanol baseline using a conducting polymer electronic nose system

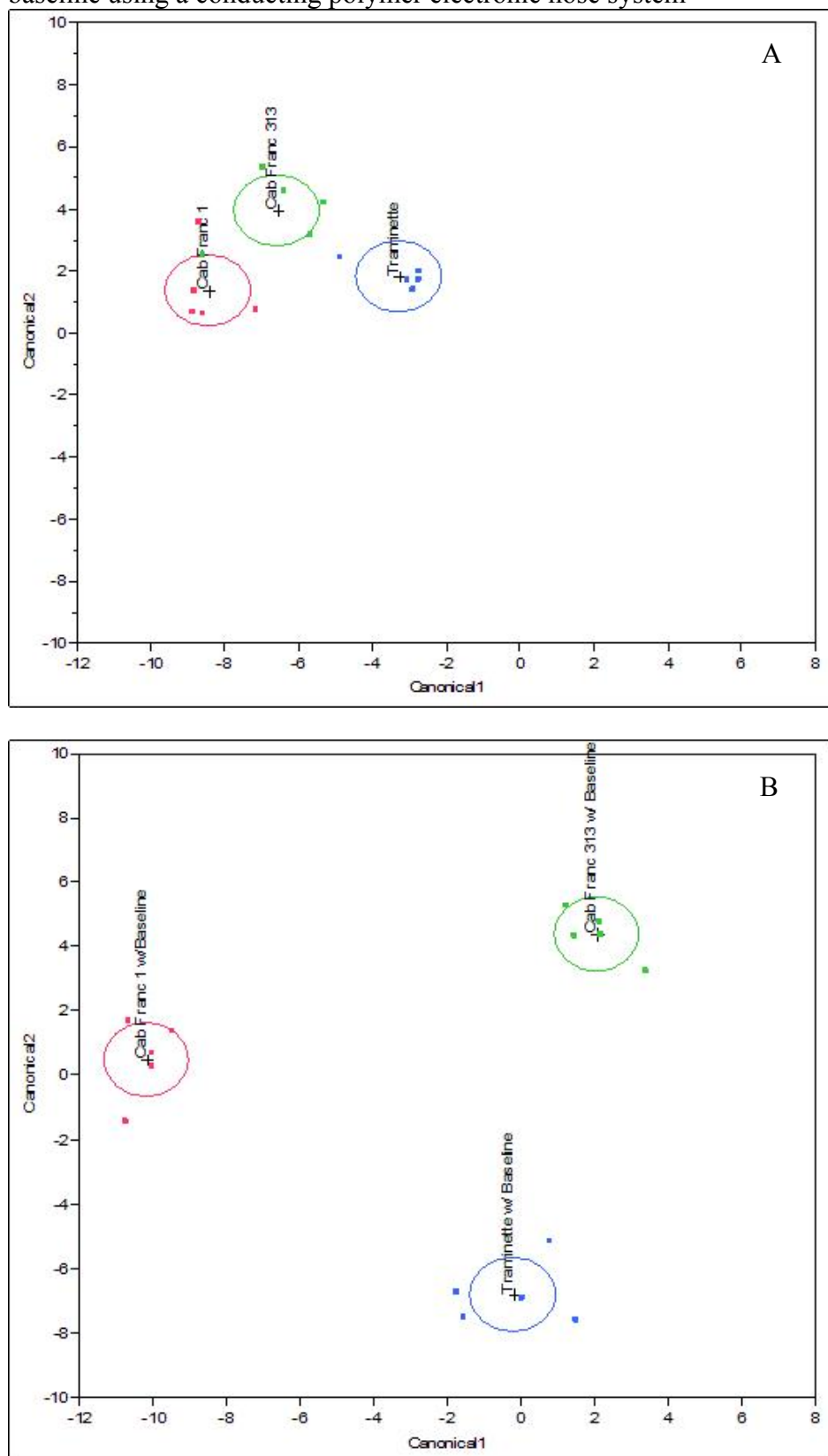


Table 7: Wine (A) pH, titratable acidity (TA), ethanol concentration, volatile acidity (VA), phenol free glycosyl-glucose (PFGG), and total glycosyl-glucose (TGG) and (B) absorbance (280 nm, 420 nm, 520 nm) and color (hue and intensity) of 2007 and 2008 Cabernet Sauvignon

A.	Treatment	pH	TA (g/L tartaric)	% Ethanol	VA (g/L)	PFGG (μM)	TGG (μM)
Vintage							
2007	Control	3.66 $\pm$ 0.00 ^a	8.47 $\pm$ 0.09 ^a	12.1 $\pm$ 0.1 ^a	0.35 $\pm$ 0.02 ^a	133 $\pm$ 8 ^a	755 $\pm$ 45 ^a
2007	Cold Soak	3.69 $\pm$ 0.00 ^b	7.69 $\pm$ 0.09 ^b	12.3 $\pm$ 0.1 ^a	0.35 $\pm$ 0.02 ^a	130 $\pm$ 8 ^a	696 $\pm$ 45 ^a
2008	Control	3.61 $\pm$ 0.00 ^a	9.44 $\pm$ 0.05 ^a	12.9 $\pm$ 0.0 ^a	0.25 $\pm$ 0.02 ^a	191 $\pm$ 41 ^a	1153 $\pm$ 86 ^a
2008	Cold Soak	3.59 $\pm$ 0.00 ^a	8.73 $\pm$ 0.05 ^b	13.1 $\pm$ 0.0 ^b	0.35 $\pm$ 0.02 ^b	195 $\pm$ 41 ^a	1395 $\pm$ 86 ^a

B.	Treatment	A_{280nm}	A_{420nm}	A_{520nm}	Color Hue	Color Intensity
Vintage						
2007	Control	2.07 $\pm$ 0.13 ^a	0.16 $\pm$ 0.01 ^a	0.26 $\pm$ 0.01 ^a	0.63 $\pm$ 0.01 ^a	0.43 $\pm$ 0.02 ^a
2007	Cold Soak	1.96 $\pm$ 0.13 ^a	0.15 $\pm$ 0.01 ^a	0.23 $\pm$ 0.01 ^a	0.66 $\pm$ 0.01 ^a	0.38 $\pm$ 0.02 ^a
2008	Control	4.32 $\pm$ 0.05 ^a	0.63 $\pm$ 0.04 ^a	0.95 $\pm$ 0.04 ^a	0.67 $\pm$ 0.02 ^a	1.58 $\pm$ 0.08 ^a
2008	Cold Soak	3.76 $\pm$ 0.05 ^b	0.59 $\pm$ 0.04 ^a	0.70 $\pm$ 0.04 ^b	0.83 $\pm$ 0.02 ^b	1.29 $\pm$ 0.08 ^a

Values that have different letters indicate significant differences within a column ($\alpha = 0.05$) between same vintage year samples

Figure 9: PCA distribution of (A) 2007 and (B) 2008 Cabernet Sauvignon control and cold soak wine chemistries determined by pH, TA, ethanol concentration, volatile acidity, PFGG, TGG, absorbance (280 nm, 320 nm, 420 nm, 520 nm) and color (hue and intensity)

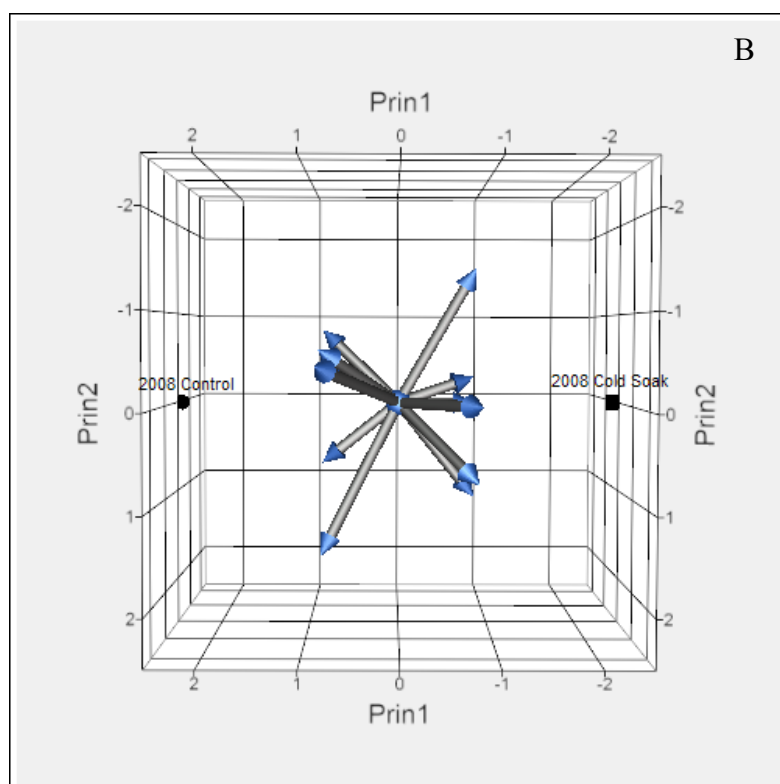
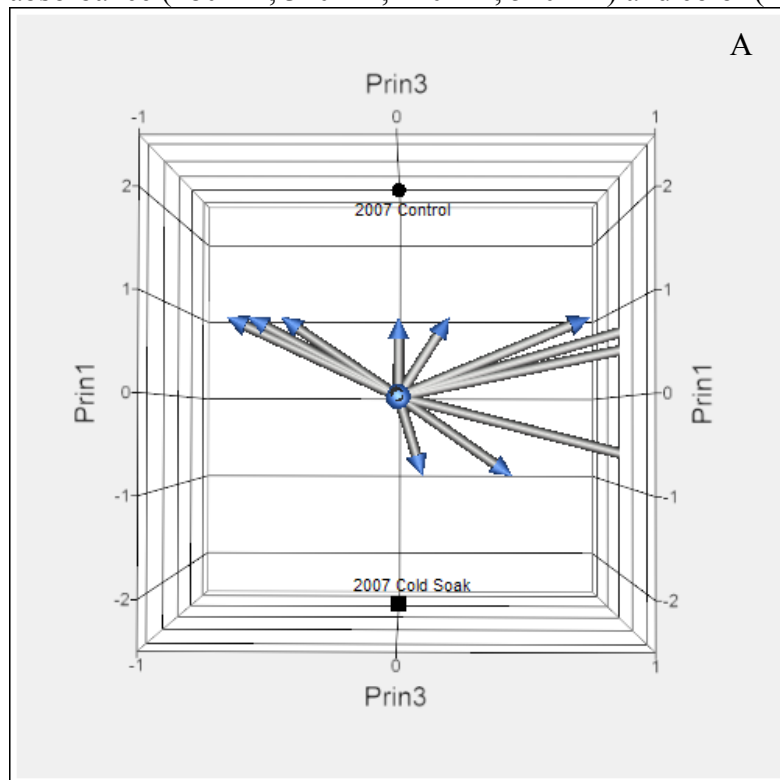


Table 8: Cabernet Sauvignon wine aroma (sample temperature 19°C) triangle difference sensory results (n=54) of control vs. cold soak treatments per vintage year ($\alpha = 0.05$, $\beta = 0.10$, $\rho_{\max} = 30\%$, 25 correct responses needed for significant difference)

Sample 1	Sample 2	Correct Responses	Total Responses	Significant Difference Between Treatments
2007 Control	2007 Cold Soak	14	54	No
2008 Control	2008 Cold Soak	22	54	No

Table 9: Aroma units of select volatile compounds in 2007 and 2008 Cabernet Sauvignon control and cold soak wines

Compound	Aroma ^a	Aroma/Flavor Units*			
		2007 Control	2007 Cold Soak	2008 Control	2008 Cold Soak
1-octanol	burnt, citrus	0.25 – 0.30	0.19 – 0.23	0.39 – 0.47	0.43 – 0.51
2-dodecanone	citrus, floral	0.00	0.00	NP	NP
3-methyl butanol	burnt	3.37	3.12	2.97	3.06
benzaldehyde	almond	0.00 – 0.02	0.01 – 0.08	0.00 – 0.03	0.01 – 0.06
benzyl alcohol	flower, berry, cherry, walnut, grapefruit	0.05	0.06	0.05	0.06
β-damascenone	honey	58.00	57.80	64.40	90.60
cis-3-hexenol	fresh	0.02	0.03	0.25	0.28
citronellol	rose, geranium	0.45	0.36	0.24	0.33
ethyl acetate	pineapple, anise, ethereal	8.50	10.31	7.57	8.03
ethyl heptanoate	berry, plum, melon	1.63	0.96	0.56	0.49
ethyl hexanoate	fruit, apple, ethereal, banana	44.32	25.20	52.34	41.80
ethyl octanoate	fat, apricot, pineapple	947.40	528.05	831.45	754.65
ethyl palmitate	waxy	0.05	0.04	0.03	0.03
hexanoic acid	fruit, cheese	0.53	0.46	0.70	0.89
hexyl acetate	herb	0.68	0.70	6.35	4.52
isoamyl acetate	banana	164.50	133.38	248.69	227.18
isovaleric acid	rancid, animal, cheese	4.25 – 24.81	2.83 – 16.53	1.80 – 10.47	2.47 – 14.39
methyl salicylate	peppermint	0.34	0.26	0.24	0.23
n-hexanol	resin	0.03	0.03	0.32	0.38
octanoic acid	sweat, oily	0.30	0.27	0.58	0.70
phenethyl acetate	honey, rose	4.85	4.65	2.90	3.00
phenethyl alcohol	honey, rose	47.12	48.37	52.92	55.89

*Aroma unit calculated by detected concentration of compound divided by odor threshold concentration. Values >1.00 are contributing compounds to sample aroma.

^aAroma descriptors given from Flavornet (<http://www.flavornet.org/>) and Sigma-Aldrich Flavors & Fragrance Catalogs (Aldrich Catalog, Sigma-Aldrich, Milwaukee, WI)

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Summary

This project reviewed the use of a conducting polymer electronic nose (ENose) for wine analysis. The first study evaluated the method behind using a commercial ENose during wine evaluation, by training the system to minimize ethanol interference of sensor responses. It was found that ethanol concentrations could have minimal influence on sensor response if a standard ethanol solution was purged into the machine prior to sampling wine. This was shown by a dip in sensor response before sample volatiles were drawn into the ENose. Additionally, 30°C was shown to be an optimal sampling temperature of red and white wines, providing the greatest degree of discrimination in canonical distributions, and adequate sensor response during evaluation.

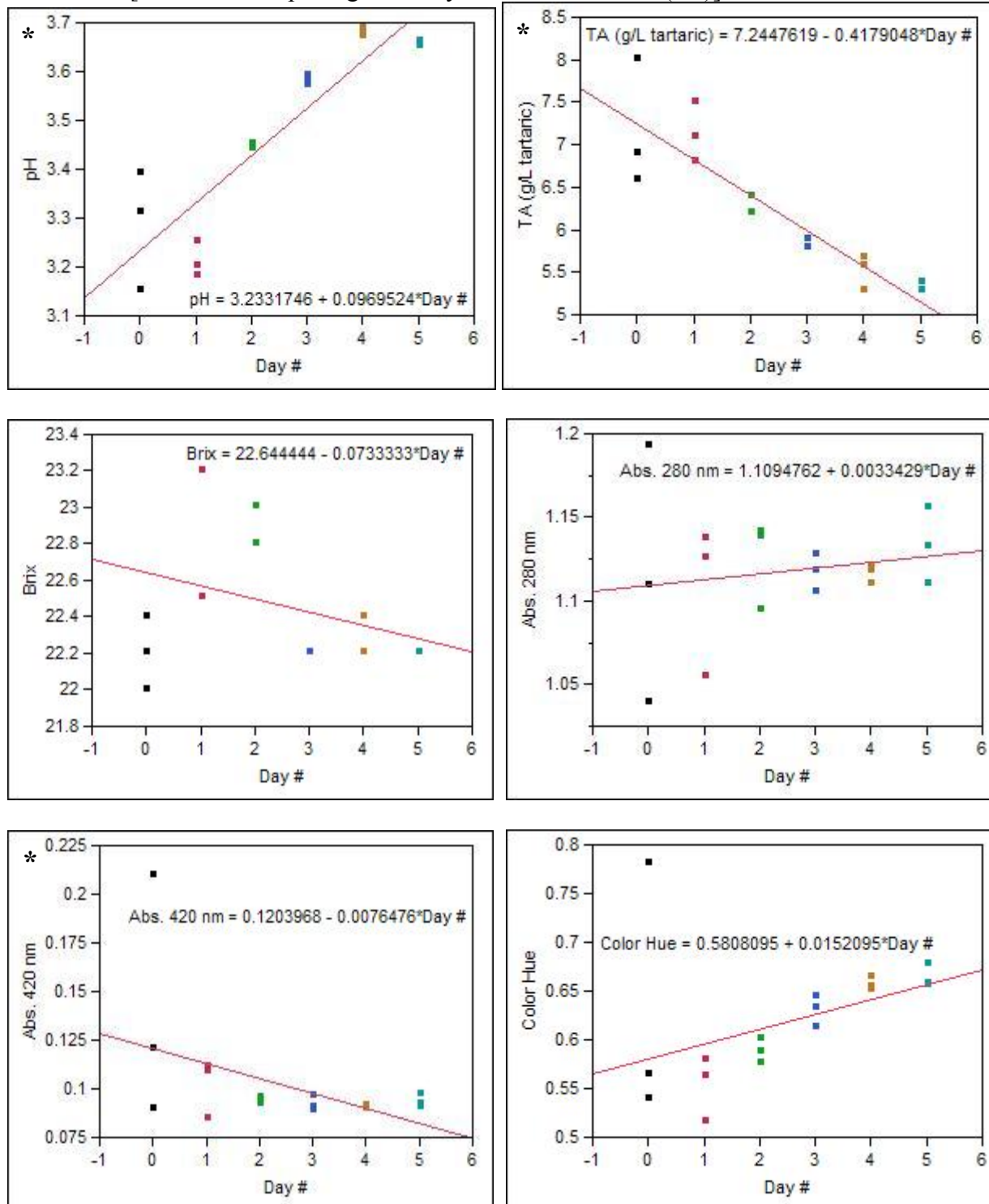
The second study focused on the use of a commercial ENose to monitor grape volatiles through a five day cold soak prior to fermentation and to discriminate wine volatiles based on alterations in processing in a two year evaluation. It was found that the ENose detected differences in grape volatiles during the cold soak period. These findings were compared with changes in juice chemistry throughout cold soak. A 51% decrease in PFGG in 2008 may have been due to possible hydrolysis during cold soak, releasing free volatile compounds. Control and cold soak wines also had different volatile components, as detected by the ENose and demonstrated in canonical and PCA distributions. These results were compared with GC-MS, which showed differences in individual volatile concentrations based on treatment. In 2007, ethyl esters had higher concentrations in control wines than cold soak, with the exception of ethyl palmitate and ethyl acetate. In 2008, control wines had higher concentrations of ethyl palmitate while cold soak wines were higher in diethyl succinate concentrations. Concentrations varied between years emphasizing seasonal variation and possible extraction differences, but separation of control versus treatment was apparent in PCA distributions based on volatile concentrations. Sensory analysis of control versus cold soak wines found no significant difference based on treatment. These results were contributed to use of a consumer panel with inherent variability, variations of wine ethanol concentrations which alters the perception of certain volatile compounds, and possible synergistic or antagonistic effects of volatile aromas during sensory evaluation.

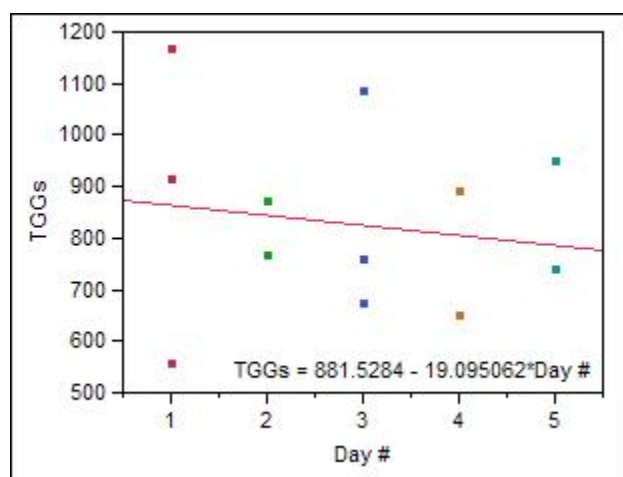
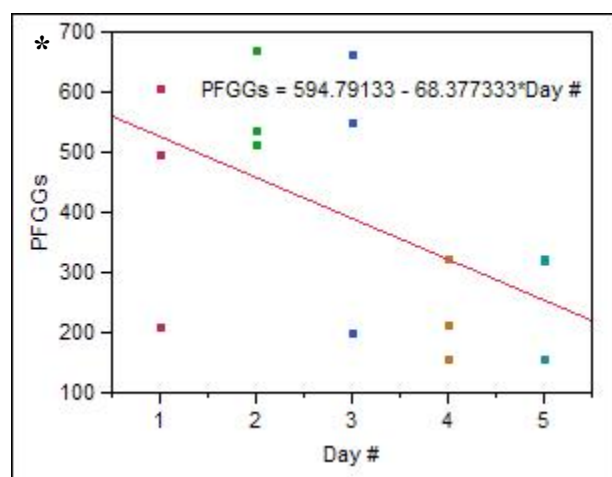
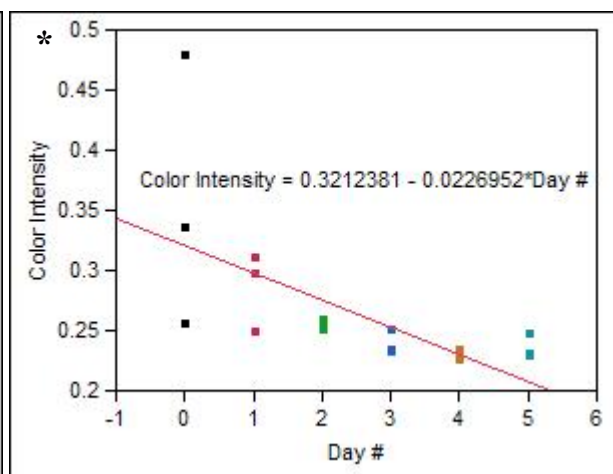
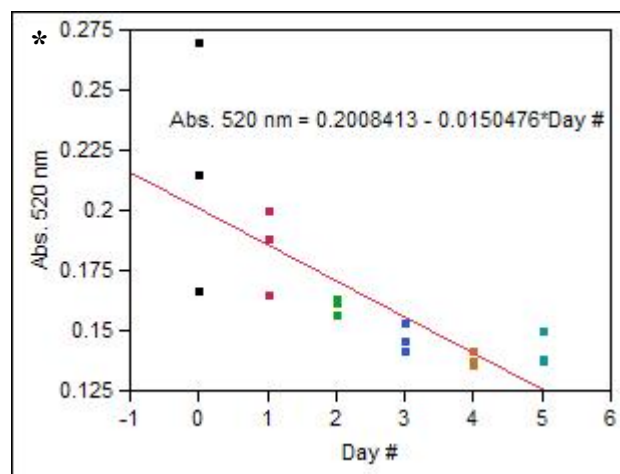
Additional research using this ENose unit is needed. Possible studies on ethanol variations in water/ethanol solutions and wines may provide insight into the discrimination

power of the ENose. However, of particular interest is the use of the ENose as a practical tool during wine evaluation. For wine varieties that have known dominate volatiles, standard model solutions of those varieties can be made, evaluated, and compared to wines of those varieties made during production. This would assess the ability of the ENose to classify wines based on their characteristic volatile component and act as a quality assurance tool for the wine industry.

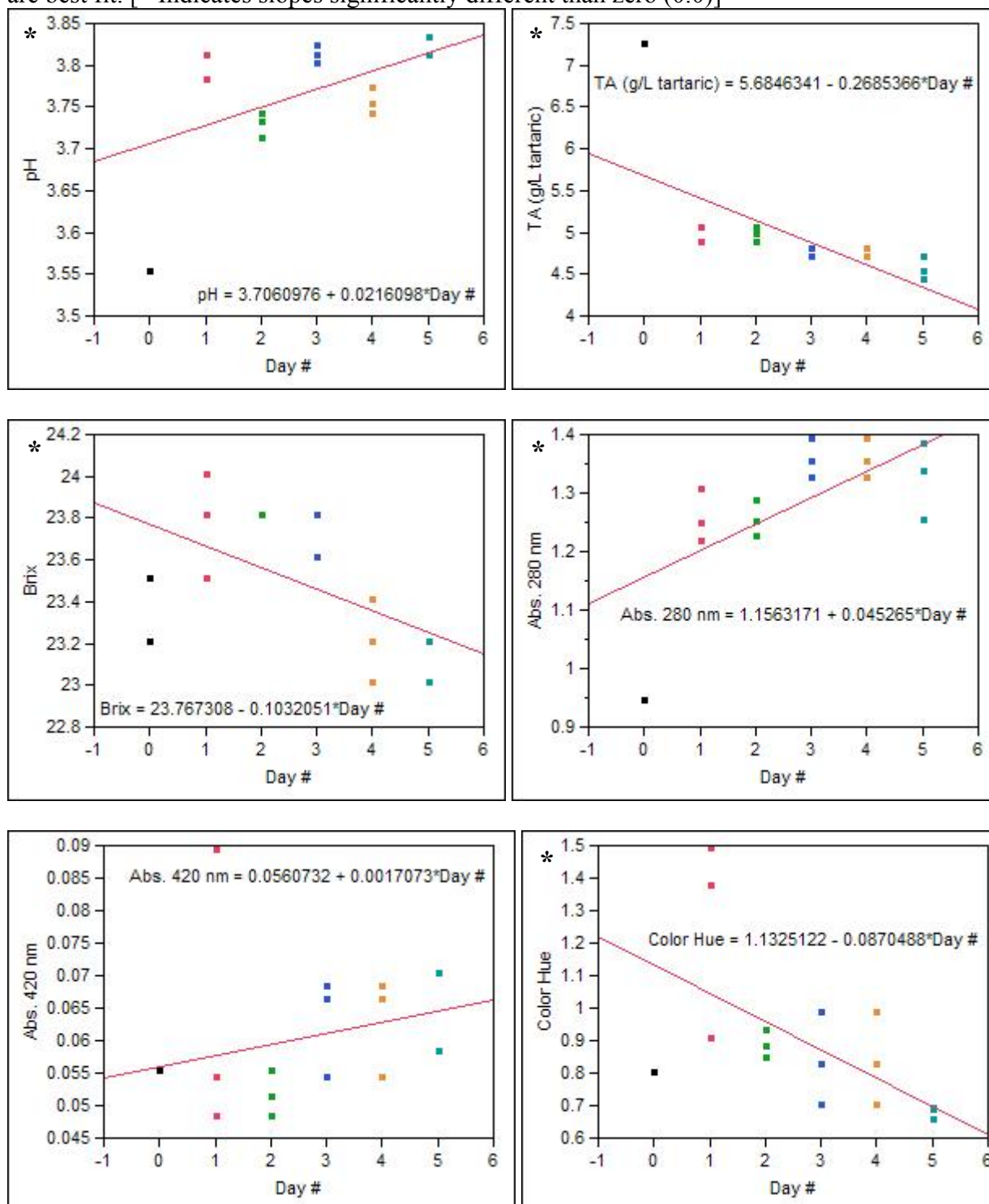
Appendix

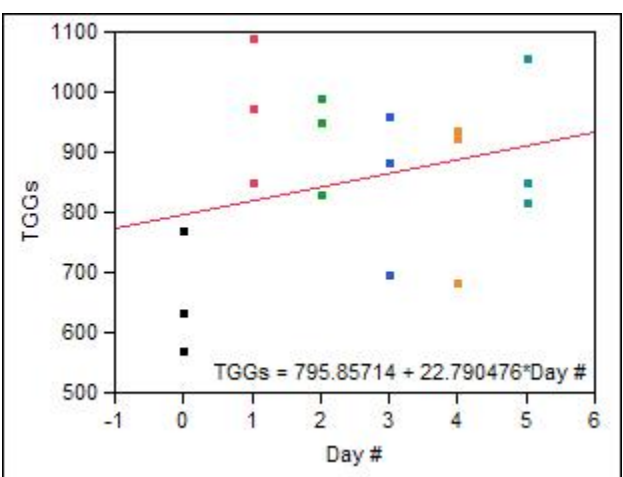
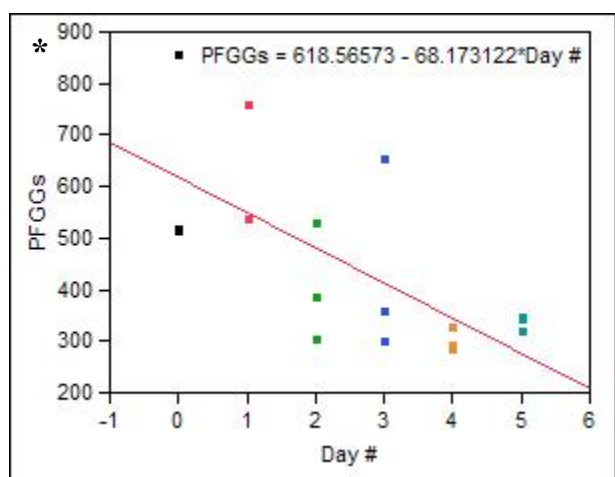
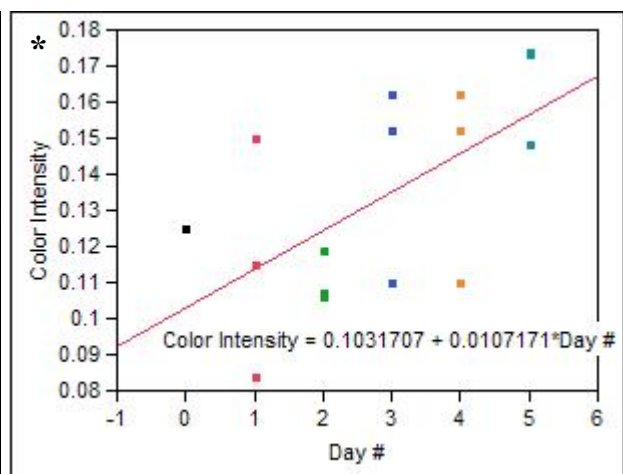
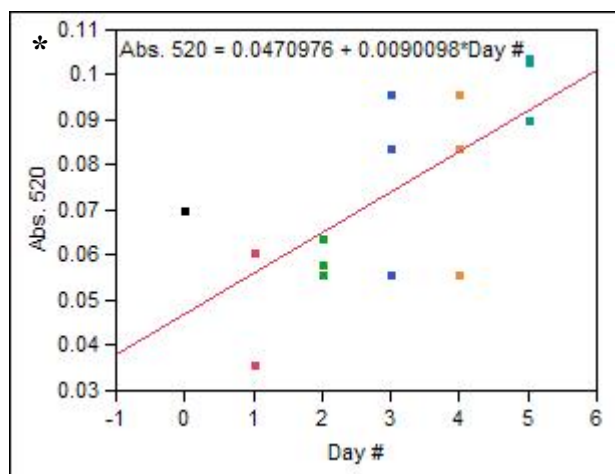
A.1: Regression Data for 2007 Cabernet Sauvignon Juice During Cold Soak: Lines chosen are best fit. [* Indicates slopes significantly different than zero (0.0)]





A.2: Regression Data for 2008 Cabernet Sauvignon Juice During Cold Soak: Lines chosen are best fit. [* Indicates slopes significantly different than zero (0.0)]





A.3: IRB Approval Documentation



VirginiaTech

Office of Research Compliance
Carmen T. Green, IRB Administrator
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24061
540/231-4358 Fax 540/231-0959
e-mail: cgreen@vt.edu
www.ibr.vt.edu
FRA00030573, expires 10/03/10
IRB # is IRB00300907

DATE: January 29, 2009

MEMORANDUM

TO: Susan E. Duncan
Denise Gardner

FROM: Carmen Green 

SUBJECT: **IRB Exempt Approval:** "Effects of Prefermentation Cold Soak of *Vitis vinifera* L. cv. Cabernet Sauvignon Grape and Wine Volatiles", OSP #448085, IRB # 09-090

I have reviewed your request to the IRB for exemption for the above referenced project. The research falls within the exempt status. Approval is granted effective as of January 29, 2009.

As an Investigator of human subjects, your responsibilities include the following:

1. Report promptly proposed changes in the research protocol. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.
2. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

cc: File
OSP

Invent the Future

VIRGINIA POLYTECHNIC INSTITUTE UNIVERSITY AND STATE UNIVERSITY

An equal opportunity, affirmative action institution

A.4: IRB Sensory Consent Form

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants in Research Projects Involving Human Subjects (Sensory Evaluation)

Title of Project: Effect of Prefermentation Cold Soak of *Vitis vinifera* L. cv. Cabernet Sauvignon Grape and Wine Volatiles

Investigators: Denise M. Gardner and Bruce W. Zoecklein, Ph.D.

I. Purpose of this Research/Project

You are invited to participate in a sensory evaluation on red wine aroma. The purpose of this study is to evaluate if consumers can detect differences in red wine aromas based on wine processing variations.

II. Procedures

You will be given 3 samples of red wine in wine glasses, covered with Petri dishes. Please sniff the aromas of each sample in the order of which they are given to you. Once you have sniffed all 3 samples, indicate on the sheet, which sample you believe to be different. You must choose 1 of the 3 samples.

If you or your family members are sensitive to certain foods such as sulfites, please inform the investigator.

III. Risks

There are no more than minimal risks for participating in this study. If you are aware of any allergic reactions to sulfites please inform the investigator.

IV. Benefits

Your participation on this study will provide valuable information about consumer awareness and ability to perceive aroma differences in red wines. Results from this study will likely be published. If you would like a summary of the research results, please contact the researcher at a later time.

V. Extent of Anonymity and Confidentiality

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by a code number for data analyses and for any publication of the results.

VI. Compensation

You will not be compensated for participating in this study. You will receive a treat for participating.

VII. Freedom to Withdraw

If you agree to participate in this study, you are free to withdraw from the study at any time without penalty. There may be reasons under which the investigator may determine you should not participate in this study. If you have allergies to sulfites, you are asked to refrain from participating.

VIII. Subject's Responsibilities

I voluntarily agree to participate in this study. I have the following responsibilities:

- 1) Smell red wine samples.
- 2) Complete a feedback survey to submit to the investigator.

IX. Subject's Permission

I have read the Consent Form and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent:

_____ Date _____
Subject signature

Should I have any pertinent questions about this research or its conduct, and research subjects' rights, and whom to contact in the event of a research-related injury to the subject, I may contact:

Susan Duncan, Faculty/Investigator (540) 231-8675;
duncans@vt.edu

Denise M. Gardner, Graduate Research Assistant, Investigator (540) 231-9843
dmg1214@vt.edu

A.5: Copyright Permission Letter from Smiths Detection

smiths detection
bringing technology to life

Smiths Detection
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T: +1 410 612 4000
F: +1 410 580 9491
www.smithsdetection.com

May 8, 2009

Ms. Denise M. Gardner
Graduate Student
Virginia Tech University
Wine Grape Chemistry Group
FST Bldg., Blacksburg, VA 24061
Via e-mail: dmg1214@vt.edu

RE: Use of Cyrano® 320 Diagram

The purpose of this letter is to provide authorization for Ms. Denise M. Gardner, graduate student, Virginia Tech University, to include the attached diagram of the Smiths Detection Cyrano® 320 in her thesis titled "Characterization of Cold Soak on *Vitis vinifera* L. cv. Cabernet Sauvignon Grape and Wine Volatiles Using an Electronic Nose System."

The purpose of Ms. Gardner's thesis is to measure volatile compounds in Cabernet Sauvignon grapes and wine using the Cyrano® 320, and to view changes in the volatile component based on a wine processing treatment. An alternative focus of her research was to minimize sensor response of ethanol fluctuations of various wine varieties.

The diagram shall be labeled, "Representation of change in resistance due to sensor swelling during volatile absorption in Cyrano® 320" and will be annotated with an acknowledgement that the graphic was provided courtesy of Smiths Detection.

Sincerely,

Jill M. McClune

Jill McClune
Director, Contracts & Compliance
Smiths Detection
410-612-2612
Jill.mcclune@smithsdetection.com

410-612-2612 and 410-580-9491 are company

Smiths Detection Inc.

smiths detection

