

WINE DISCRIMINATION AND ANALYSIS USING QUARTZ MICROBALANCE BASED ELECTRONIC NOSE TECHNOLOGY

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Thesis submitted to the Faculty of
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science

In

Biological Systems Engineering

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January 18, 2007

Blacksburg, VA

Keywords: chemosensory, wine evaluation, ethanol spray, wine aroma

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ABSTRACT

Wines are composed of numerous compounds that are complex, making them difficult to analyze. Wine evaluation and discrimination is typically done through chemical and human sensory evaluation. Unfortunately, both of these methods are time consuming and expensive. Therefore a new rapid analysis technique for wine discrimination and analysis is desired. The electronic nose has been suggested as an alternative to current wine discrimination techniques.

In this study, a quartz microbalance-based electronic nose system was utilized to analyze the overall volatile components of wine. The electronic nose was optimized for Cabernet Sauvignon and Mouvédre wine to gain maximum sensor response from the sensors. Response surface methodology was used to determine the optimum sensor response by varying three experimental parameters: sensor temperature, sample temperature and equilibrium time. The maximum sensor response occurred at an equilibrium time of 20 min for each varietal and at a sample temperature of 55°C and 56°C for Cabernet Sauvignon and Mouvédre, respectively. The optimum sensor temperature selected for this study was 40°C for both varietals.

Using the optimum sensor settings, the electronic nose was used to analyze Cabernet Sauvignon wines. Grapes were treated with ethanol spray (5%, and 10%) 13 weeks post-bloom, which has been shown to affect the overall quality of the final wine product. Wine samples were evaluated using chemical analyses, human sensory evaluation and electronic nose. Significant differences between the wines were observed based on pH, percent alcohol, and color intensity only. A consumer sensory panel consisting of 81 panelists was unable to differentiate amongst sample treatments. However, the electronic nose was able to differentiate between the control group and the treated samples 100% of the time. Canonical discriminant analysis of the data placed the 5% ethanol treatment as a sub-set of the 10% ethanol treatment. The results indicate that the electronic nose can be used as a discriminatory tool for assessing wines.

ATTRIBUTION

Author Amanda M. Martin is the major contributor and writer of the manuscripts in chapter three and chapter four of this thesis. Co-author Dr. Parameswarakumar Mallikarjunan was the Committee Chair. Co-authors Drs. Bruce W. Zoecklein and Dr. Susan W. Gay were committee members. Drs. Mallikarjunan and Zoecklein provided supervision, advice and laboratory support. Dr. Gay provided literary advice and constructive criticism during thesis revisions.

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ACKNOWLEDGEMENTS

I would first like to thank my major advisor, Dr. Kumar, for assisting me with my degree. His guidance and support have been greatly appreciated during my studies. Thank you, Dr. Zoecklein, for giving me the opportunity to participate in this study, and for the use of your laboratory and equipment. Thanks also to my final committee member, Dr. Susan Gay. I am grateful for your support, guidance and advice during my academic career (both undergrad and graduate!)

I would like to thank Lisa Pélanne and Sandy Birkenmaier from the Grape-Enology group for their assistance in the laboratory and winery. I learned a great deal from both of you!!! I would also like to thank the FST faculty and staff that assisted me during my research - Dr. Susan Duncan, Kim Waterman and John Chandler.

Special thanks to my whole BSE family for your support. The faculty, staff and students of the department (I think) are the best on this campus, and I've been happy to call BSE home for the past six years! Special thanks to Tameshia, Ahmad, Rhonda, Les and the entire HH crew. Woo! Geaux BSE Foodies!!

A great deal of thanks and appreciation goes to Dr. Bevlee Watford for her extensive and unfailing support since I set foot on this campus. I would not have made it through undergrad OR grad school without you. I greatly appreciate the CEED office, and the CoE Academic Affairs Office. There is no way that I can express how much you all mean to me, and how much I appreciate the support and friendship that has come from all of you.

Thank you Dean DePauw for your insight and for all of the opportunities you have given me. I am grateful for your vision and your dedication to the Graduate School and its students. Thanks for everything, Momma Goose!

I cannot begin to express my thanks to you, Nick, for everything you have given me. Your love, support and advice is the reason that I survive every day. Thank you for being exactly who you are.

Finally I would like to thank Mom, Dad, Gordon, MamaRe, Papo, GJo, GLee and the rest of my family. I am so fortunate to have such an amazing, loving, caring and devoted family that has supported my decisions and given me a reason to succeed. You have made me who I am today, and I owe all of my achievements to you!

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CHAPTER 1

INTRODUCTION

Smell is the most important sense used in wine tasting. Humans can perceive only four tastes – sour, bitter, sweet and salt – but can typically identify more than two thousand different scents (Zraly 2005). The overall aroma, or bouquet, of any given wine consists of approximately two hundred compounds. In contrast, the basic flavor depends on 20 or more compounds. Several compounds have been identified in the aroma profile of wine, including but not limited to soluble solids, alcohol, esters, acids, carbonyl compounds, ethers, sulfur compounds, nitrogen compounds, phenolic compounds and other trace metals (Baldy 1997; Zoecklein and others 1999; Garcia and others 2006b).

Each varietal has a unique composition of aroma and flavor compounds. However, discriminating between varietals is often difficult due to the complex nature of wines. Therefore, the complete analysis of wine aroma is a time consuming and expensive endeavor. Two methods are commonly used to assess the aroma quality of wine; trained human sensory panels and instrumental analytical techniques. Sensory panels are widely used in wine classification and analysis, but are subject to errors due to training quality, standardization, and the stability and reproducibility of the wine evaluation. Instrumental analytical techniques such as gas chromatography can identify and quantify the volatile compounds in the headspace of most food and beverage products. Although this method is more reliable and reproducible than sensory panels, it has a higher cost per analysis.

Electronic nose systems are an alternative to analyzing wine aroma profiles. The electronic nose consists of an array of gas sensors with different selectivity, a signal-collecting unit and pattern recognition software (Van Deventer 2001; Abbey and others 2003; Garcia and others 2006c; Buratti and others 2007). This technology was developed in the 1980s, and typically uses metal oxide semiconductor sensors, surface acoustic wave sensors and quartz resonators (Van Deventer 2001; Garcia and others 2006b). The advantages of electronic nose systems are portability, cost-effectiveness, and reliability (Garcia and others 2006b). These systems are particularly useful for analyzing the

headspace of liquid or solid food samples. In the past few years, there have been numerous attempts to use the electronic nose for classification of wines (Guadarrama and others 2001; Kallithraka and others 2001; Buratti and others 2004; Garcia and others 2006b; Buratti and others 2007). However, such analysis can be difficult due to the non-specificity of the sensor arrays in the electronic nose due to the presence of high ethanol and water concentrations in the wines (Garcia and others 2006b).

1.2 SIGNIFICANCE AND RATIONALE

In the wine industry, there is a great need for cost effective systems that perform like a biological nose, but with reliability. The electronic nose is cost-effective because the system requires less extensive preparatory measures than human sensory and instrumental analysis. Evidence suggests that electronic nose sensor technology can determine characteristic recognition patterns of different wines and has the potential as a discriminatory analysis tool (Lozano and others 2005; Garcia and others 2006b; Buratti and others 2007). Recently, metal-oxide sensors have been used in wine discrimination. However, extensive review of the literature found no analysis using a quartz-microbalance system. Quartz microbalance-based systems have been reported to produce stable results. Therefore, this research will focus on the optimization of a quartz-microbalance electronic nose system and the implementation of this system for wine discrimination and analysis.

1.3 HYPOTHESES AND OBJECTIVES

This study will address the following research hypotheses and objectives for the HKR QMB6 electronic nose system:

Hypothesis 1: An electronic nose system based upon quartz-microbalance technology can effectively discriminate between Cabernet Sauvignon and Mouvèdre wines.

Objective 1: To optimize the electronic nose for adequate detection levels of volatiles from Cabernet Sauvignon and Mouvèdre wines.

Hypothesis 2: An electronic nose system based upon quartz-microbalance technology can be effectively used to discriminate wines produced using different viticulture practices such as ethanol spray.

Objective 2: To test the ability of the electronic nose to accurately classify Cabernet Sauvignon wines made from grapes subjected to three different ethanol spray treatments (0, 5 and 10% v/v).

Hypothesis 3: The electronic nose will produce comparable results to that of a consumer sensory panel.

Objectives 3: To comparatively assess the electronic nose system against a sensory panel based on its ability to discriminate between Cabernet Sauvignon wines made from grapes subjected to three different ethanol spray treatments.

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CHAPTER 2

LITERATURE REVIEW

2.1 SUMMARY

Electronic nose systems are comprised of multi-sensor arrays that measure aroma compounds from a limitless variety of samples. Therefore electronic nose chemosensory systems have the potential to discriminate between wine varieties and between wines made from the same grape variety that have been subjected to different treatments. The electronic nose may be used by the wine industry to compliment sensory panel and instrumental chemical analyses. The role of a quartz-microbalance headspace sampler as an additional wine discrimination tool is reviewed in this chapter.

Keywords: chemosensory, wine evaluation, ethanol spray

2.2 INTRODUCTION

Americans are drinking more wine than ever before. Impact Databank's 2006 report on the U.S. wine market, published by M. Shanken Communications, shows a 45 percent increase in wine consumption over 30 years. Wine sales in the U.S. increased by 12 percent in quality and 5 percent in quantity, which reflects increased demand for a quality product (USDA 2006). Increased consumption has been fueled by improved access to a wider selection of varieties and the availability of lower-priced wines. Consumer interest in wine has led to the improved quality of restaurant wine lists and the hiring of full-time wine writers to the staffs of major newspapers including *The Wall Street Journal* and *USA Today*. The United States has been projected to be the world's biggest wine consumer by the end of the decade (Zraly 2005).

Winemakers rely on sensory and chemical analyses to produce wine that is acceptable to the increasingly knowledgeable consumer base. Sensory analysis is essential to wine discrimination; chemical analysis is valuable for determining the chemical composition of the final wine product. Although sensory and chemical analyses are accepted practice by the wine industry, both methods are time-consuming and

expensive. A faster, cost-effective method of discrimination and analysis would be a welcome addition to the current analysis techniques used in wine evaluation.

Electronic nose systems have been introduced as a new method for wine discrimination and analysis. The perception of aroma volatiles by the electronic nose involves the reactions of aroma compounds with an array of sensors. Statistical methods are used to compare the electronic nose results to a known database. The main advantage of electronic nose systems is reduced preparation and processing time and subsequent costs, compared to sensory or chemical analyses. Electronic nose systems are based on several different technologies. This study investigated the use of a quartz-microbalance based as a tool for wine discrimination.

2.3 GRAPE VARIETALS

Grape variety is the single most important element of wine flavor. Wines can be made from one grape variety or a blend of two or more varieties. For this study, two red wine varieties, Cabernet Sauvignon and Mouvédre, were used during the optimization of the electronic nose system. These varieties were chosen due to their versatility in winemaking. Cabernet Sauvignon, referred to as the “king of reds”, is the premier red wine grape in the world and is native to the Bordeaux region of France. This variety has small berries with a thick, tough skin. Skin toughness makes the grapes fairly resistant to disease, spoilage and rain damage. The Cabernet Sauvignon variety produces distinctive, tannic wines that can have long aging potential (LaMar 2005). Although commonly bottled as a single variety, Cabernet Sauvignon can also be blended with other varieties to increase the overall complexity of the final wine. The taste characteristics of Cabernet Sauvignon are dark cherry, cedar, tobacco and black currant. Grapes grown in cool climates may yield green pepper or olive notes (Baldy 1997). The combination of berry characteristics and flavor appeal has made Cabernet Sauvignon one of the most popular red wine varieties worldwide.

The Mouvédre wine variety originated in Spain where it is called *monastrell*. Mouvédre is the foremost red variety in the five appellations that are found on Spain's southeastern Mediterranean coast (LaMar 2005). The Mouvédre grapes variety is slow ripening, and tends to bud and ripen late. Because the grapes form tight bunches that

require ventilation, Mouvédre grapes grow best in hot, windy climates. This varietal is rarely bottled as a single variety, but has desirable blending characteristics such as color, high acidity and high tannin content. Mouvédre is most recognized in blended wines from the South Rhone valley (Zrally 2005). The taste characteristics of Mouvédre are meaty, rustic, blackberry, leather, herbs and spice.

2.4 WINEMAKING

Winemaking is the craft of producing an alcoholic beverage by fermenting the juice of grapes or other fruits. Grapes are ideal for winemaking because they contain the proper amount of sugar, acid, yeast, and other compounds to ferment completely. Other fruits, such as plums and cherries, require extra ingredients to produce wine. Most grapes contain enough sugar to produce a dry wine with 12 to 13.5 percent alcohol.

The overall style, flavor and quality of the wine are controlled by the winemaker. The winemaker is also responsible for the variations between wines produced from the same grape variety in the same region. The basic winemaking process begins when the winemaker decides when to harvest the grapes based on taste and sugar content. Once the grapes are harvested, they are destemmed and crushed, and the juice, skins, and seeds are poured into a stainless steel fermentation vat. Because most winemakers prefer cultured yeasts, sulfur dioxide is added to the grape juice to kill the natural yeasts. Cultured yeasts then added to the vat and fermentation begins.

To produce a quality wine, the proper temperature must be maintained during the fermentation process. If the temperature is too high, the yeasts will stop metabolizing the fermentable sugars into alcohol, and bacteria may convert the half-fermented juice into vinegar. A fermentation temperature that is too low will prevent the extraction of the grapes' full flavor and color. Another important key to the fermentation process is maintaining a moist cap of grape skins on the surface of the vat. The winemaker mixes the fermenting juice and grapes on a regular basis to keep the grape skin cap from drying.

During fermentation, Brix readings are taken daily to assess the sugar content of the juice. The fermentation process is terminated when the residual sugar is less than 2.0 g/L. The wine is then drawn off and the solids are placed in a press to extract any remaining liquid. The free-run wine and pressed wine are then racked into containers

(stainless steel, epoxy-lined cement tanks, wooden vats or barrels) that are completely filled to prevent oxidation. In some regions, varietals may be blended together at this point. Wines are typically transferred to different tanks from time to time to prevent the wine from growing stale. The time between fermentation and bottling varies from a few weeks to a few years.

Wines may be fined to remove any unwanted elements such as phenolics and yeast and grape solids left over from the winemaking process. Fining agents are grouped into the following categories: earths (bentonite, kaolin), proteins (gelatin, caseins, albumens, yeasts), polysaccharides (gum arabic, alginates), carbons, synthetic polymers, silica gel, tannins and others (blue fining, metal chelators, enzymes) (Zoecklein and others 1999). Wines may also be filtered to remove bacteria and solids. Some winemakers prefer to skip these processes because they may remove flavor. Once wines are bottled, they are discriminated and analyzed by sensory and chemical means.

2.5 CHEMICAL ANALYSIS

Numerous chemical analyses of wine are used in the evaluation of wine. The wine industry typically analyses wine and determines its quality using the following: pH, percent alcohol, titratable acidity, °Brix, tartaric and malic acids, total glycosides, phenol free glycosides (PFG), total phenols, color intensity and hue, total anthocyanins, pigment cofactors and polymers.

Most wineries recognize pH as the most important indicator of grape juice or wine quality (Zoecklein and others 1999). Typically wines have pH values between 2.5 and 4.0 (Baldy 1997). Low pH values are important to wine quality for two main reasons. First, wines with low pH values are more resistant to oxidation and microbial spoilage requiring less sulfur dioxide for preservation, thus saving money (Baldy 1997). Second, red wine color intensity is enhanced at lower pH values. Red-colored anthocyanins are mainly responsible for the color of red wine. In equilibrium, these anthocyanins exist in either colored or colorless form. However, a low pH causes the equilibrium to shift towards the colored form.

The percent alcohol (ethanol) in wine can be determined by numerous methods including ebulliometry, gas chromatography and hydrometry. Ebulliometry measures the

boiling point of the wine relative to the boiling point of water. The difference between the two boiling points is related to the percent ethanol in the wine. Gas chromatography separates ethanol from other wine components and quantifies the amount of ethanol. Hydrometry is used to measure the specific gravity of distilled wine from which percent ethanol can be determined (Zoecklein and others 1999).

Titrateable acidity is a measure of the total acid content of a solution. Wine is titrated with sodium hydroxide (NaOH), and the pH increases until the equivalence point is reached. This is the point where the amount of NaOH added is equal to the amount of acid originally present in the solution. The concentration of the acid in the wine sample can be calculated from the volume and concentration of NaOH solution needed to reach the equivalence point.

°Brix is used to determine the amount of soluble solids in the wine during fermentation. °Brix is measured using hydrometry or refractometry. Tartaric and malic acid can be determined using a spectrophotometer or HPLC unit. A key factor in aroma and flavor analysis is the phenol-free glycosides. The literature states that increased phenol-free glycosides may reflect an increase in the aroma and flavor compounds in the wine (Zoecklein and others 2000). Finally, total phenols, color intensity and hue, total anthocyanins and pigment cofactors are determined using UV spectrometry. These analytical chemical analyses offer a way for winemakers to further understand the product that they have produced. Familiarization of these procedures is important for winemakers to identify problems during production and thus produce a quality wine.

2.6 HUMAN SENSORY EVALUATION

Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Lawless and Heymann 1999; Zoecklein and others 1999). This definition is accepted by sensory evaluation committees within the Institute of Food Technologists (IFT) and the American Society for Testing and Materials (ASTM). Sensory evaluation is another way to discriminate between wines and assess their quality.

Humans tend to perceive the attributes of a food item in the following order: appearance, aroma, consistency and flavor (Meilgaard and others 1999). Aroma is one of the best ways to evaluate a wine sample, and a well-trained sensory panel can detect subtle and major aroma differences between wines.

The human sense of smell is more complicated than the other senses in regard to the mechanisms responsible for the primary reaction to an external stimulus (Lozano and others 2005). Airborne odors are sensed by the olfactory epithelium located in the roof of the nasal cavity. The human olfaction system uses approximately 10,000 biological receptors in the nose to produce signals that the brain interprets and recognizes as a particular aroma (Hodgins 1996). This system is also able to detect aromas from compounds at concentrations in the sub-ppb range (Payne 1998). Based on the sensitivity and selectivity of the human nose, sensory panels have a great advantage over chemical analyses. Unfortunately, sensory panels have limited availability, high cost, associated fatigue, time limitations and inherent subjectivity.

Sensory testing can occur in a number of locations depending on the desired data being collected. However, the physical setting must be designed to minimize bias, maximize sensory sensitivity, and eliminate variables from products that are not being tested. Private booths are a popular choice for sensory testing because booths reduce the amount of distraction from other panelists. Lighting in the booths is planned to permit adequate viewing of the samples (Meilgaard and others 1999). Red, green and blue lighting is sometimes used to mask differences between samples in difference tests.

Sensory tests are chosen based on the question that the sensory analyst is attempting to answer. Overall difference tests determine if there is a sensory difference that exists between the samples. Attribute difference tests attempt to discover how a particular attribute such as saltiness or sweetness differs between samples. Triangle difference testing is often used during wine sensory panels as a form of discrimination. In this testing method, panelists are presented with three samples that have been given 3-digit random codes. Two of the samples are the same, and one sample is different. The panelist is asked to identify the different sample in the group based on one particular sense; smell, sight, taste or touch.

Sample presentation is an important aspect of sensory evaluation. The order of presentation should allow each sample to appear in a given position an equal number of times. For example, if you are using three different products, possible presentation combinations are:

ABC – ACB – BCA – CAB – CBA – BAC

For this example, the tests should be set up in multiples of six so that each combination is presented an equal number of times (Meilgaard and others 1999). Coding of the samples should be completely random, and should not have any identifying features to prevent bias. Sensory analysts typically select codes from a table of randomized triple-digit codes.

2.7 ELECTRONIC NOSE TECHNOLOGY

The electronic nose is an instrument consisting of an array of electronic chemical sensors capable of detecting and identifying aromas. These systems were developed to mimic the human olfactory system. Electronic noses became commercially available in 1995 and have been used extensively in the food industry for quality and discriminatory analysis. Electronic nose technology has been successfully used to discriminate quality and flavor of various products including ham (Garcia and others 2006a), tomatoes (Gomez and others 2006), wine (Guadarrama and others 2001), oil (Cosio and others 2006; Hai and Wang 2006), onions (Abbey and others 2001), apricots (Natale and others 2006), apples (Pathange and others 2006) and strawberry ice cream (Miettinen and others 2002). Electronic noses have also been used in the research of food safety (Arora and others 2006), potable water (Catarina Bastos and Magan 2006), perfumes (Poprawski and others 2006), fermentation processes (Bachinger and Mandenius 2001), fuel qualification (Sobanski and others 2006), and fire detection (Scorsone and others 2006). These systems are considered to be reliable, fast and easy to use (Payne 1998).

The electronic nose is not intended to be a direct substitute for the human sensory panel. However, it can be used as a rapid, automated and objective tool for detecting, monitoring and measuring aroma compounds (Pathange 2003). Electronic nose systems are classified according to the material from which the sensor is made. Most commercially-available systems are based on conducting polymers, quartz microbalance

or metal oxide. Each system uses a number of individual sensors which have overlapping whose selectivities for different compounds. The response from a chemical sensor is measured as the change of some physical parameter such as mass, conductivity or current. The response times for electronic nose systems range from seconds to up to a few minutes (Lozano and others 2005).

2.7.1 Conducting Polymer Sensors

Conducting polymer sensors consist of a conducting polymer, a counter ion and a solvent. These components “grow” from a solution onto an electrode to form a resistor. As a result, this type of electronic nose measures changes in resistance. Sensors made from polymers based on aromatic compounds are sensitive to many volatile compounds, and have the greatest range and balance between selectivity and sensitivity. This type of electronic nose can be used at moderate temperatures, so problems associated with the breakdown of volatiles at the sensor surface at increased temperatures is avoided (Payne 1998). The ability to perform at moderate temperatures factor also allows this type of electronic nose system to be used as a portable device. The main disadvantages associated with electronic nose systems based on conducting polymer are greater sensitivity to water vapor and higher production costs.

2.7.2 Metal-Oxide Sensors

Metal-oxide semiconductor (MOS) sensors consist of a ceramic substrate heated by a wire and coated by a metal oxide semiconducting film. MOS sensors have a low sensitivity to moisture and are robust; therefore, they typically operate at temperatures ranging from 400 to 600 °C. In general, these sensors are not sensitive to nitrogen- or sulfur- based odors, but are sensitive to alcohols and other combustibles (VanDeventer 2001).

2.7.3 Quartz-Microbalance Sensors

Quartz-microbalance (QMB) sensors evolved from a larger group of piezoelectric crystal sensors. These sensors use crystals that can vibrate in either a surface acoustic mode (SAW) or a bulk acoustic mode (BAW). Quartz-microbalance sensors are made

from thin discs composed of quartz, lithium niobate or lithium tantalite. The discs are typically coated with materials which are in gas chromatographic (GC) stationary phases. However, any non-volatile compounds that are chemically and thermally stable can be used (Roussel and others 1999). When exposed to aroma, the volatiles adsorb onto the GC phase coating of the sensor, which causes a mass change. This change causes a measurable change of the oscillating frequency of the sensor. QMB sensors are a particularly useful electronic nose technology because they produce stable results (Roussel and others 1999).

The discriminatory power of any electronic nose system is based upon its ability to produce a measured response from aroma components and to respond differently to aromas with varying components. The fundamental response of each sensor is affected by the chemical nature and concentration of the volatiles in an aroma, system parameters and sample preparation (VanDeventer 2001). Optimizing the experimental parameters of the system ensures maximum sensor response and allows the electronic nose to become a useful analytical tool. Making electronic sensors more discriminating for particular aromas could increase the applicability in the food and beverage industry and other fields.

2.8 STATISTICAL ANALYSIS

Statistical analysis is the key to understanding the sensor response of any electronic nose system. Response Surface Methodology is used during the optimization of the electronic nose system. Multivariate discriminant analysis is performed on the data generated from the electronic nose in order to illustrate the discriminatory power of the system.

2.8.1 *Response Surface Analysis*

The electronic nose must be optimized for maximum sensor response during testing in order to ensure that the maximum sensor response is reached. Roussel and others (1999) performed sensor optimization while examining the influence of experimental parameters on the multi-sensor array measurements. Response surface designs are generated from electronic nose data to develop a model of the multi-sensor response. Response surface analysis involves the investigation of linear and quadratic

effects of two or more factors. The fundamental principle of response surface analysis is to develop a simple mathematical expression that approximates the relationship between response and examined factors. The response surface analysis procedure uses the method of least squares to fit quadratic response surface regression models. These models identify where optimum estimated response values occur.

2.8.2 *Multivariate Analysis*

Multivariate techniques are used to summarize large amounts of data. Some of the most common techniques are Principle Component Analysis (PCA), Factor Analysis (FA) and Canonical Correlation Analysis (CCA). Techniques used for comparing group means are Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA). Predicting grouping of data can be completed using Discriminant Analysis (DA), Canonical Discriminant Analysis (CDA) and Cluster Analysis (CA) (Pathange 2003).

2.8.2.a *Principle Component Analysis*

PCA is an exploratory multivariate technique that attempts to determine the transformation of a set of predictable variables (possibly correlated) into a set of new uncorrelated variables called principle components. In discrimination analysis, the objective of PCA is to reduce the number of variables to detect structure in the relationship. PCA assesses similarities between samples and the relationships between variables and attempts to identify clusters present in the data.

2.8.2.b *Canonical Discriminant Analysis*

Multivariate discriminant analysis, also known as canonical discriminant analysis is the most common analysis method used by electronic nose systems to separate classes of observations in a database. CDA is a dimension reduction technique that creates new canonical variables by taking linear combinations of the original response variables. The fundamental purpose of CDA is to provide a visual representation of the data observations that have been classified into different categories.

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CHAPTER 3

OPTIMIZATION OF CHEMOSENSORY SYSTEM FOR WINE DISCRIMINATION

(Manuscript in preparation for submission to Journal of Food Science)

Optimization of Chemosensory System for Wine Discrimination

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Running Title: Optimizing Chemosensory Discrimination of Wines

3.1 ABSTRACT

The specific objective of this study was to optimize the response of an electronic nose to volatiles from two red varietals of wine, Cabernet Sauvignon and Mouvédre. A quartz-microbalance based system containing a series of six unique nonselective sensor elements was used for this study. Response surface methodology was used to determine the optimum sensor response by varying several experimental parameters: sensor temperature (40, 60, and 80 °C), sample temperature (40, 60, and 80 °C) and equilibrium time (10, 15, and 20 min). The maximum sensor response occurred at an equilibrium time of 20 min for each varietal and at a sample temperature of 55 °C and 58 °C for Cabernet Sauvignon and Mouvédre, respectively. The optimum sensor temperature was 40 °C for both varietals. The results indicated that the electronic nose can be used as a tool for wine discrimination and analysis using the sample temperature and equilibrium time parameters found in this study.

Keywords: electronic nose, response surface, Cabernet Sauvignon, Mouvédre

3.2 INTRODUCTION

Current wine discrimination and evaluation techniques employ sensory and chemical analyses, which require extensive preparation and expertise in interpreting the results (Buratti and others 2004). The electronic nose offers an additional analysis technique to compliment current methods of wine discrimination techniques. These automated systems were developed to mimic the human olfactory system and can be used to provide rapid and objective responses to volatile analysis (Pathange 2003). Therefore, the electronic nose is an alternative tool wine discrimination analysis. The electronic nose was used to evaluate two wine varieties for this study.

Cabernet Sauvignon, sometimes referred to as the “king of reds”, is the premier red wine grape in the world. From the Bordeaux region of France, this varietal produces distinctive, tannic wines that have a long aging potential. Cabernet Sauvignon is commonly bottled as a single varietal, but is also blended with other varieties to increase the overall complexity of the final wine. Cabernet Sauvignon taste characteristics are dark cherry, cedar, tobacco and black currant. Cool climate growth can give green pepper or olive flavors (Baldy 1997).

Mouvédre wines, native of Spain, are medium-bodied, deeply-colored and full of cherry and berry fruit. This varietal is rarely bottled as a single variety because of its desirable blending characteristics including color and high acid and high tannin content. Mouvédre is best known for its blending role in the southern Rhone valley (Zrally 2005). Mouvédre taste characteristics are meaty, rustic, blackberry, leather, herbs and spice.

The specific objective of this study was to optimize a quartz-microbalance electronic nose (QMB6) for maximum sensor response to the volatiles of two red varieties, Cabernet Sauvignon and Mouvédre. System parameter optimization was performed for the QMB6 electronic nose system to optimize the sensor responses and enhance their discriminatory power. The three experimental parameters optimized were equilibrium time, sensor temperature and sample temperature. The QMB6 system was chosen because of its low sensitivity to water and ability to produce stable results.

The electronic nose is an instrument that can evaluate the overall aroma of wine and provide reproducible discriminatory results faster than current wine evaluation methods. However, the electronic nose must be optimized for maximum sensor response

before it can be used to evaluate wine samples. The response of each sensor and the overall discriminatory power of the electronic nose system are affected by the volatiles in the wine aroma, sample preparation and system experimental parameters (e.g. equilibrium time and sensor temperature). Research has been conducted to improve testing methodologies at optimum parameter settings and to examine the influence of various experimental parameters on maximum sensor response (Natale and others 1997; Hansen and Wiedemann 1999; Roussel and others 1999; Van Deventer 2001). Extensive work has been completed to optimize metal oxide electronic nose systems for a wide variety of applications in the food industry. However, no optimization parameters are available for a quartz- microbalance-based system to evaluate wines. This study investigates the optimization of a quartz-microbalance electronic nose system for wine discrimination and analysis.

3.3 MATERIALS AND METHODS

3.3.1 Electronic Nose System Information

For this study, a quartz-microbalance electronic nose system (Fig. 3.1) (Model QMB6, HKR Sensorsystems, Munich, Germany), connected to an automatic headspace sampler (Model HS-40, Perkin-Elmer LLC, Norwalk, CT) was used. This electronic nose consists of a series of six unique non-selective sensor elements that are coated with gas chromatographic stationary phases of varying polarities. The interaction between the sensors and the compounds in the sample aroma is measured as a function of the change in oscillating frequency of the sensors. The system evaluates volatiles in the headspace of the 21-mL headspace vials and passes it through a sensor chamber using nitrogen as a carrier gas. The sensors oscillate at 10 MHz. The adsorption of volatiles from the sample headspace onto the sensors causes a change in the mass of the sensors that result in a measurable change in its oscillating frequency. Sensor resolution is ± 1 Hz (Perkin-Elmer 1999). The cumulative response from each sensor together forms a characteristic recognition pattern for that particular sample. The electronic nose must be optimized for maximum sensor response in order to be trained to evaluate samples.

3.3.2 *Sample Preparation*

Cabernet Sauvignon and Mouvédre were used for this optimization study. Samples were obtained from the Virginia Tech Research Winery in Blacksburg, Va. Each headspace vial was filled with a 250- μ L sample as instructed by the operators' manual (Perkin-Elmer 1999). Five samples per varietal were prepared for each experiment in the response surface analyses.

3.3.3 *Wine Chemical Analyses*

Each wine treatment was evaluated by measuring pH, percent alcohol, titratable acidity, color hue, color intensity and total anthocyanins. The pH was determined with a pH/conductivity meter (Accument[®] model 20, Fischer Scientific, Pittsburgh, Pa., U.S.A.). Titratable acidity was determined by titration with NaOH to an end-point of pH 8.2. Percent alcohol was calculated using an ebulliometer. Total phenols ($A_{280\text{nm}} - 4$), color intensity ($A_{520\text{nm}} + A_{420\text{nm}}$), color hue ($A_{520\text{nm}}/A_{420\text{nm}}$) and total anthocyanins ($20 \times A_{520\text{nm}}$) were estimated spectrophotometrically (Genesys 5TM, Spectronic Instruments Inc., Rochester, N.Y., U.S.A.).

3.3.4 *Parameter Optimization with Response Surface Methodology*

A centrally rotatable design with three parameters and three levels was used to examine the effects of system parameters on the sensor response levels to Cabernet Sauvignon and Mouvédre volatiles. The principle of utilizing response surface analyses is to develop a mathematical expression that approximates the true relationship between response and parameter factors as closely as possible without having to perform a full factorial design of experiments (Devineni and others 1997). The system parameters and their range of values examined in this study were selected based on volatile kinetics and consultation with the system manufacturer.

For this study, the effects of sensor temperature (40, 60, and 80°C), sample temperature (40, 60, and 80°C) and equilibrium time (10, 15 and 20min) were investigated. Table 3.1 lists the experimental design including the range for each of the examined parameters. Design Expert 7.0 (Stat-Ease, Inc., Minneapolis, Minn., U.S.A.) was used to analyze the data.

3.4 RESULTS AND DISCUSSION

This study is the first stage of performance analysis for the QMB6 model to discriminate between wine samples. Chemical analyses were completed for both wine varieties. Table 3.2 provides a summary of the results obtained from these analyses. These results indicate that the two wines are similar in chemical composition. Based on the results from the chemical analyses it is expected that the optimization parameters may be similar for both wine varieties.

Maximum response from the QMB6 electronic nose system was found for the Cabernet Sauvignon and Mouvédre wines based on three parameters: sample temperature, sensor temperature and equilibrium time. The ranges for the three parameters were chosen based on the ranges received from the literature (VanDeventer 2001). The QMB6 electronic nose system has six individual sensors. Each of these sensors has an individual and unique response based on the sensitivity and selectivity of the sensor. Prior to the study, it was determined from the specifications of the users' manual that if the response of any of the sensors were less than 20Hz, that particular sensor would be deemed unacceptable for use in the study. Similarly, the sensor would be deemed unacceptable at a frequency change higher than 5000 Hz. Analysis of wine samples caused a noted overload in sensor 3, whose responses were well over the stated acceptable range of 20-5000 Hz. Because of the overloaded response from sensor 3 and recommendations from the system manufacturer, this sensor was not used during the optimization study.

As previously mentioned, each sensor has a unique and individual response. An example of two sensor responses for the Cabernet Sauvignon wine can be seen in Figure 3.2. In this figure, the individual sensor responses from sensor 1 and sensor 5 can be seen. The maximum response from sensor 1 during Cabernet Sauvignon analysis was 152.658, and the maximum response from sensor 5 was 522.413. Based on these responses, it can be seen that sensor 5 was much more sensitive to the Cabernet Sauvignon wine samples than sensor 1. In fact, Table 3.3, which displays all of the individual responses for each sensor, indicates that sensor 5 gave the highest response for Mouvédre as well.

For the purposes of this study, the overall response from all six sensors is needed. The models for all of the sensors were significant, and were therefore all used for the generation of the overall sensor response plots. The coefficient values for each of the system parameter linear terms and whether they were significant are given in Table 3.4. In examining the original plots generated for each sensor and verified by the magnitude of the linear coefficients in Table 3.4, it was seen that with the exception of sensor 6 (Mouvédre), the sensor temperature was the parameter that most affected the overall sensor response. For sensor 6 (Mouvédre), the parameter making the greatest contribution was found to be sample temperature. For sensors 1, 2, 4 and 6, the least contributing parameter is the equilibrium time, and for sensor 5, it is shown that the sample temperature is the least contributing parameter. All of the information from the individual sensors was used to generate the overall sensor response plots for each wine.

The overall sensor response for the parameter optimization of Cabernet Sauvignon can be seen in Figure 3.4. The maximum sensor temperature and sample temperature were 40 °C and 55 °C, respectively. The equilibrium time was selected to be 20 min. Figure 3.4 displays the overall sensor responses from the QMB6 system for the parameter optimization of Mouvédre wine. The maximum sensor response was obtained at a sensor temperature of 40°C and a sample temperature of 58°C. The equilibrium time was also set at 20 min for this varietal.

Each parameter was examined in order to verify the optimum settings for wine analysis. When choosing the equilibrium time, the overall sensor response was examined. At 15 min, the sensor response was at a minimum. The sensor response increased as the equilibrium time increased. Because of the need for a rapid analysis tool, equilibrium times above 20 min were not considered. Sample temperature was also optimized for the two varietals. The overall sensor response reached a maximum value around a sample temperature of 55 °C to 57 °C for both wines. Lastly, sensor temperature was optimized for the two wines. In the case of both varietals, the overall response from the electronic nose continued to increase with decreasing sensor temperature. In fact, a higher response could have been obtained by using a sensor temperature lower than 40 °C. Sensor temperatures below 40 °C could not be obtained without the addition of an external cooling unit.

Table 3.4 provides a summary of the optimization results for Cabernet Sauvignon and Mouvédre wines. The comparison of these results indicates no significant difference between the parameter settings for the electronic nose. This is comparable to the chemical analyses data obtained for these wine samples. Therefore, based on the data found in this study, both of these wines require the same parameter settings in order to receive maximum sensor response from a QMB6 electronic nose system.

3.5 CONCLUSIONS

Optimum settings for sample temperature and equilibrium time have been determined for wine analysis using a quartz microbalance-based electronic nose system. Due to a slight difference in the optimum sample temperature for the two wines, additional tests are needed to determine if the sample temperature parameter is significant based on wine varieties. Lower sensor temperatures should be tested to determine optimum sensor temperature. In this study, lowering the sensor temperature below 40 °C could not be accomplished because the system would require an external cooling unit. The purpose of this study was to optimize the QMB6 unit in its original state. In the future, optimization of the QMB6 electronic nose system should be completed for additional varieties, including white wines, in order to assess the optimum operating parameters for a variety of wines. Once this is completed the electronic nose may be utilized as a tool for wine discrimination and analysis.

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Table 3.1 Experimental design of response surface analysis experiments for the HKR Sensorsystems QMB6 using three factors and three levels

Experiment number	Sensor Temperature °C	Sample Temperature °C	Equilibrium Time Min
1	60	80	15
2	80	40	20
3	40	80	10
4	60	60	15
5	80	60	20
6	60	60	15
7	60	40	15
8	60	40	10
9	80	60	10
10	40	40	20
11	60	60	15
12	40	40	10
13	40	60	15
14	40	80	20
15	60	40	15
16	80	80	10
17	60	60	15
18	60	60	15
19	60	60	20
20	80	60	15

Table 3.2 Chemical analyses data for Cabernet Sauvignon and Mouvédre wine samples used for QMB6 electronic nose optimization study.

Varietal	pH	% Alcohol	TA	Intensity	Hue	Anthocyanins
Cabernet Sauvignon	3.73	11.4	2.81	0.515	0.775	28.5
Mouvédre	3.75	12	4.17	0.794	0.711	32.8

Table 3.3 Individual sensor responses from QMB6 system for Cabernet Sauvignon and Mouvédre wine

Sensor	Cabernet Sauvignon Sensor Response	Mouvédre Sensor Response
1	152.658	160.968
2	333.194	320.995
4	300.121	344.262
5	522.413	541.259
6	169.182	146.22

Table 3.4 Coefficients of the linear terms from the models for the HKR Sensorsystems QMB6 sensors obtained from the response surface methodology experiments.

	Sensor	R ²	Intercept	Equilibrium Time	Sensor Temperature	Sample Temperature
Cabernet Sauvignon	1	0.5329	93.28	-4.23*	-36.76*	5.32
	2	0.6027	194.15	18.79	-73.40	-31.36
	4	0.5270	175.87	-8.77*	-92.09	27.58
	5	0.5531	305.00	56.75	-107.39	-49.70
	6	0.6600	62.69	10.46	-44.14	-35.73
Mouvédre	1	0.5829	87.56	5.14*	-34.73	4.24*
	2	0.6421	197.69	17.28	-65.86	-40.45
	4	0.5846	172.76	9.44	-103.90	29.78
	5	0.5907	304.79	61.96	-111.97	-45.38
	6	0.7638	55.21	4.06	-39.84	-41.45

* Values found to be not significant ($\alpha = 0.05$)

Table 3.5 Sensor temperature, sample temperature and equilibrium time optimum settings for a QMB6 electronic nose system for Cabernet Sauvignon and Mouvédre wine discrimination analysis.

	Sensor Temp (°C)	Sample Temp (°C)	Equilibrium Time (min)
Cabernet Sauvignon	40	56	20
Mouvédre	40	55	20



Figure 3.1 HKR Sensorsystems QMB6 electronic nose system used for optimization analysis of Cabernet Sauvignon and Mouvédre wines

Design-Expert® Software

Sensor 1

● Design points above predicted value

○ Design points below predicted value

200.8

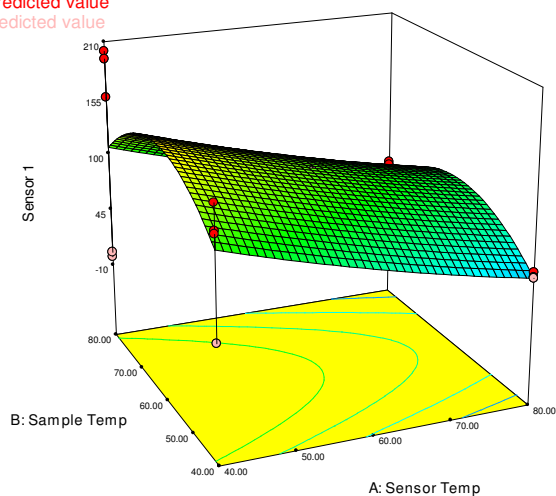
-2

X1 = A: Sensor Temp

X2 = B: Sample Temp

Actual Factor

C: Equil Time = 20.00



(a)

Design-Expert® Software

Sensor 5

● Design points above predicted value

○ Design points below predicted value

571.8

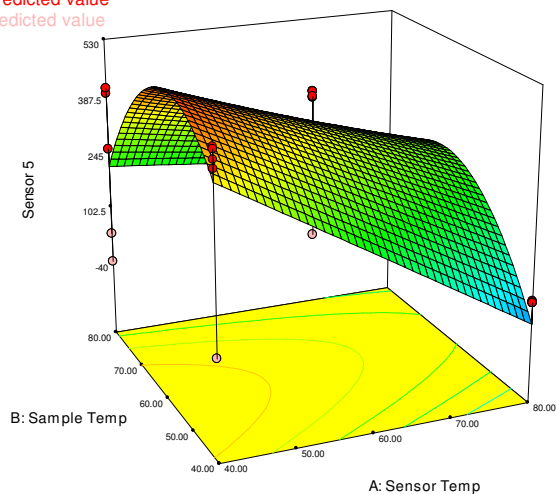
-146.9

X1 = A: Sensor Temp

X2 = B: Sample Temp

Actual Factor

C: Equil Time = 20.00



(b)

Figure 3.2 Responses of (a) sensor 1 and (b) sensor 5 from QMB6 electronic nose system for Cabernet Sauvignon wine optimization study.

Design-Expert® Software

Desirability

● Design points above predicted value



X1 = A: Sensor Temp

X2 = B: Sample Temp

Actual Factor

C: Equil Time = 20.00

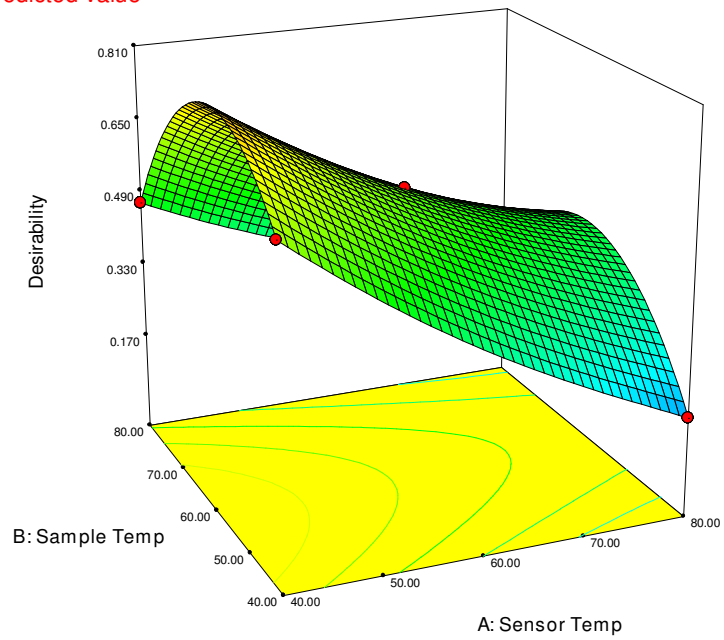


Figure 3.3 QMB6 sensor response for Cabernet Sauvignon wine

Design-Expert® Software

Desirability

● Design points above predicted value



X1 = A: Sensor Temp
X2 = B: Sample Temp

Actual Factor
C: Equil Time = 20.00

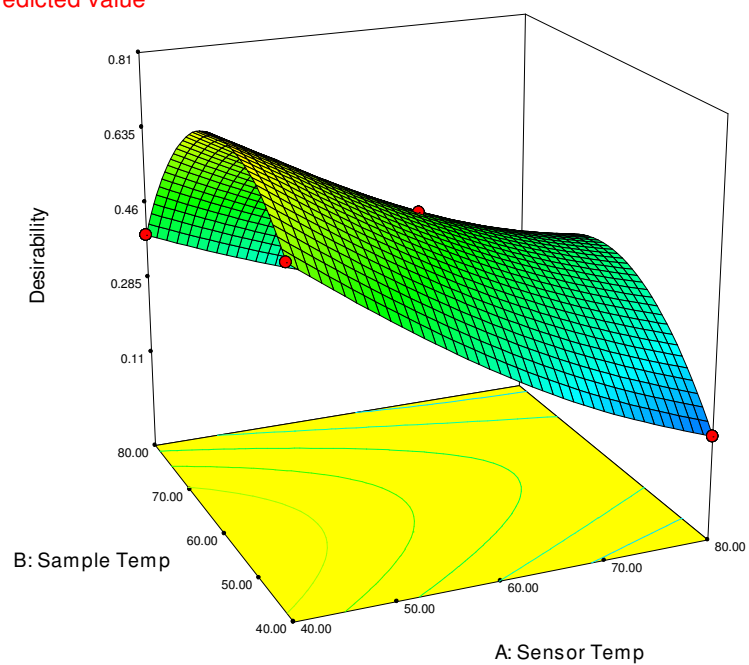


Figure 3.4 QMB6 sensor response for Mouvédre wine

CHAPTER 4

CHEMOSENSORY DISCRIMINATION OF WINES PRODUCED FROM CABERNET SAUVIGNON GRAPES TREATED WITH AQUEOUS ETHANOL POST-BLOOM

(Manuscript in preparation for submission to the Journal of Food Science)

**Chemosensory Discrimination of Wines Produced from Cabernet Sauvignon Grapes
Treated with Aqueous Ethanol Post-Bloom**

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Chemosensory Discrimination of Wines...

Food Engineering and Physical Properties

4.1 ABSTRACT

The ability of a quartz microbalance based electronic nose system to classify wines made from Cabernet Sauvignon (*Vitis vinifera* L.) grapes that received different ethanol spray treatments during growth was investigated in this study. Aqueous ethanol at concentrations of 5% and 10% v/v was sprayed on grape clusters at 13 wks post-bloom. Discriminatory analysis of the wines produced from these grapes was completed through objective (electronic nose, chemical analysis) and subjective (human sensory evaluation) testing. Results from chemical analysis and human sensory evaluation were compared with analysis of volatiles from the electronic nose. Chemical analyses including pH, percent alcohol, and color intensity indicated significant differences between the wines produced. Other chemical analyses including titratable acidity, total and phenol-free glycosides, hue or total anthocyanins showed no significant difference between the treatments. The consumer sensory panel consisting of 81 panelists was unable to differentiate amongst sample treatments. However, the electronic nose was able to differentiate between the control group and the treated samples 100% of the time. Canonical discriminant analysis of the data placed the 5% EtOH treatment as a sub-set of the 10% EtOH treatment. The results indicate that the electronic nose can be used as a discriminatory tool for assessing wines.

Keywords: electronic nose, wine evaluation, wine discrimination, sensory evaluation, ethanol

4.2 INTRODUCTION

By the end of this decade, the United States is projected to be the world's biggest wine consumer (Zraly 2005). Increased wine consumption has been fueled by improved access to a wider selection of varietals and the availability of lower-priced wines. Wine sales increased by 12 percent in value and 5 percent in quantity, which reflects increased demand for a quality product (USDA 2006). Wine quality is a subjective judgment that depends on the degree to which the wine is satisfying, balanced, and reflects the character of the grape. To consistently ensure a high quality product, the wine industry must perform discrimination and quality analysis of their wines.

Current methods for wine discrimination and quality analysis can include chemical analysis and human sensory evaluation. Chemical analyses can accurately discriminate between wines, but can be time intensive and expensive (Buratti and others 2007). Some of the most common properties evaluated during chemical analysis of wines include pH, alcohol, titratable acidity, °Brix, tartaric and malic acids, total glycosides, phenol free glycosides, total phenols, color intensity and hue, total anthocyanins, pigment cofactors, and polymers.

Sensory evaluation is also widely used to discriminate between and assess the quality of wines. Although sensory evaluation is perhaps the most reliable method of assessing wine quality, there are some key limitations to its use including the requirement of a highly trained sensory panel, extended preparation time and the need for a sensory evaluation specialist to analyze the data (Buratti and others 2007). Furthermore, sensory evaluation is subjective which makes it difficult to replicate and correlate the sensory data with data obtained from chemical analyses. Therefore, the identification of an objective, rapid-analysis technique for wine discrimination could save the wine industry time and money (Garcia and others 2006b).

The electronic nose is a relatively new technology that has gained popularity in the food industry for a number of applications (Lozano and others 2005; Garcia and others 2006b; Moens and others 2006) including food analysis (Natale and others 1997) and bioprocess monitoring (Bachinger and Mandenius 2001). Electronic nose systems have a multisensor array that is used to measure aroma compounds much like the human olfactory system (VanDeventer 2001; Miettinen and others 2002; Pathange 2003; Garcia

and others 2006b). The unique feature of the electronic nose system is that its response accounts for all the characteristic features (chemical and physical) of a sample with one single measurement (Hai and Wang 2006).

Electronic noses with metal-oxide sensors have been used to classify different types of wine with different denominations of origin (Kallithraka and others 2001; Buratti and others 2004). Garcia and others (2006) used a metal-oxide electronic nose to classify four wines of the same varietal which come from the same cellar. The wines differed in the evolution that occurred after the fermentation of the wine (aging length and barrel material). Penza and Cassano (2004) characterized Italian wines using thin-film multisensor arrays and artificial neural networks. Discrimination of wine aroma using an array of conducting polymer sensors with solid-phase micro-extraction was studied by Guadarrama and others (2001). This study used a quartz-microbalance electronic nose system to expand upon current wine discrimination research, and to see if an electronic nose system could be used to discriminate between wines produced under different vineyard managements. To date, applications of the quartz-microbalance-based electronic nose systems include edible oils, spice mixtures, marinades, terpenes in citrus products, and food packaging materials (Perkin-Elmer 1999; VanDeventer 2001).

The overall objective of the study was to determine if a quartz-microbalance-based electronic nose system is capable of discriminating between wine samples made from grapes treated with 5% and 10% (v/v) concentrations of aqueous ethanol, and to correlate the objective measurements to those obtained from a consumer sensory panel. Aqueous ethanol sprayed at 8 to 13 weeks post bloom on Cabernet Sauvignon (*Vitis vinifera* L.) grape clusters increased the anthocyanin content in berry skins, juice and wines (El Kereamy and others 2002). This evidence suggests that aqueous ethanol may impact fruit maturity and subsequent wine quality.

4.3 MATERIALS AND METHODS

4.3.1 Experimental Design

Sixteen vines per treatment were randomly selected within a Cabernet Sauvignon (*Vitis vinifera* L.) vineyard grown at the Winchester Field Research Facility in Winchester, Va. Treatments consisted of a control (water) and aqueous ethanol (5% or

10% v/v) sprayed on grape clusters in the field 13 weeks post bloom. Ethanol concentrations were chosen based on previous work completed by the Enology Laboratory group at Virginia Tech. Each treatment had 16 vines for a total of 48 Cabernet Sauvignon vines available for the experiment. Commercial harvesting standards for Cabernet Sauvignon were used for this study. The grapes were made into wine using three fermentation replications for each treatment for a total of 9 experimental units. Wine chemical analyses commonly utilized by the wine industry were completed for this study and are discussed below. Electronic nose evaluation was conducted 5 months post-fermentation for wine discrimination and compared to the results of sensory evaluation.

4.3.2 Winemaking

Approximately 87 kg of fruit were harvested per treatment, with an average of 1.8 kg of grapes per vine. Fruit was transported to the Virginia Tech Research Winery (Blacksburg, Va.) and stored at 7 °C processing. Rotted fruit was removed from each lug to improve the quality of fruit to be processed. The grapes were destemmed and crushed with 70% berry breakage using a (Model type-A2, Wottle) destemmer/crusher. Potassium metabisulfate (10 mg/L) and 100 mL/ton pectinase (ColorX, Scott Labs, Petaluma, Calif., U.S.A.) were added post-crush. Twenty-two kilograms of each treatment was transferred to separate cylindrical tanks and cold soaked at 7 °C for 4 days with an addition of 200 mg/L dimethyl dicarbonate (DMDC) (Velcorin™, Bayer Corporation, Pittsburgh, Pa., U.S.A.) at day 4. Must was mixed once a day during the cold soaking. Post cold-soak, each lot was capitalized to 22 °Brix and inoculated with 120 g/L *Saccharomyces cerevisiae* (D-254, Scott Labs, Petaluma, Calif., U.S.A.). Fermentation was conducted in cylindrical tanks at 27 °C with hand cap punching 3 times daily, 5 h apart. At dryness (≤ 2.0 g/L residual sugar), wines were dejuiced, cold settled at 7 °C for 24 h, and racked into 5 L glass carboys in an anaerobic environment. An addition of 25 mg/L sulfur dioxide and 200 mg/L dimethyl dicarbonate were added to the racked wines. The wines were racked and stored at a constant temperature of 7 °C. Wines were bottled by hand into 750 mL screw-capped bottles and stored at 7 °C until the sensory and electronic nose evaluation.

4.3.3 *Wine Chemical Analysis*

Each wine treatment was evaluated by measuring pH, °Brix, percent alcohol, titratable acidity, total and phenol-free glycoside concentration, color hue, color intensity and total anthocyanins. The pH was determined with a pH/conductivity meter (Accument[®] model 20, Fischer Scientific, Pittsburgh, Pa., U.S.A.) and °Brix was determined using a temperature-compensating refractometer (Model 10419, American Optical, Buffalo, NY, U.S.A.). Titratable acidity was determined by titration with NaOH to an end-point of pH 8.2. Percent alcohol was calculated using an ebulliometer. Total glycoside concentration was determined as described by the literature (Iland and others 1996), but modified with 200 mg polymeric reverse-phase extraction cartridges (Model HLB, Strata X[™], Phenomenex, Torrance, Calif., U.S.A.) (Zoecklein and others 2000). Phenol-free glycosides were analyzed as described by Zoecklein and others (2000) using a hydrophilic, lipophilic balance (Oasis[™] HLB, Waters, Milford, Mass., U.S.A.). Total phenols ($A_{280\text{nm}} - 4$), color intensity ($A_{520\text{nm}} + A_{420\text{nm}}$), color hue ($A_{520\text{nm}}/A_{420\text{nm}}$), total anthocyanins ($20 \times A_{520\text{nm}}$), and polymers were estimated spectrophotometrically (Genesys 5[™], Spectronic Instruments Inc., Rochester, N.Y., U.S.A.).

4.3.4 *Electronic Nose Analysis*

Ten samples from each wine treatment were evaluated using the quartz-microbalance-based electronic nose system (Model QMB6, HKR Sensorsystems, Munich, Germany) as described by the user's manual (Perkin-Elmer 1999). The QMB6 system (Fig. 4.1) was connected to an automatic headspace sampler (Model HS-40, Perkin-Elmer LLC, Norwalk, Conn., U.S.A.). The system consists of six non-selective sensors coated with gas chromatographic stationary phases of varying polarities oscillating at a frequency of 10 MHz. Nitrogen served as the carrier gas. Each 21-mL vial was filled with 250 μL of wine as instructed by the operators' manual (Perkin-Elmer 1999). During the electronic nose evaluation, a sample headspace was generated and passed through a sensor chamber by pressurizing the headspace with the nitrogen carrier gas. To obtain maximum sensor response, operating parameters for the electronic nose were optimized for the wine samples (Martin 2006). The optimized parameters were equilibrium time, sensor temperature, and sample temperature. Equilibrium time was set

at 20 min, sensor temperature at 40 °C, and sample temperature was set at 56 °C (Martin 2006). Data were analyzed using the QMB6 software (QMBSOft v. 1.22).

4.3.5 *Sensory Evaluation*

Wines were evaluated 5 months post-fermentation at the Food Science and Technology Sensory Laboratory at Virginia Tech (Blacksburg, Va.) using triangle difference testing as described by Meilgaard and others (1999). Each panelist conducted two triangle tests per session, one concerning aroma and one concerning flavor. Eight panelists participated in each sensory session for a total of 16 sets of samples per session. A total of 81 panelists were utilized for the sensory evaluation for a total of 11 sessions. Panelists were given oral instructions at the beginning of each session and written instructions during each test. Panelists were allotted 10 min to determine differences in aroma and flavor during the same session. Clear, standard ISO glasses, 3-digit random coded, were filled with 15 ml of wine at 20°C and presented to each panelist in a red light setting using the sensory evaluation booths. The panelists were asked to identify the odd sample out of each test. After marking their choice on the scorecard, they could choose to make comments on each of the samples.

A triangle test was used to compare the control samples to those treated with 5% EtOH. The following statistical parameters were set for the evaluation: $p_d = 0.30$, $\alpha = 0.05$, and $\beta = 0.20$. A total of 48 responses were collected. A total of 22 correct responses were needed for the two treatments to be statistically significant. A triangle test was also used to compare the control samples to those treated with 10% EtOH. The following parameters were set for the evaluation: $p_d = 0.30$, $\alpha = 0.05$, and $\beta = 0.30$. A total of 33 responses were collected. A total of 17 correct responses were needed for the two treatments to be significantly different.

4.3.6 *Statistical Analysis*

Multivariate discriminant analysis was performed using the statistical software package PC-SAS (SAS Inc., Cary, N.C., U.S.A.). The GLM procedure was run to generate ANOVA tables, generate plots and t-tests on the data. STEPDISC, CANDISC and PRINCOMP procedures were used to identify significant variables, canonical

discriminate analysis and principle component analysis, respectively. In discrimination analysis, the objective of PCA is to reduce the number of variables in order to detect structure in the relationship. PCA assesses similarities between samples, the relationships between variables and attempts to identify clusters present in the data. The PROC STEPDISC in SAS was used to rank electronic nose sensor data based on the contribution to the discriminatory power of the system. In addition to SAS, the electronic nose data was evaluated using QMBSoft v. 1.22 (QMB6 software). Evaluation of the sensory data was completed using statistical tables from Meilgaard and others (1999).

4.4 RESULTS AND DISCUSSION

4.4.1 Wine Chemical Analyses

ANOVA tests were completed for the chemical analyses data to compare the different tests and identify significant differences in the three treatment samples. A relative comparison of the chemical analyses is presented in Figure 4.2. No significant difference was found between the three treatments based on titratable acidity, total and phenol-free glycosides, hue or total anthocyanins (Fig. 4.2). Significant differences were found between the pH of control samples (3.89 ± 0.05) and those treated with 5% EtOH (3.97 ± 0.004). Percent alcohol was significantly different for control (12.8 ± 0.001), 5% EtOH (12.4 ± 0.05) and 10% EtOH (12.2 ± 0.05). Color intensity found to be significantly different between 5% (4.52 ± 0.25) and 10% EtOH (3.85 ± 0.25) only. Total anthocyanins were not affected by the treatment in this study. However, work from El Kereamy and others (2002) showed that spraying Cabernet Sauvignon grapes with 5% ethanol increases anthocyanin accumulation. Similarly, work from Baki (2003) also indicated that there were significant differences between treatments based on anthocyanins. The discrepancy with this finding between this study and previous work by Baki (2003) could be due to processing differences, seasonal differences and changes in the grape maturity at the time of harvest. On the other hand, the primary objective of this study was not evaluate the effect of ethanol treatment on wine quality but to evaluate the applicability of electronic nose system to discriminate wines from different viticulture practices, like aqueous ethanol treatment.

As a source of comparison, data from previous work (Baki 2003) using ethanol treatments for grape maturity and wine quality was used. Table 4.1 displays a summary of the data obtained from this study. Significant differences were found between total anthocyanins and total phenols (Baki 2003). Unlike the data obtained from this study Baki (2003) stated that percent alcohol and pH did not differ between the wine treatments. Also, in contrast to the data from this study, Baki (2003) indicated no significant difference between 5% and 10% based on color intensity. The discrepancies of these results indicate a need for more research to be completed to evaluate the effect of aqueous ethanol treatment on wine quality.

Principle component analysis (PCA) was completed to verify cluster structures within the chemical analyses data. The PCA plot in Fig. 4.3 illustrates the distinct separation between treatment groups. Therefore, the chemical analyses were successful in discriminating between the three treatments even though the only significant chemical analyses were pH, percent alcohol and color intensity.

The CANDISC procedure determined that alcohol and pH were the most significant in separating the treatments. Based on this procedure, alcohol and pH were used for canonical discriminant analysis of the chemical analyses data. A canonical plot (Fig. 4.4) was created to illustrate the separation of the three treatments. Cross validation using the data from all chemical measurements resulted in 100% discrimination between the treatments.

4.4.2 Sensory Evaluation

The sensory evaluation responses for the comparison of the control group to the 5% EtOH treatment were analyzed. Fourteen correct responses were received for aroma and 15 correct responses were received for taste. Because 22 correct responses were required for both aroma and taste, the 0% EtOH treatment and the 5% EtOH treatment were not significantly different in either taste or aroma. The sensory evaluation responses for the comparison of the control group to the 10% EtOH treatment were also analyzed. Fourteen correct responses were received for aroma and 11 correct responses were received for taste. Thirty-three correct responses were required for both aroma and taste. Therefore, aroma and taste for the wine treated with 0% EtOH and the wine treated

with 10% EtOH was not significantly different. Furthermore, a significant difference between 5% and 10% EtOH treatments would not occur based on this data, so sensory evaluation between these treatments was not conducted.

Sensory evaluation from Baki (2003) was investigated for a basis of comparison. Like the results from this study, the panel was not able to identify a difference between the control group and 10% ethanol treatment based on aroma or flavor. Similarly, the panel did not identify a difference between the control group and the 5% ethanol treatment based on flavor. However, unlike this study, Baki (2003) indicated that the panel was able to discriminate between the control group and the 5% ethanol group based on aroma.

The results from the sensory evaluation revealed that an untrained consumer panel was not able to discern any differences between the wines based on aroma and taste. Consumer acceptance of wine is important because they are the end-user of the product. Based on the sensory evaluation from this study, consumers were not able to distinguish between wines created from grapes that received aqueous ethanol during growth.

4.4.3 Electronic Nose Evaluation

Figure 4.5 is a plot of the data obtained from the electronic nose. This plot indicates that the electronic nose was able to discriminate between the control and treated samples. The CANDISC procedure was performed to produce a canonical discrimination plot (Fig. 4.6) for the electronic nose data, which indicates a similar separation between the control and the treated samples.

The discriminant analysis cross-validation for this information (Table 4.3) identifies the number of observations and the percent classified into each treatment by the electronic nose. This table shows that the electronic nose was successful in categorizing the control group and the 5% EtOH treatment group 100% of the time. The electronic nose identified no separation between the 5% and 10% EtOH treatments (Fig. 4.5), where the 5% EtOH treatment appears to be a subset of the 10% EtOH treatment.

The electronic nose is not designed to discriminate between samples based on the intensity or concentration of a particular element: rather, the responses of each of the sensors form a characteristic recognition pattern for a particular sample. Therefore, this

system is able to give an overall objective evaluation of a sample, causing it to be more sensitive to the overall aroma of the wine. The literature states that increased phenol-free glycosides may reflect an increase in the aroma and flavor compounds in the wine (Zoecklein and others 2000) due to the liberation of α -glycones, which would allow the electronic nose data to correlate well with PFGG results. However, the PFGG results indicated no significant difference between treatments. In contrast, a significant difference in percent alcohol was found between the samples, despite negligible variations in the data. Because of its design, the electronic nose could not discriminate between different concentrations of alcohol in each treatment, even though 100% discrimination was observed using chemical analysis data.

Volatile analysis results found from Baki (2003) indicated that there was not a significant difference between treatments based on a large percentage of the volatile components in the wine as can be seen from Table 4.2. In fact, the only volatile components that indicated a significant difference in the previous study were ethyl decanoate and ethyl dodecanoate. Based on this information, further investigating the selectivity of the electronic nose sensors could help to indicate which sensor is most sensitive to these particular volatile components. In addition, chromatographic analysis could also reveal the differences between the samples. However, GC analysis was not completed for this study as that was not the primary objective for this study.

From the results of the electronic nose, there is a distinct difference between the control group and the treated samples. Based on this information, the application of aqueous ethanol during grape growth causes a change in the final wine product that can be detected by the electronic nose which supports the application of the electronic nose to wine discrimination testing.

4.5 CONCLUSIONS

The purpose of this study was to determine if electronic nose technology could accurately discriminate between wines made from Cabernet Sauvignon grapes that received different aqueous ethanol treatments during growth. The electronic nose data was compared to chemical analyses data and results from a sensory panel.

Multivariate discrimination based on the chemical analyses was able to effectively discriminate between the three samples. These results are important because they verify that the wine samples being evaluated can be discriminated and grouped. However, discrimination was due to only three significant parameters: color intensity, percent alcohol and pH. Despite the fact that there was clear separation between groups shown during the chemical analysis, the sensory panel was not able to discriminate between the control group and the treated wine samples. The electronic nose was successful in grouping the control group separately from the other treatments evaluated in this study. The treated samples were grouped in the same area, with a distinct separation from the control group.

The electronic nose can be used as a discriminatory tool for control and treated wine samples in an ethanol spray study. However, more research is needed to determine if the electronic nose can be utilized for discriminating the treated samples. Further analysis using gas chromatography would be required to validate the electronic nose findings. Further chemical and volatile analysis would also help to identify significant parameters that differ between the treated samples.

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Acknowledgments

Saccharomyces cerevisiae (D-254) was donated by Scott Labs, Petaluma, CA

Table 4.1 Effect of ethanol sprays (control, 5%, 10% v/v) on wine color, anthocyanins, TFGG and PGG of Cabernet Sauvignon post-bloom treatments (Baki 2003)

	0% EtOH	5% EtOH	10% EtOH
Total Anthocyanins (AU₅₂₀)	1.60 ^c	1.63 ^b	1.71 ^a
Color Intensity (AU₄₂₀₊₅₂₀)	0.594 ^b	0.629 ^a	0.629 ^a
Color Hue (AU_{420/520})	0.475 ^b	0.479 ^b	0.484 ^{ab}
TGG	1183 ^b	1727 ^a	1490 ^{ab}
PFGG	56 ^b	51 ^b	73 ^a

Mean values of three replicates

^{a, b, c}. Different letters within rows denote significant difference ($p \leq 0.05$) of treatment means.

Table 4.2 Effect of ethanol sprays (control, 5% v/v, 10% v/v) on wine volatile organic compounds of Cabernet Sauvignon week-13 post-bloom treatments (Baki 2003)

	Control	5% EtOH	10% EtOH
2-Methyl Propanol (mg/L)	85.6 ^a	67.4 ^a	75.5 ^a
Isoamyl Acetate (µg/L)	3682 ^a	3797 ^a	4568 ^a
3-Methyl Butanol (mg/L)	400 ^a	322 ^a	337 ^a
Ethyl Hexanoate (µg/L)	516 ^a	540 ^a	576 ^a
Hexyl Acetate (µg/L)	109 ^a	101 ^a	116 ^a
N-Hexanol (mg/L)	3.33 ^a	3.45 ^a	3.00 ^a
Ethyl Octanoate (µg/L)	261 ^a	296 ^a	351 ^a
Acetic Acid (mg/L)	102 ^a	115 ^a	121 ^a
Ethyl Decanoate (µg/L)	116 ^b	121 ^{ab}	129 ^a
Diethyl Succinate (µg/L)	N.D.	N.D.	N.D.
Citronellol (µg/L)	30 ^a	33 ^a	31 ^a
Phenethyl Acetate (µg/L)	399 ^a	344 ^a	447 ^a
Damascenone (µg/L)	7 ^a	5 ^a	5 ^a
Ethyl Dodecanoate (µg/L)	104 ^b	105 ^{ab}	107 ^a
Benzyl Alcohol (µg/L)	659 ^a	621 ^a	635 ^a
2-Phenylethanol (mg/L)	52.2 ^a	40.8 ^a	42.7 ^a
Octanoic Acid (mg/L)	N.D.	N.D.	N.D.
Decanoic Acid (mg/L)	N.D.	N.D.	N.D.

Table 4.3 Cross-validation of the discriminate analysis of electronic nose data for wine samples made from Cabernet Sauvignon grapes treated with 0%, 5%, and 10% v/v aqueous ethanol solution. Cells indicate number and percentage of samples tested (rows) in which discriminant analysis indicated they should be categorized (columns).

		Classified as			
		Control	5% EtOH	10% EtOH	Total
Treatment	Control	7	0	0	7
		100%	0	0	100%
	5%	0	10	0	10
	EtOH	0	100%	0	100%
	10%	0	5	3	8
	EtOH	0	62.50%	37.50%	100%
		7	15	3	25
	Total	28	60	12	100



Figure 4.1 HKR Sensorsystems QMB6 system used for wine analysis

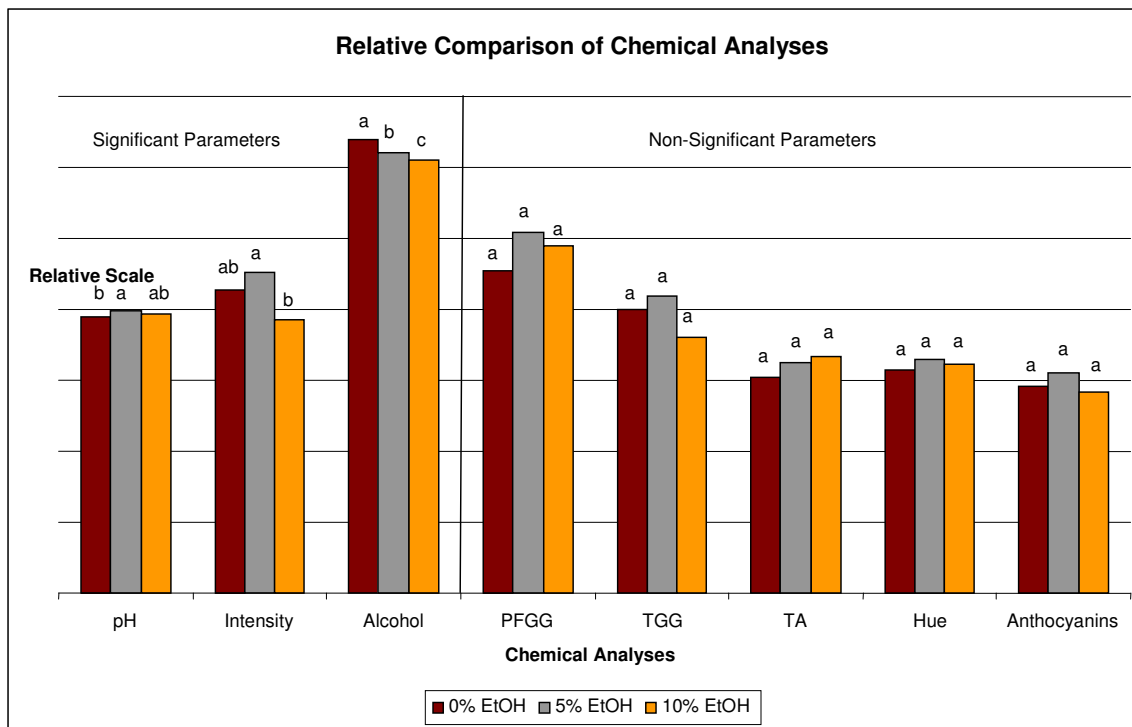


Figure 4.2 Comparison of chemical analyses based on treatment

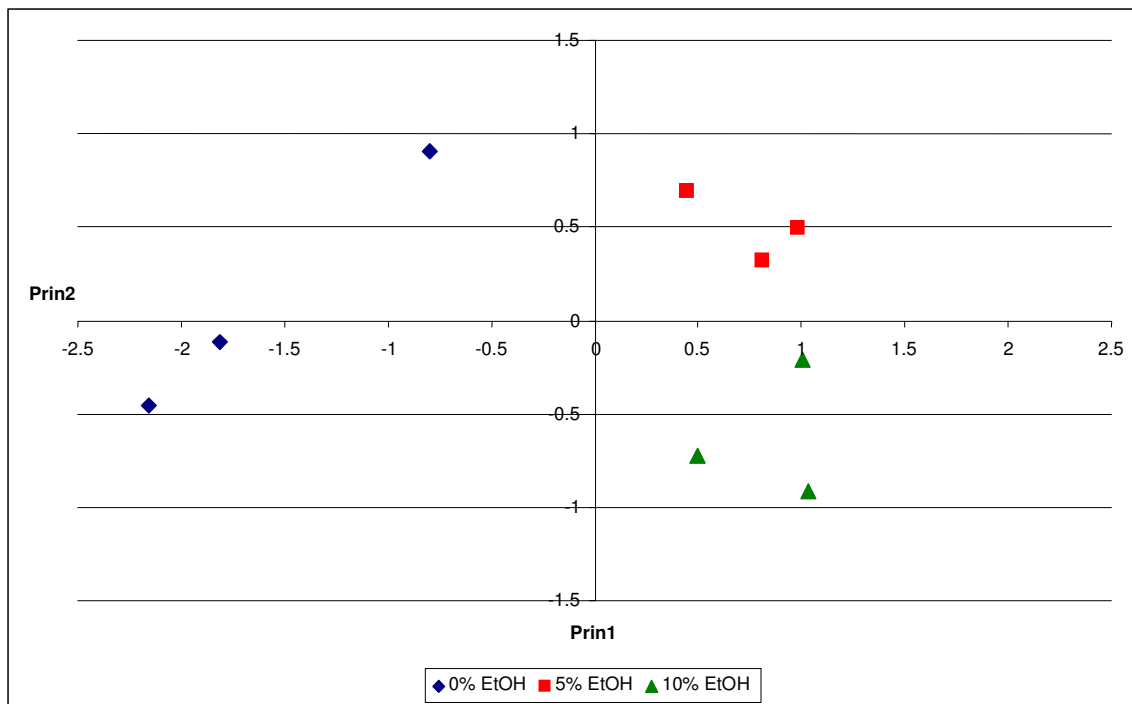


Figure 4.3 Principle component analysis plot of chemical analysis data for Cabernet Sauvignon wine samples made from grapes treated with 0%, 5% and 10% aqueous ethanol solution

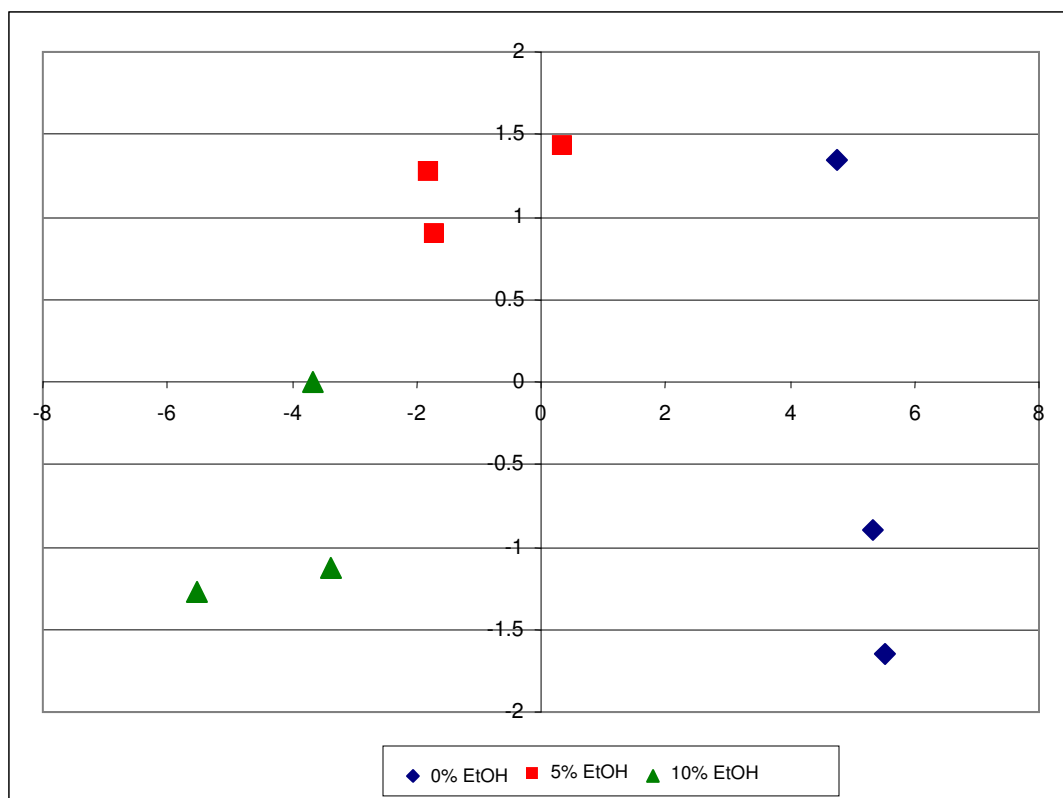


Figure 4.4 Canonical plot of chemical analysis data for Cabernet Sauvignon wine samples made from grapes treated with 0%, 5% and 10% aqueous ethanol solution

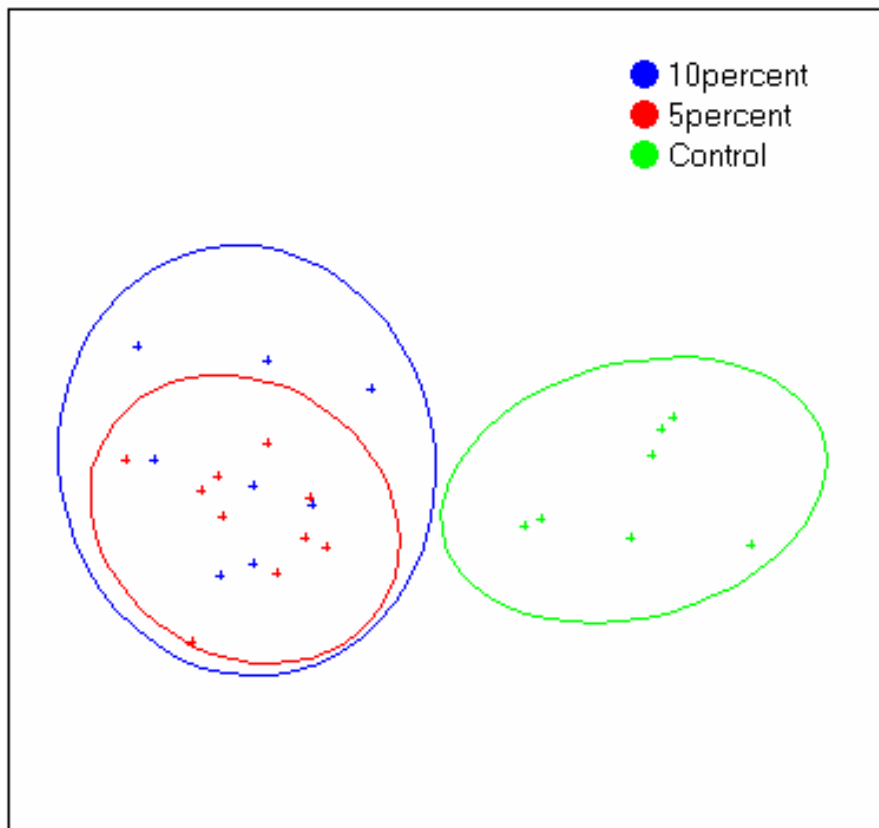


Figure 4.5 Projection plot of 0%, 5% and 10% aqueous ethanol treatments from the discriminate analysis by the QMB6 system

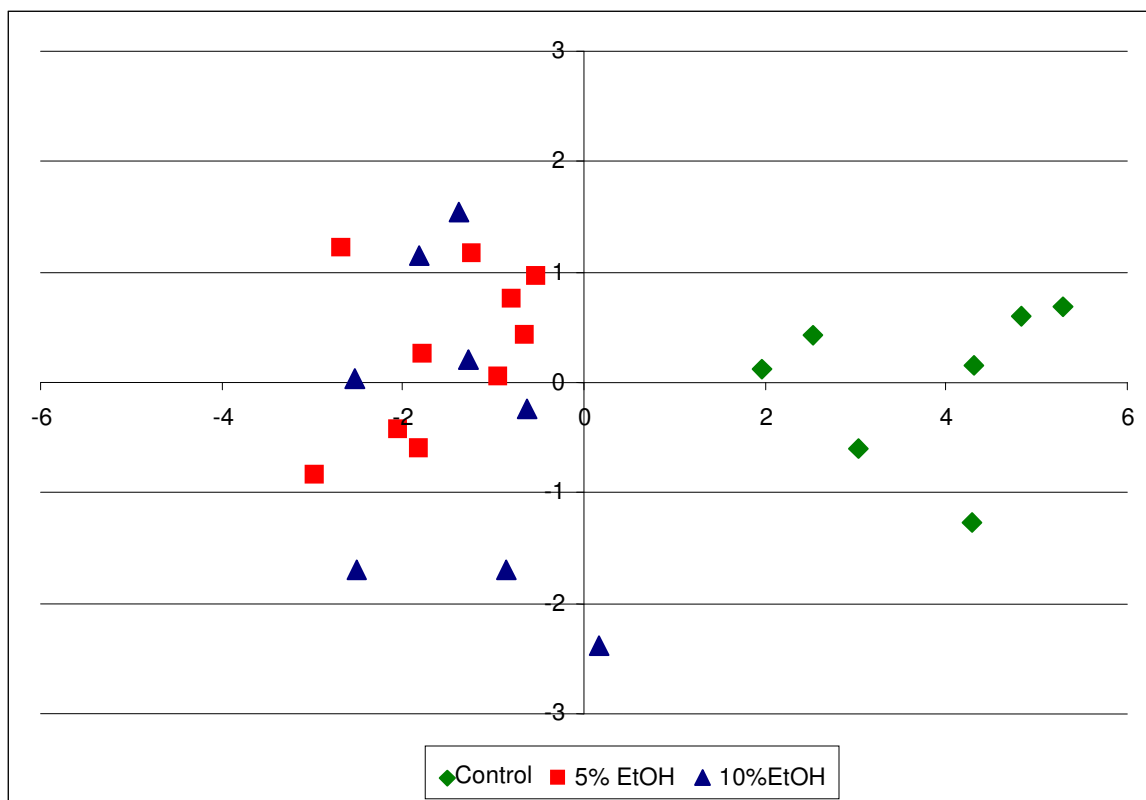


Figure 4.6 Canonical plot of electronic nose data for Cabernet Sauvignon wine samples made from grapes treated with 0%, 5% and 10% aqueous ethanol solution

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

The first study presented in this paper was an optimization study. The target of this study was to determine the optimum settings for three parameters (sample temperature, sensor temperature and equilibrium time) of a quartz microbalance-based electronic nose system for wine analysis. Based on the results of this study, the optimum settings for sample temperature and equilibrium time were determined for wine analysis using the electronic nose. A slight difference in sample temperature was detected for the two varieties used in this study. Due to this difference, sample temperature must be optimized for each variety being evaluated with the electronic nose. Lower sensor temperatures should also be tested using an external cooling unit to determine if the maximum sensor response is being obtained.

The purpose of the second study was to determine if electronic nose technology could accurately discriminate between wines made from Cabernet Sauvignon grapes that received different aqueous ethanol treatments during growth. The electronic nose data was compared to chemical analysis data and results from a sensory panel.

The chemical analyses were able to effectively discriminate between the three samples. However, discrimination was due to only three significant parameters: color intensity, percent alcohol and pH. Despite the separation shown between groups during the chemical analysis, the sensory panel was not able to discriminate between the control group and the treated wine samples. The electronic nose was successful in grouping the control group separately from the other treatments evaluated in this study. Based on these results, it was found that the electronic nose can be used as a discriminatory tool for untreated and treated wine samples. Future work should be completed to determine what component of the treated samples is effecting the sensor responses.

The hypothesis of the overall study was that a quartz microbalance-based electronic nose system could perform effectively in discriminating between different wine varieties and treatments and results from the electronic nose technology could give complimentary results to that of a sensory panel. The results of this research demonstrated that the electronic nose can be optimized for wine discrimination, and is successful in identifying different wine treatments from control groups. Additionally, the

electronic nose was able to find a difference in wine samples even though no difference was found based on a human sensory panel.

After reflection of this study, suggestions for future work were made. There are different electronic nose technologies available, but no testing has been completed to see what technology is the most effective for wine evaluation. Performing electronic nose evaluation on the must of the wine might also be able to identify differences before the final wine sample is completed. Another thought for future work would be to perform electronic nose evaluation on wines that have experienced different levels of oxidation. In this way, the electronic nose may be able to determine if a wine has oxidized without chemical or sensory testing.

APPENDIX A

JUICE CHEMISTRIES

The juice for each treatment was evaluated using pH, °Brix, titratable acidity and estimate of fermentable nitrogen. Table A.1 provides the results of each of these tests. °Brix was determined using an American Optical model 10419 temperature-compensating refractometer and pH with a Fischer (Pittsburgh, PA) Accumet® model 20 pH/conductivity meter. Titratable acidity was determined by titration with NaOH to an end-point of pH 8.2. The estimate of fermentable nitrogen was determined using formol titration.

Table A.1 Results of juice chemistry evaluation for wines made from Cabernet Sauvignon wines that received different concentrations of aqueous ethanol during fruit maturation

Treatment	pH	Brix	TA	Formol
0% EtOH	3.92	22.5	4.85	107.5
5% EtOH	3.83	21.4	4.95	133.1
10% EtOH	3.84	22	5.16	153.6

Mean values of three replicates

APPENDIX B
ANOVA PLOTS FOR CHEMICAL ANALYSES

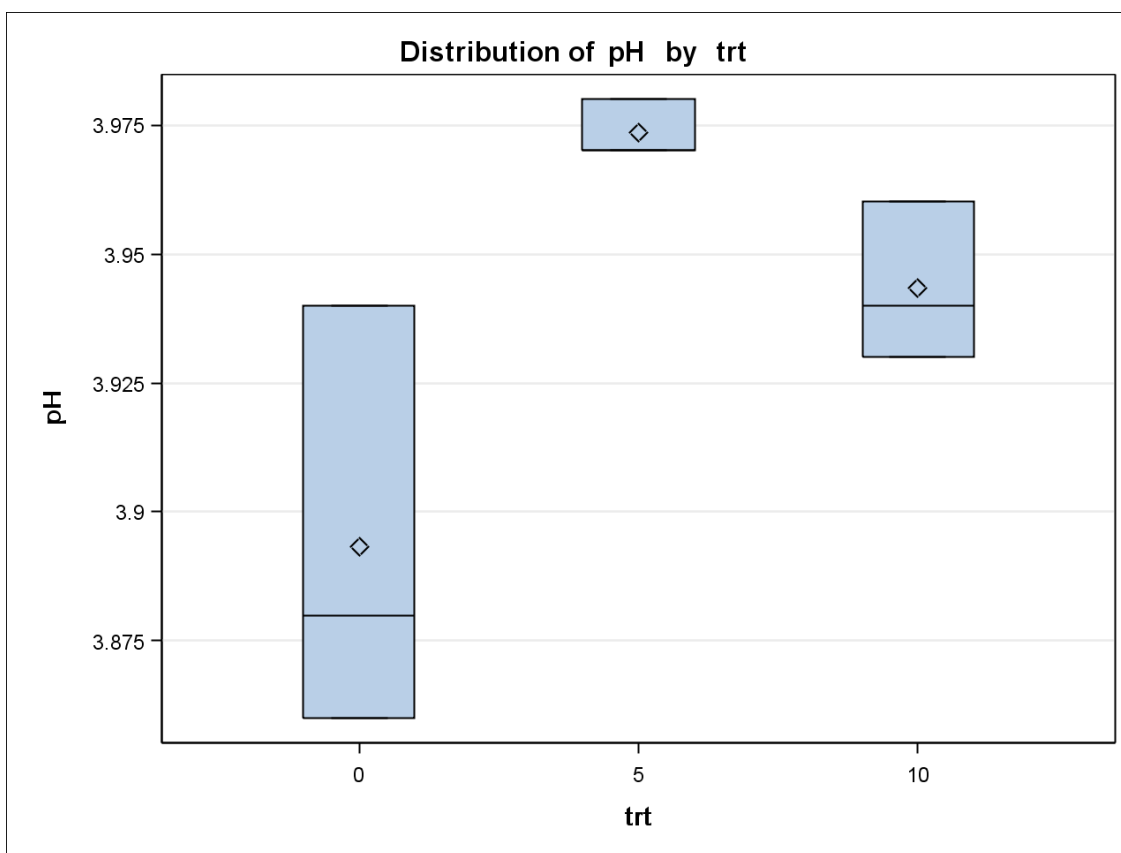


Figure B.1 Oneway analysis of pH by treatment

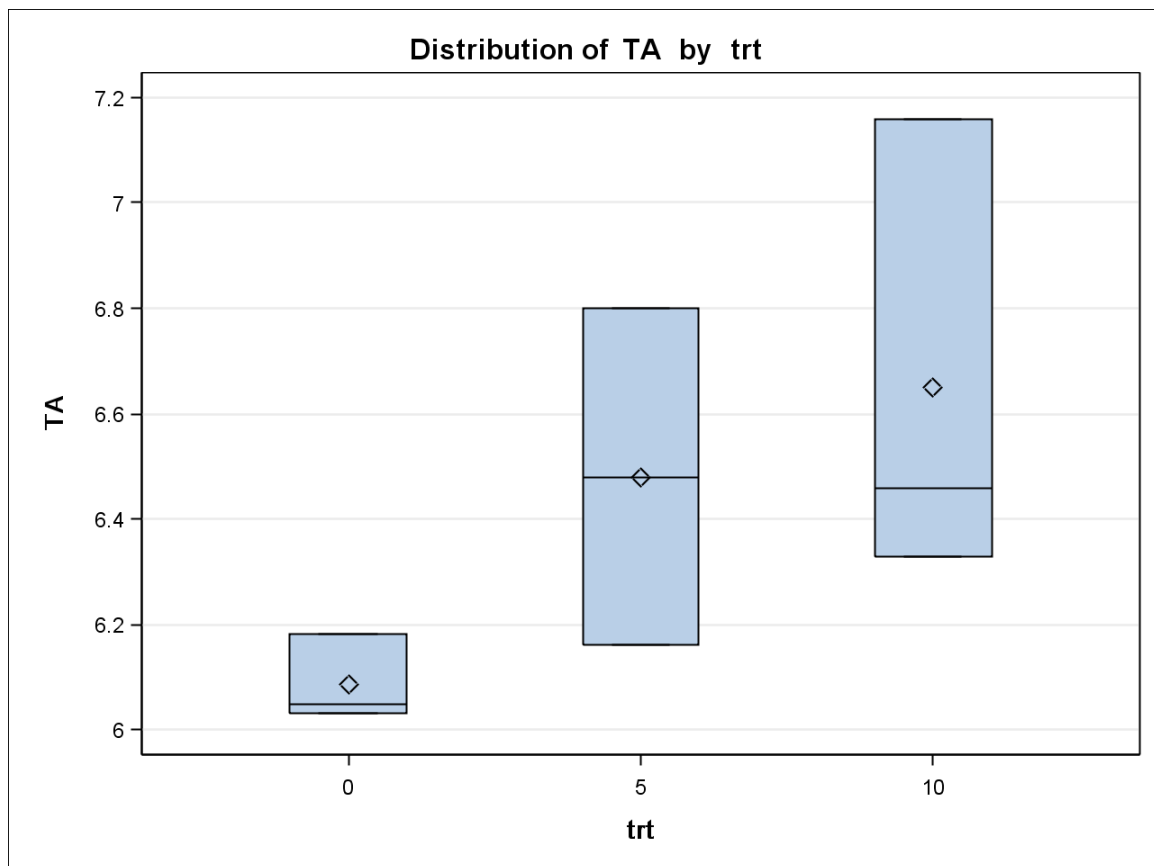


Figure B.2 Oneway analysis of total anthocyanins by treatment

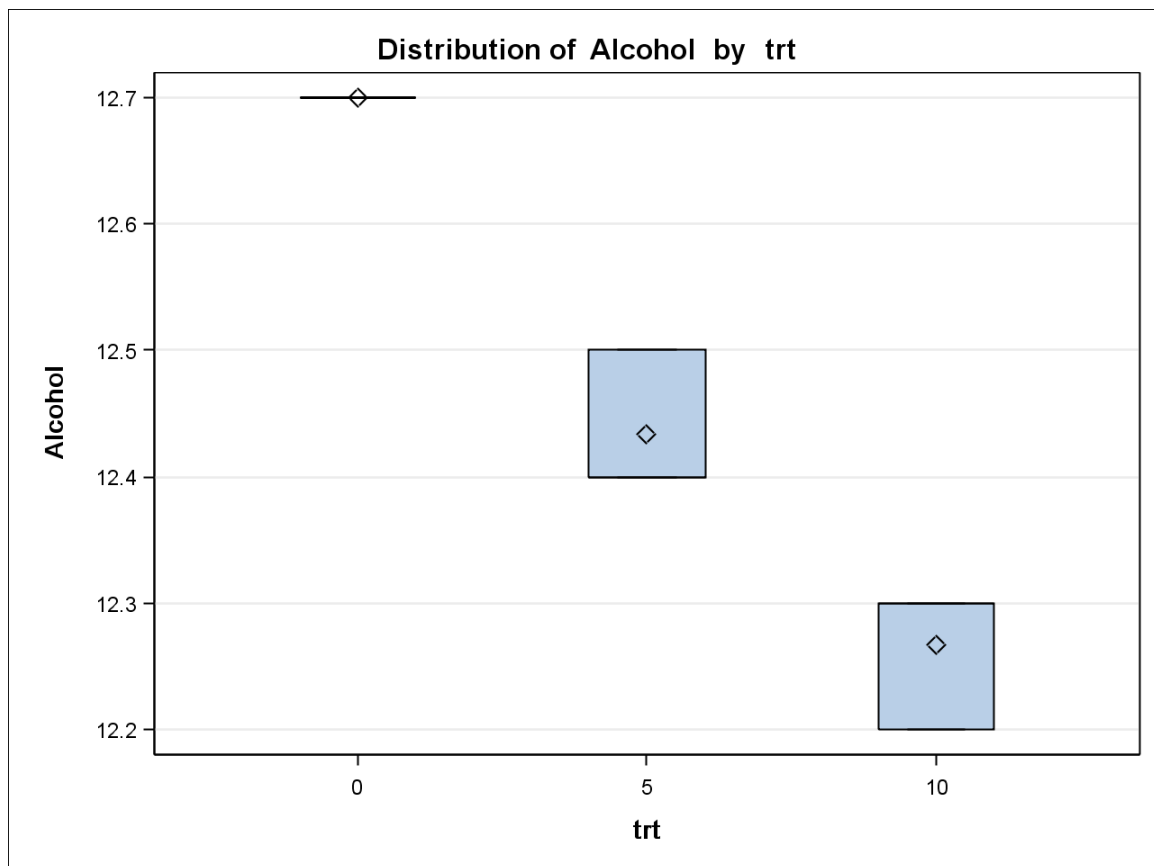


Figure B.3 Oneway analysis of alcohol by treatment

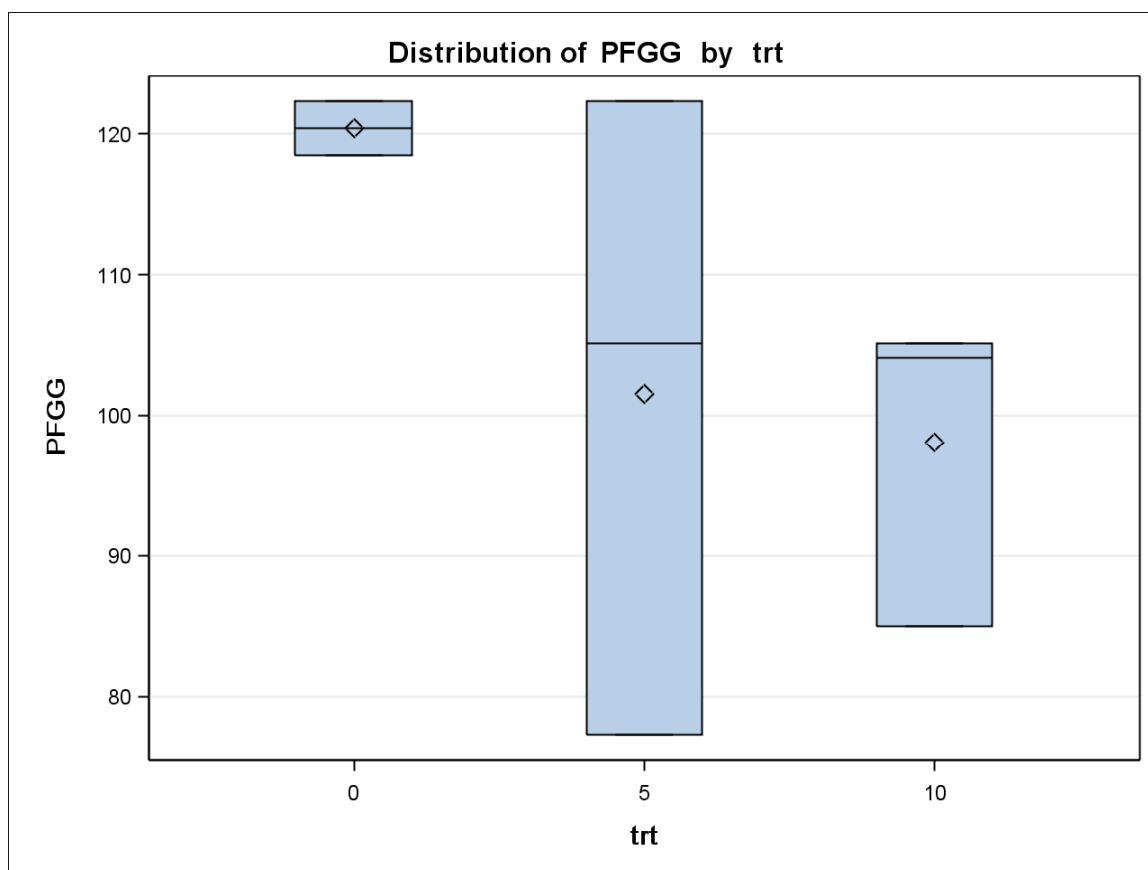


Figure B.4 Oneway analysis of phenol-free glycosides by treatment

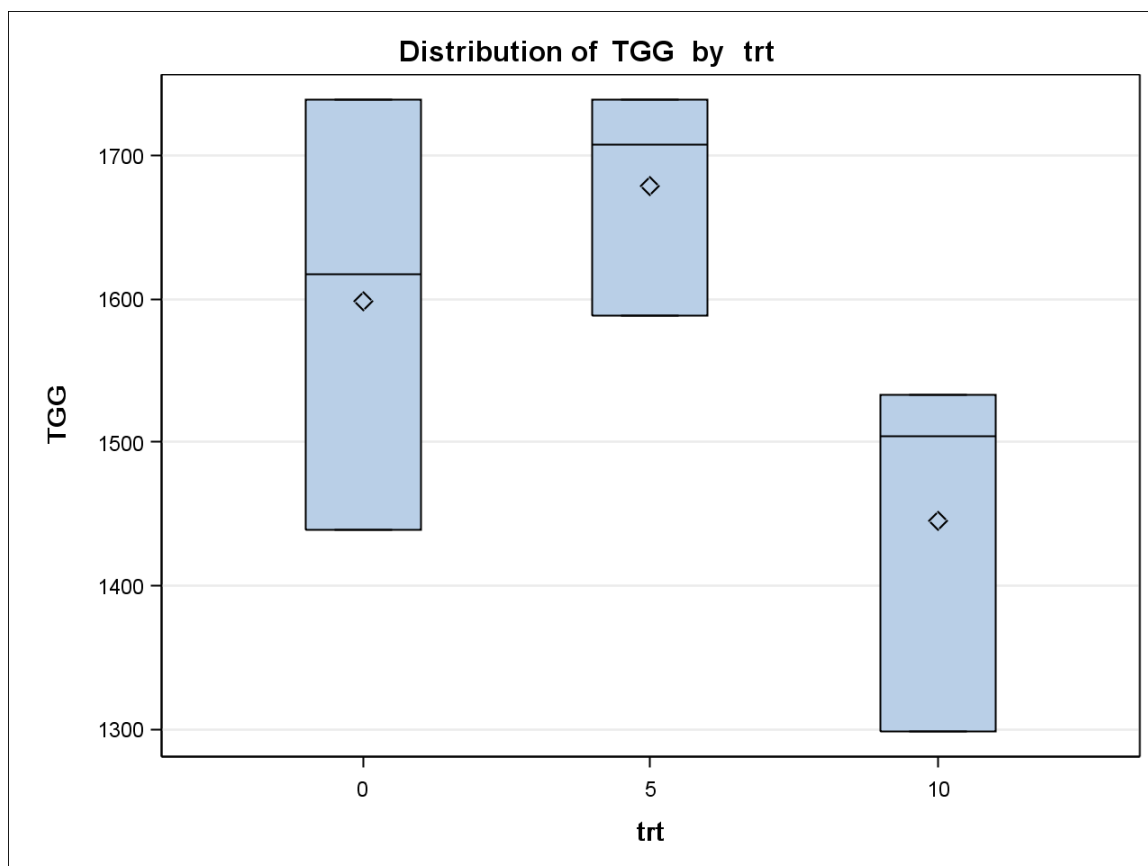


Figure B.5 Oneway analysis of total glycosides by treatment

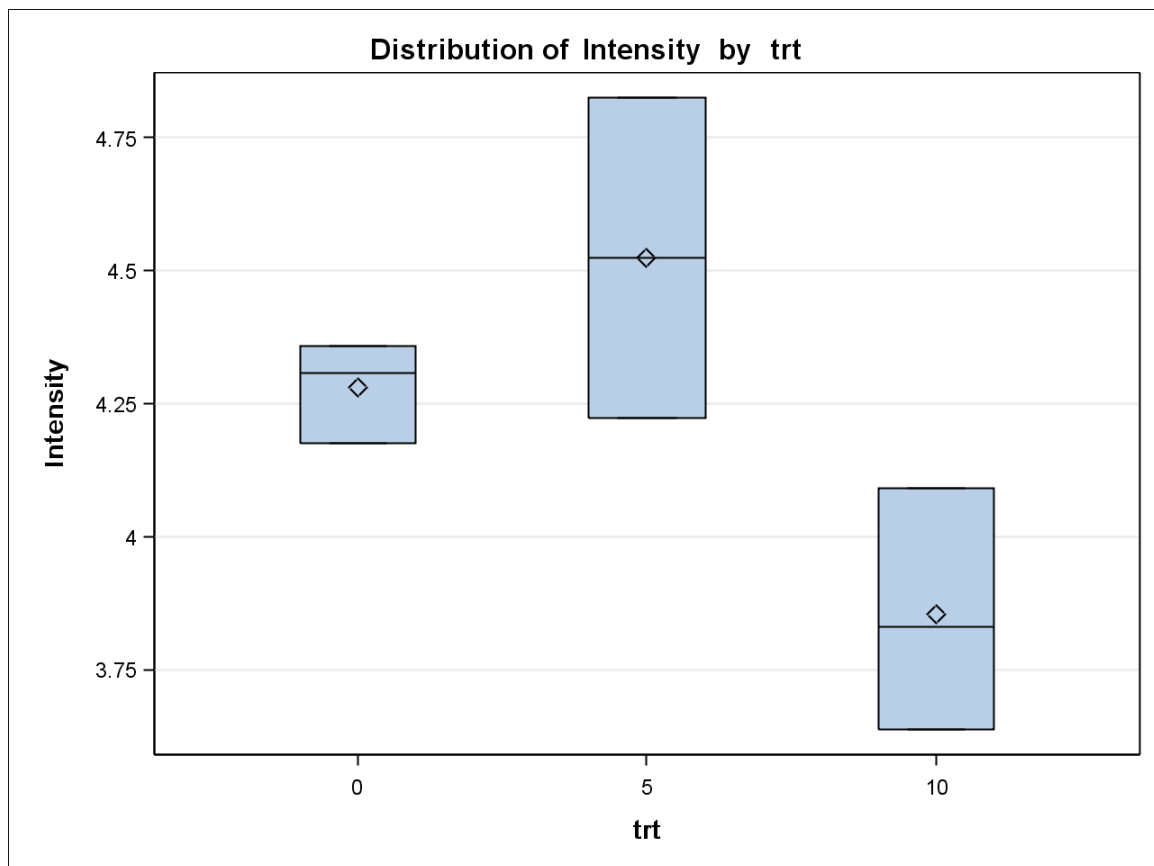


Figure B.6 Oneway analysis of color intensity by treatment

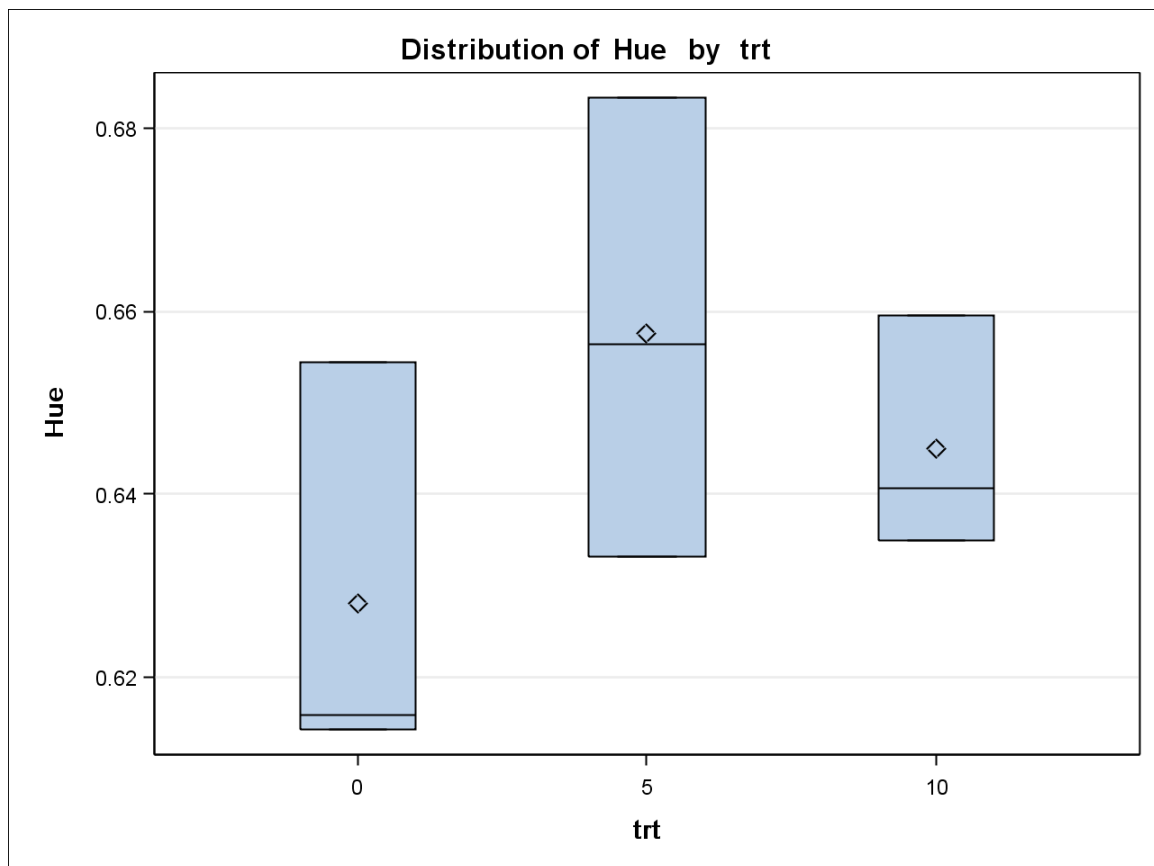


Figure B.7 Oneway analysis of hue by treatment

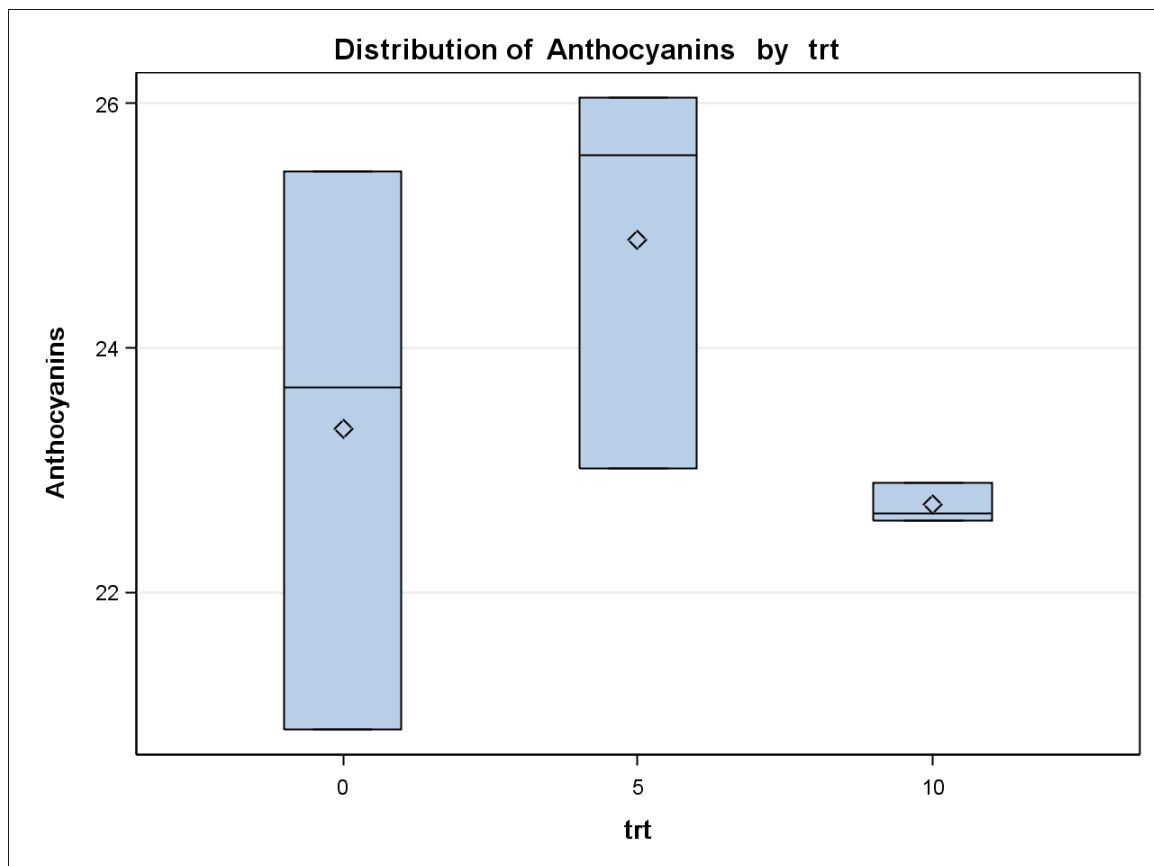


Figure B.8 Oneway analysis of total anthocyanins by treatment

APPENDIX C

HUMAN SENSORY EVALUATION: FORMS AND PREPARATION

Sensory Evaluation for Cabernet Sauvignon (Ethanol Spray Study)

Materials

The following materials will be needed for this study: 0% EtOH Cabernet Sauvignon, 5% EtOH Cabernet Sauvignon, and 10% EtOH Cabernet Sauvignon.

Sensory Evaluation

A total of 48 untrained panelists (males and females) with varying ages will be used for each session of this consumer study. Demographic information (gender, age) will be collected from each panelist before the beginning of the sensory evaluation. Panelists will be prescreened on the basis of having consumed wine. Panelists will evaluate the samples utilizing a scorecard found in Appendix A. Sample preparation will be completed using the worksheet found in Appendix B. Before starting the sensory evaluation, all panelists will sign a consent document. An example of the consent document can be found in Appendix C.

Sensory evaluation will be conducted in the Food Science and Technology Department Sensory Laboratory at Virginia Polytechnic and State University (Virginia Tech), Blacksburg, VA.

Each panelist will conduct two triangle tests per session, one concerning aroma and one concerning flavor. A total of three sessions will be completed for this study. Eight panelists will be tested per session totaling 16 sample sets per session. A total of 48 panelists will be tested for each session. Clear, standard ISO glasses, 3-digit random coded, will be filled with 15 ml of wine at 20°C will be presented to each panelist in a red light setting using the sensory evaluation booths. The panelists will be asked to identify the odd sample out of each test. After marking their choice on the scorecard, they may choose to make comments on each of the samples.

A total of 2.6 L (0.687 gal) will be needed from each treatment (0% EtOH, 5% EtOH and 10% EtOH) for this sensory study. There is approximately 9.46 L (2.5 gal) of each treatment in storage, 3.78 L (1 gal) of which is being used for chemical analyses and will not be utilized for the sensory study. For each session, a maximum of 48 people will be tested. Therefore, 144 glasses are needed per session. Glasses will be cleaned at the end of each sensory session.

The proposed dates for sensory sessions are listed below:

Session	Date	Time
1	Wednesday, March 22	11:00-14:00
	Friday, March 24	14:00-17:00
2	Monday, April 3	14:00-17:00
	Wednesday, April 5	11:00-14:00
3	Monday, April 10	11:00-14:00
	Wednesday April 12	11:00-14:00

A sign-up sheet will be passed around the Wines and Vines classes in order for the students to sign-up for the sensory sessions. An email will be sent to other individuals. They will be directed to an online survey and will be emailed with their sensory time for each session.

APPENDIX C.1 SCORECARD

Triangle Test – Aroma		
Judge #: _____ Type of Sample: Cabernet Sauvignon		
Instructions: Smell the samples on the tray from right to left. Two samples are the same and one is different. Select the <u>different/odd</u> sample and indicate on this scorecard by placing a X next to the code of the odd sample.		
Samples on Tray _____ _____ _____	Indicate odd sample <div style="text-align: center;"> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> </div>	Remarks _____ _____ _____
If you wish to comment on the reasons for your choice or if you wish to comment on the wine characteristics, you may do so under remarks.		

Triangle Test – Taste		
Judge #: _____ Type of Sample: Cabernet Sauvignon		
Instructions: Taste the samples on the tray from right to left. Two samples are the same and one is different. Select the <u>different/odd</u> sample and indicate on this scorecard by placing a X next to the code of the odd sample. Please expectorate the sample.		
Samples on Tray _____ _____ _____	Indicate odd sample <div style="text-align: center;"> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> </div>	Remarks _____ _____ _____
If you wish to comment on the reasons for your choice or if you wish to comment on the wine characteristics, you may do so under remarks.		

APPENDIX C.2

PREPARATION WORKSHEETS

Session 1 - Preparation Worksheet																								
<p>This sheet will be posted in the area where the trays are prepared. Scoresheets will be coded ahead of time, as well as glasses.</p>																								
<p>Type of samples: Cabernet Sauvignon Type of test: Triangle test - Aroma</p>																								
Sample ID	Sets with two A's	Sets with two B's																						
0% EtOH treatment	587 246	413																						
5% EtOH treatment	894	365 751																						
<p>Code serving containers as follows:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 45%; padding: 5px;">Judge #</th> <th style="width: 30%; padding: 5px;">Codes in Order</th> <th style="width: 25%; padding: 5px;">Underlying Pattern</th> </tr> </thead> <tbody> <tr> <td style="padding: 5px;">1,7,13,19,25,31,37,43</td> <td style="padding: 5px;">587, 246, 894</td> <td style="padding: 5px;">AAB</td> </tr> <tr> <td style="padding: 5px;">2,8,14,20,26,32,38,44</td> <td style="padding: 5px;">587, 894, 246</td> <td style="padding: 5px;">ABA</td> </tr> <tr> <td style="padding: 5px;">3,9,15,21,27,33,39,45</td> <td style="padding: 5px;">365, 751, 413</td> <td style="padding: 5px;">BAB</td> </tr> <tr> <td style="padding: 5px;">4,10,16,22,28,34,40,46</td> <td style="padding: 5px;">894, 587, 246</td> <td style="padding: 5px;">BAA</td> </tr> <tr> <td style="padding: 5px;">5,11,17,23,29,35,41,47</td> <td style="padding: 5px;">365, 751, 413</td> <td style="padding: 5px;">BBA</td> </tr> <tr> <td style="padding: 5px;">6,12,18,24,30,36,42,48</td> <td style="padding: 5px;">413, 365, 751</td> <td style="padding: 5px;">ABB</td> </tr> </tbody> </table>				Judge #	Codes in Order	Underlying Pattern	1,7,13,19,25,31,37,43	587, 246, 894	AAB	2,8,14,20,26,32,38,44	587, 894, 246	ABA	3,9,15,21,27,33,39,45	365, 751, 413	BAB	4,10,16,22,28,34,40,46	894, 587, 246	BAA	5,11,17,23,29,35,41,47	365, 751, 413	BBA	6,12,18,24,30,36,42,48	413, 365, 751	ABB
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3,9,15,21,27,33,39,45	365, 751, 413	BAB																						
4,10,16,22,28,34,40,46	894, 587, 246	BAA																						
5,11,17,23,29,35,41,47	365, 751, 413	BBA																						
6,12,18,24,30,36,42,48	413, 365, 751	ABB																						
<ol style="list-style-type: none"> 1. Place sticker's with panelist's number on tray 2. Select previously coded glasses and place on tray from left to right in order shown above. 3. Serve samples 																								

Session 1 - Preparation Worksheet																								
<p>This sheet will be posted in the area where the trays are prepared. Scoresheets will be coded ahead of time, as well as glasses.</p>																								
<p>Type of samples: Cabernet Sauvignon Type of test: Triangle test - Flavor</p>																								
Sample ID	Sets with two A's		Sets with two B's																					
0% EtOH treatment	216 759	327																						
5% EtOH treatment	803	492 593																						
<p>Code serving containers as follows:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 45%; padding: 5px;">Judge #</th> <th style="width: 30%; padding: 5px;">Codes in Order</th> <th style="width: 25%; padding: 5px;">Underlying Pattern</th> </tr> </thead> <tbody> <tr> <td style="padding: 5px;">1,7,13,19,25,31,37,43</td> <td style="padding: 5px;">216, 759, 803</td> <td style="padding: 5px;">AAB</td> </tr> <tr> <td style="padding: 5px;">2,8,14,20,26,32,38,44</td> <td style="padding: 5px;">216, 803, 759</td> <td style="padding: 5px;">ABA</td> </tr> <tr> <td style="padding: 5px;">3,9,15,21,27,33,39,45</td> <td style="padding: 5px;">492, 327, 593</td> <td style="padding: 5px;">BAB</td> </tr> <tr> <td style="padding: 5px;">4,10,16,22,28,34,40,46</td> <td style="padding: 5px;">803, 216, 759</td> <td style="padding: 5px;">BAA</td> </tr> <tr> <td style="padding: 5px;">5,11,17,23,29,35,41,47</td> <td style="padding: 5px;">492, 593, 327</td> <td style="padding: 5px;">BBA</td> </tr> <tr> <td style="padding: 5px;">6,12,18,24,30,36,42,48</td> <td style="padding: 5px;">327, 492, 593</td> <td style="padding: 5px;">ABB</td> </tr> </tbody> </table>				Judge #	Codes in Order	Underlying Pattern	1,7,13,19,25,31,37,43	216, 759, 803	AAB	2,8,14,20,26,32,38,44	216, 803, 759	ABA	3,9,15,21,27,33,39,45	492, 327, 593	BAB	4,10,16,22,28,34,40,46	803, 216, 759	BAA	5,11,17,23,29,35,41,47	492, 593, 327	BBA	6,12,18,24,30,36,42,48	327, 492, 593	ABB
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4,10,16,22,28,34,40,46	803, 216, 759	BAA																						
5,11,17,23,29,35,41,47	492, 593, 327	BBA																						
6,12,18,24,30,36,42,48	327, 492, 593	ABB																						
<ol style="list-style-type: none"> 4. Place sticker's with panelist's number on tray 5. Select previously coded glasses and place on tray from left to right in order shown above. 6. Serve samples 																								

APPENDIX C.3

IRB DOCUMENTATION AND HUMAN CONSENT FORM

Request for Exemption of Research Involving Human Subjects

Investigator(s): _____ Amanda
Martin _____

Department(s): _____ Biological Systems Engineering _____ Mail Code: _____ 0303 _____ E-
mail: _____ ammartin@vt.edu _____

Project Title: _____ Wine Discrimination and Analysis Using Electronic Nose
Technology _____

Source of Funding Support: ☒ Departmental Research ☐ Sponsored Research (OSP
No.: _____)

☒ All investigators of this project are qualified through completion of the formal training program or videotape program provided by the Virginia Tech Office of Research Compliance.

Note: To qualify for Exemption, the research must be (a) of minimal risk to the subjects, (b) must not involve any of the special classes of subjects, and (c) must be in one or more of the following categories. A full description of these categories may be found in the Exempt Research section of the instructions: "Application for Approval of Research Involving Human Subjects" or in the federal regulations [45 CFR 46.101(b)(1-6)]. (<http://grants.nih.gov/grants/opr/humansubjects/45cfr46.htm#46.101>)

PLEASE MARK/CHECK THE APPROPRIATE CATEGORY OR CATEGORIES BELOW WHICH QUALIFY THE PROPOSED PROJECT FOR EXEMPTION:

- ☒ 1. Research will be conducted in established or commonly accepted educational settings, involving normal educational practices [see item (1), page ____].
- ☐ 2. Research will involve the use of educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures or observation of public behavior, **unless** the subjects can be identified directly or through identifiers linked to the subjects **and** disclosure of responses could reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability or reputation [see item (2), page ____].
- ☐ 3. Research will involve the use of educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures, or observation of public behavior that is not exempt under item 2) above **if** the subjects are elected or appointed public officials or candidates for public office; **or** Federal statute(s) require(s) that the confidentiality or other personally identifiable information will be maintained [see item (3), page ____].
- ☒ 4. Research will involve the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects [see item (4), page ____].

[] 5. Research and demonstration projects designed to study, evaluate, or otherwise examine public benefit or service programs, procedures for obtaining benefits or proposed changes in such programs [see item (5), page ____].

[X] 6. Taste and food quality evaluation and consumer acceptance studies [see item (6), page ____].

Investigator(s)	Print name	Date
-----------------	------------	------

Departmental Reviewer	Print name	Date
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Chair, Institutional Review Board		Date
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Human Subjects Forms for Sensory Evaluation

Protocol for Projects of Sensory Evaluation

If the project involves sensory evaluation, please complete the following questions about the project to assist you and the Institutional Review Board in determining the risk level of the project.

Definition: Sensory evaluation is the evaluation of food or other substances by the senses including taste, touch, smell, sight and hearing.

Check all that apply:

1. The procedure for sensory evaluation in this project involves:
 - ☒ Tasting in the mouth (includes tests where the panelist is instructed to spit it out)
 - ☐ Substances applied to the skin
 - ☒ Substances smelled for odor components
 - ☐ Substances evaluated by sound when chewed
 - ☐ Substances evaluated by visual senses
2. The product/s to be evaluated are:
 - ☒ Made entirely of ingredients approved by FDA for consumption or application under approved conditions of processing
 - ☐ Made of ingredients approved by FDA but not approved for the use in the project (e.g. heating of aspartame, fat substitutes approved only as an emulsifier).
 - ☐ Made partially or entirely of experimental ingredients pending FDA approval.
 - ☐ Made partially or entirely of experimental ingredients not approved for human consumption or topical use
 - ☐ Made from materials from or altered by biotechnology
3. The processing or preparation of the product is:
 - ☒ By usual approved good manufacturing or preparation practices for that food or topical product.
 - ☐ By experimental procedures including non-good manufacturing practices. Briefly describe the procedures.
4. The packaging of the product includes:
 - ☒ Processing or storage in FDA-approved packaging materials.
 - ☐ Processing or storage in packaging materials not approved by FDA.
5. Describe the storage protocols for the product that are necessary to maintain the product in safe condition.

The wines are being stored in a walk-in refrigerator kept at ~3°C (40 °F). Wines are stored in full glass containers (to prevent oxidation).

6. If microbiological cultures are a part of the food processing or preparation procedure, describe what cultures will be used, if they will be active on consumption, and give evidence that these cultures are known to be safe for human consumption.

7. Allergies

No Are any ingredients to be used potentially allergenic as consumed or by topical application? If yes, describe. Have panelists been made aware of these ingredients?

When you have completed this form, indicate the risk level to the panelists of this project. Complete the appropriate form; for "not at risk", the Certificate of Exemption form; for "at minimal risk", the Request for Approval form.

Virginia Polytechnic Institute and State University
Informed Consent for Participation in Sensory Evaluation

Title of Project: Wine Discrimination and Analysis Using Electronic Nose Technology

Principal Investigator: Amanda Martin

I. THE PURPOSE OF THIS PROJECT

You are invited to participate on a sensory evaluation panel about Cabernet Sauvignon. The purpose of this research is to determine if using electronic nose technology is an acceptable method to evaluate wine. Sensory analysis will be compared with the electronic nose analysis.

II. PROCEDURES

There will be 3 sessions over a period of one month involving about 20 minutes at each session. You will be presented with approximately 6 samples at each session. As a panelist, it is critical to the project that you attend each session. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to other samples.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, list them in the following space.

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide information to the investigator that will assist in the purpose of the project. You may receive the results or summary of the panel when the project is completed.

IV. 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 code for analyses and in any publication of the results.

V. COMPENSATION

Course Credit

You may receive extra credit for the Wines and Vines to be determined by the instructor of the course.

VI. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after

reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

VIII. SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study.

Signature/Date

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes.

Address _____

Phone _____

------(tear off)-----

IX. SUBJECT'S PERMISSION (provide tear off for human subject to keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study which will require: (list sessions to be attended or other requirements.)

Signature

Should I have any questions about this research or its conduct, I should contact:
Amanda Martin/231-4301

Investigator/Phone

Dr. Bruce Zoecklein/231-5325

Faculty/Phone

Chair, IRB/Phone for Research Division

(540)231-6077

VITA

Amanda Martin was born on December 10, 1981 in Charleston, S.C. She graduated with a Bachelor of Science in Biological Systems Engineering from Virginia Polytechnic Institute and State University in December 2004. She continued at Virginia Tech to pursue a Master of Science in Biological Systems Engineering under the direction of Dr. P. Kumar Mallikarjunan. She completed the final exam for the degree in January 2007.