Discriminating the Effects of Vineyard Management Practices on Grape and Wine Volatiles from Cabernet Franc and Merlot Grape Varieties Using Electronic Nose Systems

By

Yamuna S. Devarajan

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Committee:
Kumar Mallikarjunan (Chair)
Bruce W. Zoecklein (Co-chair)
Sean O' Keefe
Robert "Bobby" Grisso

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Abstract

Vineyard management practices are known to affect fruit composition and resultant wines, in part, by altering fruit volatiles. Methods currently used to evaluate the impact of vineyard practices on grape/wine composition include measuring physico-chemistry indices and performing wine sensory analyses. These activities are both time-consuming and destructive. Two electronic nose (ENose) systems: a hand-held conducting polymer-based and a portable surface acoustic wave-based systems were investigated as grape monitoring tools. Vineyard treatments included the effect of canopy side (East vs. West and North vs. South), cluster thinning (unthinned, 1 cluster/shoot, and 1 & 2 cluster/shoot) and ethanol spray (5% v/v) on Cabernet franc, Merlot and both varieties respectively. ENose data were obtained in the field (over two growing seasons for canopy side and in 2008 for cluster thinning and ethanol spray) and laboratory (2007 for canopy side), across different sampling dates and compared with nine-grape/eight-wine chemistry assays, GC/MS (cluster thinning) and wine aroma sensory evaluations (triangular difference testing). ENose results demonstrated 100% significant differences between all Cabernet franc and Merlot treatments. Grape/wine chemistry indices, for both Cabernet franc and Merlot, did not differ among treatments (except ethanol treatment) across sampling dates or growing seasons and vineyard management practices. Wine aroma sensory evaluations demonstrated only limited differences (3 out of 8 comparisons: East vs. West, 1 cluster/shoot vs. 1 & 2 clusters/shoot and 1 cluster/shoot and 1 & 2 clusters/shoot). The high level of discrimination by ENose systems may provide opportunities to enhance the understanding of vineyard management activities.

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Introduction and Justification

Wine quality is determined by considering several attributes of grapes, fruit maturity being critical among them. Vineyard management practices are known to affect fruit maturity. Considerable research has been conducted in the past on the effect of viticultural practices (crop exposure (Bergqvist et al. 2001, Weaver and McCune 1960), Crop level (Bravdo et al. 1985, Dami et al. 2006), leaf area to crop ratio (Kliewer and Dokoozlian 2005) and ethanol treatment (Chervin and El-kereamy 2001, Chervin et al. 2004)) on grapes and it has been found these practices has an impact on grape maturity and subsequently the chemical composition in grapes. Sensory and physical chemistry analysis are currently used to determine harvest maturity. However, these methods are time consuming and are also invasive, which prevents testing of the same sample over a period of time (Coombe 1992, Kasimatis and Vilas 1985). The other disadvantage is that grape testing and analysis are often carried out after harvest rather than on the vine itself (Coombe 1992). Hence there is a need for a new tool that has the potential to determine the changes in grape components over time (Herrera et al. 2003) in the field. Also, recent studies show that the need for advanced tools to make harvest decisions is of foremost interest to the grape producers and wine makers (Herrera et al. 2003).

Electronic nose is an advanced technology that is composed of multi-sensor arrays and an appropriate pattern recognition system that measures volatiles similar to the human nose. It is widely used in a variety of applications in the food industry: testing apple maturity (Pathange et al. 2006), wine (Garcia et al. 2006, Martin et al. 2008), grapes (Athamneh 2006, Watkins and Wijesundera 2006), and vegetable oils (Gan et al. 2005). The electronic nose is non-invasive, mobile and fast, making it a potential tool in the laboratory and in field studies. Studies have been conducted on the use of conducting polymer electronic nose for the evaluation of fruit maturity in a particular variety of grape (Cabernet Sauvignon) (Athamneh 2006) but to our knowledge, no sources are available on the applicability of the electronic nose for the Merlot and Cabernet franc varieties as yet.

In this study, we have evaluated the following viticultural practices: crop exposure, crop level and ethanol treatment. The effect of difference in canopy side (for two growing seasons in 2007 and 2008) and ethanol treatment on Cabernet franc variety, and the effect of cluster thinning and ethanol treatment on Merlot variety were evaluated using two electronic noses (conducting polymer-based and surface acoustic wave-based). The Enose data obtained was

compared with standard grape maturity analyses and sensory analysis. In this study we sought to determine the differences in grape and wine volatiles occurring due to changes in viticultural practices using electronic noses.

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Chapter I: Review of Literature

In this chapter, the changes in grape physico-chemistry indices and volatiles with respect to maturity and vineyard management practices are reviewed. Several electronic nose systems available, their operation and their selectivity and sensitivity towards different products and applications are also discussed.

Grape Maturity and Berry Development

Three major tissues of grape berry are skin, flesh and seed. In a study by Coombe (1960), grapes follow a double-sigmoidal growth pattern. Conde and his group (2007) have found that grape berry growth occurs in three phases. Where, the first growth phase after fruit set causes cell division and expansion within the berry. They found that the berry remains small, hard, green and acidic at the end of this phase. In most varieties the first phase is followed by a lag phase in which the vegetative character of the grape is reduced while berry growth pauses. Veraison (onset of ripening and color distinction) occurs at the second growth phase. The berry enhances in volume, softness and sweetness, while reducing in acidity by cell expansion. An acid/sugar balance occurs at this phase and it is responsible for the development of flavor and aroma compounds. Coombe and McCarthy (1997) have identified that this aroma accumulation is not restricted to the increase in sugars and this rapid development and accumulation of aroma and flavor compounds in the grape berry has been coined as engustment, The increase in sugars per berry comes to a steady state after it reaches 25-26°Brix (Singleton 1966). The sugar level, acidity, anthocyanins, phenols and weight are the factors that are to be considered while deciding the harvest maturity (Herrera et al. 2003). However, it is the engustment stage that determines the varietal character of a grape variety and hence affects the greatest importance to berry maturity.

Physiochemical maturity indices

Sugars and Berry weight

The accumulation of sugar indicates ripening. Berry weight and Brix are used as indexes of grape maturity. The sugar content is critical at harvest because it determines the final alcohol content of wines fermented to dryness, and is an essential substrate for the fermentation process

to occur (Herrera et al. 2003). McCarthy and Coombe (McCarthy and Coombe 1999) have found that berry weight tends to change throughout the ripening process. This is due to changes in water and sugar concentrations. In the initial growth phases, the berry increases in size due to inflow of water through xylem and/ or phloem. However, over time the berry decreases in size due to the transpirational loss of water, and reduction of sugar flow out of the berry. Hence any increase in sugar content at the later stages of the berry development is due to shrinkage, not because of development or translocation of sugar in the berry. This decrease in berry weight could be measured and used to identify harvest. Though sugars correspond to the alcohol content, delaying the harvest to increase the sugar content beyond the optimum concentration could result in a poor quality, unbalanced wine (Suresh and Ethiraj 1987). It could be thought that measuring sugar content is useful as an index for maturity level rather than for finding optimal maturity. The sugar level can be either expressed as '°Brix or sugar per berry', but the latter is more appropriate to represent maturity because it takes berry weight into consideration (Zoecklein et al. 1999).

Acids

Onset of ripening involves a rapid decrease in acid and an unrelated corresponding increase in sugars (Conde et al. 2007, La Rosa 1955) .The titrable acidity and/or pH are used to determine the acid content. The major organic acids of grapes are malic acid and tartaric acid, and their levels in grapes vary with climate, maturity and variety (Zoecklein et al. 1999). Conde and his co-workers (2007) have found that citric, lactic, succinic and acetic acids are also present in minor amounts. Investigations from their lab show that malic acid concentration is negatively correlated with temperature. They also concluded that the level of these organic acids in grapes determines the quality and shelf stability of wine.

Potassium, sodium and titrable ions together form the total hydrogen ions of final acid composition and only 80% of this is utilized during titration (Boulton 1980b). Therefore, titrable acidity does not provide a better picture of the organic acid content (Boulton 1980a). La Rosa (1955) has identified that pH value is a measure of active acidity. It is also significant in determining optimum maturity because it increases constantly during grape growth. However, pH keeps increasing even after reaching maturity. By establishing a critical pH that can retain all required attributes of each grape variety, the danger of overripe fruit could be avoided.

Phenols

Conde and co-workers (2007) show that balanced level of phenols is also an important for wine quality. These compounds act as growth inhibitors, possess anti-oxidant activity and provide color, flavor and astringency to wine. They are classified into the chemical classes of flavonoids and non-flavonoids. Flavonoids occupy a major portion in grapes, mainly in skin and seeds. The major flavonoids in grapes are tannins and anthocyanins, in which tannins provide astringency and anthocyanins contribute to red color. Tannins exist in the seeds and skin of grapes. Phenols, except for anthocyanins, tend to change in concentration during ripening, following a standard maturation pattern. Tannin concentration shows only small changes from veraison until harvest, and these changes are tracked as an indicator of grape maturity (phenolic ripeness). Non-flavonoids are not of much importance because they mainly exist in extremely low concentrations in grapes and wine.

Aroma and Aroma-precursors of Grape and Wine

Aroma and flavor are considered to be the most important factors that determine wine quality. Wine consists roughly of about 600 – 800 aroma compounds which can be classified into four categories:

a. Primary – those from the grapes themselves,
b. Secondary – these are developed during crushing,
c. Fermentation aroma – those formed during alcoholic fermentation
d. Maturation aroma – result from the aging process (Rapp 1998).

Aroma compounds from grapes (primary group) vary in quantities with variety and are used for varietal characterization because they remain mostly unchanged in wine (Maria Joao et al. 2006). While comparing different varieties it has been noted that aroma compound quantity is affected more than type (Maria Joao et al. 2006), and the factors affecting them most are grape maturity, environmental conditions, fermentation conditions, wine production process and wine maturation or aging (Rapp 1998). The aroma related secondary metabolites of grapes exist in free and conjugated forms as glycoconjugates/glycosidic compounds (Abbott 1989). Most of the secondary metabolites exist in low concentrations in grapes and glycoconjugates constitute a major proportion of them (Sefton and Francis 1996). Glycoconjugates are a group of compounds

in which one or more sugar units are bound to an aglycone unit, some of which include aroma and flavor compounds (Abbott et al. 1991)). They also act as aroma precursors in which the aglycone unit is released during acid or enzyme hydrolysis to release aroma or flavor free volatiles. Upon hydrolysis of these glycosidic compounds, equal moles of D-glucose and non-sugar aglycones are produced (Williams et al. 1995). The acid hydrolysis of the aroma precursors is a mild reaction and is dependent on the structure of the non-sugar moiety, temperature and pH (Pogorzelski and Wilkowska 2007). The glycosyl-glucose (GG) analysis provides an indirect measure of the glycosidically bound compounds. It is believed that some of the aglycones are aroma and flavor compounds, including terpenes, sesquiterpenes, norisoprenoids, and shikimic acid metabolites, which can potentially be released from the precursor form, during fermentation and wine maturation (Zoecklein et al. 2000). *Saccharomyces cerevisiae*, which is the principal yeast in wine making, also possesses glucosidase activities but acts slowly under fermentation condition of 20°C (Delcroix et al. 1994). The aroma precursors are not very chemically reactive and also act slowly during acid hydrolysis resulting in some unhydrolyzed glycosides remnants in the wine. Hence the use of exogenous enzymes could be a viable option to improve the volatile recovery (Pogorzelski and Wilkowska 2007).

Aroma and flavor aglycones are often represented by monoterpenes, C13-norisoprenoids, benzene derivatives and long-chain aliphatic (fusel) alcohols (Pogorzelski and Wilkowska 2007). Depending on the quantity of free and bound forms, grape varieties are classified as neutral or aromatic, in which aromatic varieties have higher levels of terepenic compounds (Maria Joao et al. 2006). Previous studies have shown that these compounds provide varietal aroma to wine (Rapp 1998). Monoterpenes, for example, exist both in free and glycosidic conjugates in grapes, but the bound form are always greater in concentration than free terpenes (Dimitriadis and Williams 1984).

Other important constituents of wine aroma are C13-Norisoprenoids and they are mostly related to the wine aroma of non-aromatic grape varieties (Maria Joao et al. 2006). They are commonly present in most *Vitis vinifera* varieties and develop from enzymatic and photochemical degradation of skin and pulp carotenoids (Lee et al. 2007). Other major classes of compounds that contribute to the aroma of wine includes esters, fusel alcohols and acids (Etievant 1991). The fusel alcohols are formed during fermentation and they constitute to 50% of the aroma compounds in wine (Rapp 1998). They are also known to affect wine quality due to

their typical unpleasant odor (Etievant 1991). Esters are the major constituents of wine that contribute a fruity aroma to the wine (Etievant 1991).

Effect of Vineyard Management Practices

Crop Thinning

Crop/cluster thinning (CT) is widely accepted as a beneficial vineyard management practice (Reynolds et al. 2007) though it involves increased labor and lower yield. It is essentially done to adjust the crop load in order to attain the highest sustainable yield, desired fruit maturation with varietal character and better wine quality (Keller et al. 2005, Reynolds et al. 2007). The level of cropping should be carefully decided, because over-cropping delays fruit maturation (Petrie and Clingeleffer 2006, Weaver et al. 1957, Winkler 1954) and lowers the wine quality, color, pH and acidity (Weaver et al. 1957). Previous studies on CT show inconsistency in its effectiveness. For example, Dami and his group (2006) have shown that CT has an effect on the grape composition while in other cases, Keller and coworkers (Keller et al. 2005) show that the difference is negligible. Investigators have found that the cluster thinning increases the soluble solids and pH thus enhancing the juice composition (Dami et al. 2006). Petrie and Clingeleffer (2006) have found that this increase in the soluble solids is because of the enhanced fruit maturity rather than the increased rate of sugar accumulation, which occurs as part of the change in berry growth phase. The treatment is also known to increase the number of berries per cluster and the cluster weight (Reynolds et al. 2007) compensating the yield loss to some extent. The crop-level adjustment to low yields is known to increase the color of red wine (Bravdo et al. 1984). This finding is supported by the another study that shows an increase in anthocyanins concentration in cluster thinned vines (Petrie and Clingeleffer 2006). Wine made from these vines have a higher amount of acetyl ester and lower secondary alcohol giving it better aroma characteristics (Sinton et al. 1978). CT treatment enhances the monoterpene (the chemical class of compounds which are known to impart a distinctive wine aroma) levels in the fruit and this alteration also provides the desirable aroma and flavor attributes to the wine (Reynolds et al. 2007). There still exist other studies that show that there is little or no difference in the wine sensory properties due to CT despite the difference in grape composition observed (Bravdo et al. 1985, Ough and Nagaoka 1984, Reynolds et al. 2007).

Canopy Side

Depending on the canopy side, the sunlight exposure of the grape varies. Sunlight has a complex effect on grape composition (Crippen and Morrison 1986a). Activity of many biochemical pathways in grape are dependent on both light and temperature (Bergqvist et al. 2001). Grape composition is altered by sunlight by at least two mechanisms – temperature and solar radiation (Spayd et al. 2002). The relationship of light exposure and temperature is critical for grape composition and metabolic process but is hard to determine (Bergqvist et al. 2001). Few studies show that berry temperature increases linearly with sunlight exposure (Bergqvist et al. 2001, Smart and Sinclair 1976). Increased exposure to sunlight delays the ripening process by inhibiting sugar accumulation (Bergqvist et al. 2001, Kliewer 1977) and also tends to ripen the berries more unevenly compared to the clusters growing in shade (Kliewer and Lider 1968). In contrast, some studies show that the percentage of soluble solids is not affected much by light exposure (Crippen and Morrison 1986a, Spayd et al. 2002). Exposed clusters have been shown to have lower titrable acidity (Bergqvist et al. 2001, Crippen and Morrison 1986a, Kliewer and Lider 1968) and pH compared to the shaded clusters (Bergqvist et al. 2001). This reduction in titrable acidity was suggested to be because of the increase in the degradation of malic acid (Kliewer and Lider 1968) and that of the pH being because of the elevated temperature rather than the light exposure (Kliewer 1977). Exposure to sunlight, with no increase in temperature (potted vines under controlled growth conditions) causes an increase in the anthocyanin concentration because high temperature is considered detrimental for color formation (Kliewer 1977). Phenols are among the most important red wine components, which are responsible for wine color, bitterness, astringency and some odors and flavors. Research from Kliewer's (1977) lab shows that sunlight exposure causes an increase in the phenol content and increased temperature causes a decrease in the phenols. The contradictory results shown in literature on grape composition is suggested and not limited to be because of the seasonal variation, location and variety (Crippen and Morrison 1986a).

Ethanol Treatment

Because grapes are non-climacteric (produces only little or no ethylene) fruits, the role of ethylene hormone is not very significant in their ripening as compared to other fruits (Coombe

and Hale 1973). Though not very dominant in grapes, ethylene is found to have triggering effects in the ripening process of tomatoes (Beaulieu and Saltveit 1997). The levels of endogenous ethylene in grapes are very low (Shulman et al. 1985) since the hormone which is high at bloom, reduces rapidly as well as gradually from fruit set until ripening (Weaver and Singh 1978). Enhanced production of ethylene at the onset of ripening is required for attaining the desired ripeness for most of the fruits (Barry and Giovannoni 2007), hence there is a need for the application of exogenous compounds (Ethanol, Ethephon, Ethrel, BOA, etc.,) that could stimulate the internal ethylene production in grapes. Several studies show that ethylene not introduced at the right time and amount affects the maturation process. Investigations on finding when and what concentration of ethanol has a considerable effect on the ripening process of grapes, show that ethanol retards (6mg/g) or hastens (≤3mg/g) the ripening process, depending on the concentration (Beaulieu and Saltveit 1997). Some reports also indicate that Ethephon (2-Chloroethyl-phosphonic acid), a commercially available growth regulating hormone, delayed maturation if applied before the onset of ripening (veraison) (Hale et al. 1970). Spraying ethanol between 8-13 weeks post bloom is also found to enhance color of the fruit and wine and decrease acidity (Chervin and El-kereamy 2001, El-Kereamy et al. 2002). Increase in color (mostly anthocyanins) and decrease in acidity are indicators of maturity and flavor/aroma development (Martin et al. 2008). But though there are certain reports showing increase in anthocyanin concentration (El-Kereamy et al. 2002), no significant difference in the taste and aroma characteristics of wine has been found due to ethanol treatment (Martin et al. 2008).

Electronic Nose

Electronic nose (Enose) is a chemosensor-array-based technology consisting of a series of sensors, and works in a manner similar to that of the human olfactory system. On exposure to chemical vapor, the sensor undergoes a physical-chemical change and produces results similar to sensory analysis (Mallikarjunan 2005). It is capable of being used in online detection of drift/defect in any process, but in most cases it does not provide information on the origin or nature of the problem (Mielle 1996). Hence, to obtain information about the individual components of a volatile, there is a need for validation of the ENose results with traditional methods (sensory, GC, etc.). Electronic noses are gaining a lot attention in recent days and are being used for a variety applications in the food industry (Mielle 1996) like:

1. Monitoring cooking
2. Quality assurance of raw/finished products
3. Process monitoring
4. Study of storage conditions
5. Monitoring maturation and aging in wine
6. Product package interaction

Systems and Sensors

The major types of electronic nose, classified on the type of sensor, are conducting polymer (CP), metal oxide semiconductor (MOS), quartz micro balance (QMB) and surface acoustic wave systems (SAW). These systems are broadly classified into hot and cold types; MOS comes under hot category and the others under cold (Mielle 1996). All systems consist of three major parts: sensors, system controls and data processing units (Mallikarjunan 2005). The sample introduction into these systems could be by headspace sampling or injection.

Conducting polymer based (e.g. Cyranose 320) systems consist of an array polymer sensors. Each of them respond differently to a particular vapor and depending on the change in resistance across sensors a "smell print" is formed (non-quantitative). These systems can be operated at room temperature. This system needs to be trained before the identification process. Then, when an unknown volatile sample is introduced into the system it tries to compare it with the database of smell prints and identifies it the substance with the closest print (Cyrano Sciences Inc 2000). A drawback of this system is that it provides poor batch to batch reproducibility (Mielle 1996).

The **surface acoustic wave sensor based** system (e.g. Znose), which is marketed as a 'fast GC', provides data similar to, but faster than that of a GC. This is because Znose analysis does not involve long sample preparation procedures and runtime, making it take only a few minutes compared to a GC. (Though these claims of a faster analysis in a few minutes are usually made, they, however, do not take into account the additional cleaning and purging times required upon use.) In this system, the volatile gets adsorbed on the surface of the sensor resulting in a frequency shift and this corresponds to the amount of the material deposited, and the time taken by the substrate to reach the sensor gives information about the type of compound itself (Electronic Sensor Technology™ 2001). The data obtained could be analyzed either using chromatographic or spectroscopic approaches (Mallikarjunan 2005).

According to Mallikarjunan (2005), the **metal oxide semiconductor based** system consists of sensors made of metal-oxide-semiconducting-film-coated ceramic substrate, which are heated by a wire. These systems are less sensitive to water vapor due to their high operating temperature (Mielle 1996). They also found that coating of the sensors could be modified and used for reducing as well as oxidizing volatile compounds during analysis.

The **quartz crystal microbalance based** system comes under the category of piezoelectric crystal sensors and is made of quartz crystal, lithium niobate or lithium tantalite covered by a coating a gas chromatographic stationary phase, usually non-volatile compounds that are chemically and thermally stable (Mallikarjunan 2005). In this system, volatiles get adsorbed on the stationary phase coating and depending on the amount adsorbed, a measurable change in resonant frequency occurs (Mielle 1996).

Precision and Accuracy

Precision and accuracy of an electronic nose depends on several characteristics including the sensor selectivity, operating temperature, humidity, sensor drift, and sensitivity to a particular compound. Mielle (1996) has carried out extensive studies on these aspects and compared several types of sensor systems. According to these studies, none of the available sensors to date meet all optimum specifications of the precision, accuracy or consistency. Hence the use of a particular sensor could be limited to certain applications. He also explains that CP, SAW and QMB are the most used electronic noses today due to a lesser effect of sensor drift over time but the downside to these sensor systems is that they tend to have poor reproducibility. According to him, the MOP offers poor selectivity, but better sensitivity compared to CP because they are coated with robust materials and resilient to environmental humidity and sensor aging. Their lack of sensitivity towards water makes them potentially useful for high moisture foods. Other drawbacks are that MOP sensors are not suitable for sulfur containing compounds possibly due to the poisoning effect (irremediable or partial destruction of the material) are highly sensitive to ethanol that can mask the identity of other compounds in a food matrix. The sensitivity of QMB is related to its operating frequency (10-30 MHz) and its selectivity is affected by humidity and operating temperature. The sensitivity of the SAW is also dependent on the operating frequency but is affected by background noise caused by higher operating sensor frequency of sensor (Mielle 1996).

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Chapter II: Electronic Nose Evaluation: Effects of Canopy Side on Cabernet franc (*Vitis vinifera* L.cv.) Grape and Wine Volatiles

Abstract

The effect of canopy side on grapes and wine volatiles of Cabernet franc was evaluated using two electronic nose systems (conducting polymer-based and surface acoustic wave-based) during two growing seasons. Data from three sampling dates for each season from both electronic noses were compared with physico-chemistry and wine aroma sensory evaluations. Univariate and multivariate statistical analyses generally indicated grape physico-chemistry indices were not able to differentiate ($p>0.05$) between canopy sides consistently across growing seasons and sampling dates. Both the electronic nose systems corroborated 100% discrimination of canopy sides for grapes and wine using canonical discriminant analysis. Surface acoustic wave-based electronic nose on average was able to explain <50% of variation for grapes and <60% for wine using the first principal component compared to >80% for conducting polymer-based electronic nose. Wine aroma sensory evaluation was not able to consistently differentiate canopy sides. Considering the discriminating percentages obtained from multivariate statistical results, conducting polymer electronic nose was considered better than surface acoustic wave electronic nose in differentiating canopy sides.

Introduction

Fruits from different location of the canopy may have different maturity rates due to difference in heat and light exposure (Downey et al. 2006) altering grape composition (Jackson and Lombard 1993). Several studies have evaluated the effects of management practices on grape composition using both natural and artificial methods for manipulating light intensity (plastic sheet (Kliewer 1967), waxed bags (Weaver and McCune 1960), shade cloth (Smart et al. 1988), shaded and light exposed berries from same cluster (Price et al. 1995), different canopy sides (Bergqvist et al. 2001)). Some studies have shown increased exposure to sunlight delays ripening by inhibiting sugar accumulation (Bergqvist et al. 2001, Kliewer 1977) and may ripen berries unevenly compared to the clusters growing in shade (Kliewer and Lider 1968). In contrast, others have shown that the percentage of soluble solids is not affected by light exposure (Crippen and Morrison 1986a, Spayd et al. 2002). Berry weight was found to be higher in shaded berries in some studies (Crippen and Morrison 1986a), but not others (Crippen and Morrison 1986b). Exposed clusters have been shown to have lower titratable acidity (Bergqvist et al. 2001, Crippen and Morrison 1986a, Kliewer and Lider 1968) and pH compared to the shaded clusters (Bergqvist et al. 2001). The contradictory results shown in literature on grape composition due to light exposure suggest that the current methods of evaluation are inadequate in identifying the variation in grape composition. This causes a lack of consistency and hence creates a need for exploring alternative tools.

Electronic noses (ENose) has found its place in a variety applications in the food industry such as quality assurance of raw and finished products, process monitoring, study of storage conditions, monitoring maturation and aging in wine and product package interaction in the past (Mielle 1996). ENose is a chemosensor-array-based technology consisting of a series of sensors. On exposure to chemical vapor, the sensor undergoes physico-chemical changes in order to identify samples (Mallikarjunan 2005).

Based on the sensor, electronic noses can be classified into conducting polymer (CP), quartz micro balance (QMB), metal oxide sensors (MOS), metal oxide semiconductor field effect transistors (MOSFET)**,** and surface acoustic wave systems (SAW) based systems. All systems consist of three major parts: sensors, system controls and data processing units (Mallikarjunan 2005). Precision and accuracy of an electronic nose depends on several characteristics including sensor selectivity, operating temperature, humidity, sensor drift, and sensitivity to a particular

compound. Hence, the use of a particular sensor could be limited only to certain applications. However, from the past CP, SAW and MOS are the most used electronic noses due to lesser effect of sensor drift over time (Mielle 1996).

A CP system consists of 32 conducting polymer-based sensors, where the change in the resistance of each sensor is stored as 'smell print' during introduction of standard samples and the unknown sample is compared with the available smell prints for identification (Cyrano Sciences^{TM} Inc. 2000). In this system, sensors are made of different polymers: polyaniline, polypyrole, polythiophene, polyacetylene and polyindole at different oxidation and reduction states in order to provide selectivity to different compounds (Mallikarjunan 2005, Pinheiro et al. 2002). SAW-based systems consist of single sensor, which simulates a virtual sensor array as if consisting of a hundred orthogonal sensors (Mallikarjunan 2005). This system is a combination of sensor-based detection and GC analysis. The volatiles are adsorbed on the surface of the sensor resulting in a frequency shift, which corresponds to the amount of material deposited, time taken by the substrate to reach the sensor gives information about the type of compound (Electronic Sensor Technology^{TM} 2001). The data obtained from this system can be analyzed either using chromatographic or spectroscopic approaches (Lammertyn et al. 2004, Mallikarjunan 2005). In this study we sought to determine the ability of electronic nose systems to discriminate canopy sides compared to the traditional analyses.

Materials and Methods

Field Design

The study was performed in two grape seasons (2007 and 2008) on Cabernet franc grapes, grown on a Ballerina training system in Charlottesville, Virginia. Heat summation and average rainfall of 2007 and 2008 were recorded as 1370.6°C and 2 mm, and 1154.4°C and 3 mm, respectively. Grape vine rows with canopy side facing East/West and North/South were planted in 2004 and 2003 on 4046.86 m^2 and 16187.43 m^2 plots, respectively. The East/West facing vines were clone 4 grafted to 101-14 and North/South were clone 312 grafted to Riparia. Vines on both plots were spaced 2.13X3.05m apart. Overall research design is provided in a graphical form in Figure 1. In 2007, 10 grape vines and in 2008, five vines from the 10 vines used in the 2007 study were selected using a randomized block design for the East/West and

North/South oriented plots. Fruits were sampled on three sampling dates post-bloom (once per week) on both seasons. Sampling was carried out on week 12, 14 and 15 post-bloom in 2007 and week 14, 15 and 17 post-bloom in 2008. The last sampling dates of both seasons were the commercial harvest.

Degrees Brix by refractometer (AO Scientific instruments© 10430, New Hampshire, USA), %RH using digital hygrometer (Traceable™ 4187, Texas, USA) and temperatures using infrared thermometer (Extech instruments© 42529, Massachusetts, USA) were measured on both sides, within the vine canopy on all sampling dates, between 08:00 – 11:00 hrs. Fruits were collected from 5 neighboring vines of each canopy side for laboratory analysis. At the end of 2008 (week 17 post-bloom) growing season, approximately 80.7 kg of grapes were harvested and were frozen for processing. The components of yield determined consisting of shoots/meter, clusters/vine, cluster weight, berry weight, fruit weight/vine and fruit weight/treatment.

Lab Analysis (Berry/Juice/Wine Chemistry)

Twenty-five berries per vine were randomly selected from each frozen cluster collected from the field. Berries were weighed, thawed and crushed in a 30.97 cm^3 (15.24X20.32X0.10 cm) volume plastic sampling bags (Minigrip®, Texas, USA), which held approximately 200 grape berries. Juice was filtered through 0.45 µm syringe filters (Whatman®, New Jersey, USA). Berry weight, Brix, pH and titratable acidity (TA) were determined as described by (Zoecklein et al. 1999). Color intensity (A420+A520), hue (A420/A520) and total phenols (A280) were determined using a Genesys™ 5 spectrophotometer (Spectronic, Leeds, UK). The total glycosyl-glucose (TGG) and phenol-free glycosyl glucose (PFGG) analyses as described by Williams *et al*. (1995), and modified by Zoecklein *et al.* (2000). For wines, analyses of malic acid, fermentable nitrogen, alcohol content (v/v), residual sugar and volatile acidity were also conducted. L-malic acid was determined enzymatically (R-Biopharm AG, Darmstadt, Germany). Fermentable nitrogen was also determined enzymatically (Megazyme©, Bray, Ireland), alcohol content by FTIR (Foss WineScan™ FT 120, Minnesota, USA) and residual sugar concentration estimated by Clinitest (Bayer, Indiana, USA).

Wine samples for GC-MS analysis were prepared using 4 mL sample with NaCl salt (1.0 g) in 10 mL clear glass vials sealed with a septa (MicroLiter® Analytical Supplies, Inc., Georgia, USA). Vials had a pre-incubation time of 30 seconds at 30°C with agitation at 250 rpm. A CAR/DVB/PDMS Grey SPME Fiber (Supelco Sigma-Adrich, St. Louis, Missouri, USA) was

used to penetrate vials to a 32 mm depth. A GC-MS (Agilent Technologies, 6890N Network GC System, 5975B inert MSD) with injector temperature of 250°C, DB-Wax column (30x25x25), and helium carrier gas with a flow rate of 1 mL/min were used. Oven temperature was 40°C with a ramp rate of 6°C per minute to 230°C. Thirty-two standard compounds from each wine sample were manually integrated and quantified.

Processing and Fermentation

Harvested grapes (11.47kg) from each canopy side, frozen at –20°C were thawed for 24 hours prior to processing. Clusters were crushed and destemmed using a Wottle (Anton, Poysdorf, Austria) destemmer crusher to about 50% berry breakage, estimated visually. Berries were distributed into six open-top 60L Nalgene® fermenting bins of equal height and volume. Each bin was treated with 250mg/L Velcorin® (Scott Laboratories, California, USA) dimethyl-dicarbonate (DMDC). Bins were held for 24 hours at 7ºC cooler followed by additional 25mg/L of potassium metabisulfite (KMBS) addition. Grapes were cold soaked for 6 days at 7ºC during which time must and grapes were punched daily.

Must juice analysis was performed on day 4 of cold soak and °Brix, pH, titratable acidity (TA), and fermentable nitrogen adjustments made using sucrose, tartaric acid, FermAid®K (Lallemand, Blagnac Cedex, France) and diammonium phosphate (DAP).

Go-Ferm® (Lallemand, Blagnac Cedex, France) yeast nutrient was prepared according to manufacturer directions and added during the yeast rehydration. *Saccharomyces cerevisiae* ICV-D254 (Lallemand, Blagnac Cedex, France) yeast (20g/L) was inoculated following the cold soak treatment. After inoculation, caps were punched 3 times daily. Fermentation was monitored by hydrometer and carried out at 23±2ºC until dryness (<1% residual sugar). Following fermentation, wines were pressed using a basket press. Free run and press run fractions were separated into sanitized, carbon dioxide filled carboys. Wine was kept at 7ºC for 24 hours, racked and filled in 3.80L glass bottles.

Electronic Nose Analysis

Two electronic nose systems, a conducting polymer (CP) based, Cyranose 320™ (Smiths Detection©, Pasadena, CA, USA; both in field and laboratory) and a surface acoustic wave (SAW) based, ZNose 7300™ (Electronic Sensor Technology, Newbury Park, CA, USA) were used in this study to determine the differences between canopy sides of grapes and wine.

The CP ENose method optimized for grapes by Athamneh *et al.* (2008) is given in Table 1A. For field analysis, two clusters (1 for each canopy side) were chosen at random from each of five selected vines. Clusters were bagged with a HDPE bag and received 45 minutes equilibrium time. The same clusters were used for the ENose measurements on all sampling dates. In 2007, additional clusters from neighboring vines were collected and frozen until laboratory analysis. Frozen clusters were placed into individual 1.50L glass jars in a water bath at 30°C for 20 minutes and subsequently analyzed by CP ENose. Berry analysis was also performed in which, 50g of berries were picked from the cluster and analyzed in a 200mL glass Mason jars.

The wine evaluation method optimized for CP ENose in an earlier study for Cabernet Sauvignon was used and is provided in Table 1B (Gardner 2009). Wines were analyzed twice, once immediately post-fermentation and again 6 months post-fermentation. Five replicates of 20mL wine samples were placed in 40mL GC clear glass vials sealed with Teflon/Silicone 0.003m septa top (MicroLiter® Analytical supplies, Inc., Suwanee, Georgia, USA). The samples were analyzed following the same procedure used in laboratory for 2007 grapes.

For the SAW ENose, the default settings of DB-5 system were used, except for the sensor temperature (Table 2). Sensor temperature of 45°C was chosen using a trial and error method. The system was tuned with C6-C14 alkane standards each day. The same sampling technique of CP ENose wine analysis was applied for both juice and wine analysis in this system.

Sensory Analysis

A triangle difference test was conducted 6 months post-fermentation on wine aroma as described by Meilgaard *et al.* (2007), comparing East vs. West and North vs. South on the same day. Wines were pre-screened for sulfur-like off odors and the panelists were given three samples and asked to identify the odd sample. All wines were identified with a randomized 3-digit code. Standard ISO glasses were filled with 10mL of wine and covered with a plastic Petri dish. The wines were presented to the consumer/untrained panelists at approximately 19°C under a red light. A total of 32 panelists in the age range of 21-27 years were used (α=0.05, β=0.30, ρd=40%, 16 correct responses for significant difference). Male and female panelists in the ratio of 1:1 were chosen with a pre-requisite of wine consumption at least once a week. Analysis was performed by providing written instructions and each panelist smelling 2 sets of samples (Meilgaard *et al.*, 2007).

Data Analysis

The physico-chemistry, CP ENose and SAW ENose data of grapes and wines were analyzed and compared using univariate (one-way analysis of variance (ANOVA) and least significant difference (LSD)) and multivariate (canonical discriminant analysis (CDA) and principal component analysis (PCA)) statistical methods using SAS JMP Version 7 (SAS Institute, New Jersey, USA). The SAW ENose data was analyzed using the regular GC chromatographic approach as described by Lammertyn *et al.* (2004). For the sensory data, number of correct responses was counted.

Results and discussion

Harvest yield components were not different among any grape vine canopy sides, with the exception of berry weight (Table 3). Canonical discriminant analysis (CDA) of seven grape physico-chemistry indices (Brix, berry weight, pH, TA, color intensity, hue, and total phenols) demonstrated the ability of these to identify grape vine canopy side, increased with time (Table 4A). However, these indices did not predict 100% differences between canopy sides on any of the sampling date. Canonical discriminant scores show the number of samples correctly identified per canopy side (Table 4B). A canonical plot from CDA represents the multivariate mean of the data points as circles whose size indicates 95% confidence limit for the mean. Non-intersecting circles indicate significant differences. CDA of the physico-chemistry data for 2007 and 2008 illustrated that these indices could not differentiate both East from West and North from South (Figure 2).

The p-values for the analyses of nine physico-chemistry indices (phenol-free glycosyl glucose (PFGG) and total glycosyl glucose (TGG), in addition to the seven indices listed above), are given in Table 5. The pair wise comparison test (t-test) results are provided in Table 6A and 6B. Brix (2007), titratable acidity (2008) and pH (both seasons) values were not significantly affected by canopy side difference on most sampling dates. These results confirm a previous study using Cabernet Sauvignon (Athamneh et al. 2008). However, differences between canopy sides were evident for ºBrix (2008) and titratable acidity (2007). Berry weight was differentiating between canopy sides in both seasons for all sampling dates. Fruit color intensity showed canopy differences in 2007, but not in 2008. In general, the physico-chemistry indices did not show

consistent differences between canopy sides across both seasons, suggesting they may not be optimum gauges for predictive modeling.

Based on the ANOVA of in-field CP ENose data, most sensors were sensitive ($p<0.05$) to grape volatiles and able to differentiate between canopy sides (except sensors S19, S24 and S32 in 2008) (data not shown). Also, canonical plots illustrated differences between canopy sides at all maturity levels, in both seasons in field for CP ENose as well as in laboratory using CP ENose and SAW ENose (Figures 3, 4, 5 and 6) based on the fruit volatiles.

Principal component analysis of physico-chemistry, CP and SAW ENose data showed that for each data source, the first three components together explained 100% of the variation (Table 7). It can be noted that CP ENose explained most of the variation (>90%) in a single (prin1) axis, whereas physico-chemistry and the SAW ENose data were able to explain similar variation using prin1 and prin2. PCA is a multivariate statistical method in which the variation of data is summarized in the form of principal components. This method explains the variation in data by replacing the larger set of variables correlated with canopy side with a smaller set of uncorrelated variables. The original variables are shown as biplot rays in the PCA plot, where length indicates the relative importance of each variable in explaining the difference.

Since maximum variability in data is captured in the prin1 axis of PCA, the conducting-polymer-based system may be considered better in discriminating between canopy sides than the surface acoustic wave-based system. Observing the lengths of biplot rays for physico-chemistry data (Figure 7) in 2007 and 2008, Brix was the least affected parameter followed by TA and hue in detecting canopy side difference. For the CP ENose, except sensors S19, S24, S32 and S6 all others were associated with the variation in data in most of the sampling dates. However, sensors S30, S31, S25 and S21 were also not able to explain the canopy side difference for some sampling dates (Figure 8). These results demonstrate that PCA captured the sensitivity of sensors towards the canopy side difference better than the ANOVA, where only three of the eight (S19, S24 and S32) sensors were identified to be insensitive towards canopy side difference.

Five wine chemistry parameters (PFGG, TGG, and color intensity, hue and total phenols) were analyzed using pairwise comparisons and ANOVA (Table 8). Results of these indicate difference between canopy sides for all parameters ($p<0.05$), except TGG and color intensity (only North and South). Additional wine chemistry parameters measured were in the range of pH

(3.2-3.5), % alcohol (12.5-13.5), residual sugar (<1), TA (6.75-7.3g/L) and malic acid (0.75-1.8g/L), illustrating only slight difference between canopy sides.

Wine volatiles were analyzed both immediately post-fermentation and 6 months post-fermentation using CP ENose. CP ENose data explained most of the variation along prin1 axis (>80%) both immediately post-fermentation and six months post-fermentation. Six months post-fermentation gave better discrimination of canopy sides (prin1: 97.6%, prin2: 1.7% and prin3: 0.7%) than when performed immediately post-fermentation (prin1: 81.1%, prin2: 17.9% and prin3: 1.0%) using CP ENose. The SAW ENose analysis performed immediately post-fermentation was able to explain <50% variation using prin1 (prin1: 48.4%, prin2: 35.6% and prin3: 16.0%). However, both CP ENose and SAW ENose analysis of wine volatiles were able to explain 100% of the variation (based on CDA, and prin1, prin2 and prin3 of PCA) immediately post-fermentation (Figure 9A and 10) and six months post-fermentation (Figure 9B).

The biplot rays show that sensors S23 and S31 were sensitive to canopy side differences immediately post-fermentation and 6 months later (Figure 11). ANOVA on CP ENose sensor responses shows that most of the sensors were sensitive to canopy side differences in the wine 6 months post-fermentation (all but S17, S22, S24 and S26) whereas, only two sensors (S6 and S31) were associated with the canopy side differences immediately post-fermentation.

Volatile concentration differences were detected for 25 of 32 compounds measured across canopy sides (Table 9). CDA and PCA (prin1: 67.5%, prin2: 18.6% and prin3: 13.9%) explained 100% variation in the data across canopy sides (Figure 12). PCA biplot rays indicated that ethyl myristate, citronellol, ethyl nonanoate and hexyl acetate were the compounds most associated with the canopy side differences. The differences detected by GC/MS for individual volatile compounds (25 out of 32) support the variation detected by ENoses.

During the wine sensory evaluation of aroma in 2008, panelists were able to differentiate between East and West only (Table 10). These results may have been different if a trained panel was used, although a trained panel may not have been indicative of a consumer response. Lack of differences detected by sensory tests does not always illustrate treatment similarities since it may involve bias and variability over time (Meilgaard et al. 2007). Though the electronic noses may produce results similar to sensory analysis (Mallikarjunan 2005) it evaluates both aroma and non-aroma volatiles (Haugen and Kvaal 1998a). Additionally electronic noses have the ability to evaluate objectively, volatiles in a complex matrix such as wine. The results show that even

when comparing wine from different canopy sides, CP ENose performed better than SAW ENose, sensory and physico-chemistry analyses. The performance of SAW ENose was not comparable to the CP ENose likely due to the non-linear nature of its data (Haugen and Kvaal 1998b).

Conclusions

This study was performed in order to determine if conducting polymer-based and surface acoustic wave-based electronic noses were able to distinguish and discriminate between grape and wine volatiles across canopy sides. Results were compared with the traditional physico-chemistry and sensory analysis. The aroma sensory evaluation was able to show differences only between East and West facing canopy sides. The physico-chemistry analyses did not differentiate between canopy sides on most sampling dates and did not show trends across growing seasons, suggesting conventional methods may not be optimum. Considering time efficiency and discrimination percentages obtained, CP ENose could be considered better than SAW ENose in differentiating between canopy sides. In the future analyzing the data of SAW ENose using non-linear data analysis methods, which has been proven to provide better discrimination than multivariate statistical methods (CDA, PCA, etc.,), the percentage of variation explained by SAW ENose could be improved. The sensor sensitivity information found from this study may lead to development of electronic nose technology that could be specific for grapes/wines by eliminating sensors that were found to be insensitive. Such modifications could result in design and development of low cost technologies for a wider application in the wine industry.

A) Grape method		
Parameter/Setting	**Value**	**Level**
Baseline purge	20 sec	Medium
Sample draw 1	30 sec	Medium
Sample draw 2	0 sec	Medium
Snout removal	0 sec	Low
1st sample gas purge	0 sec	High
1st air intake purge	10 sec	High
2nd sample gas purge	60 sec	high
2nd air intake purge	0 sec	High
Digital filtering	On	
Substrate heater	On	42
Training repeat count	1	
Identifying repeat count	1	
Algorithm	Canonical	
Preprocessing	Auto-scaling	
Normalization	Normalization 1	
Identification quality	Medium	

B) Wine method		
Parameter/Setting	**Value**	**Level**
Baseline purge	30 sec	Medium
Sample draw 1	20 sec	Medium
Sample draw 2	0 sec	Medium
Snout removal	0 sec	Low
1st sample gas purge	0 sec	High
1st air intake purge	10 sec	High
2nd sample gas purge	60 sec	High
2nd air intake purge	0 sec	High
Digital filtering	On	
Substrate heater	On	40°C
Training repeat count	1	
Identifying repeat count	1	
Algorithm	Canonical	
Preprocessing	Auto-scaling	
Normalization	Normalization 1	
Identification quality	Medium	

Table 2.1: Conducting polymer-based electronic nose analysis method settings used for (A) Grape (Athamneh et al. 2008) and (B) Wine (Gardner 2009).

Category	Temperature (°C)
Sensor	45
Db-5 column	40
Valve	165
Inlet	200
Trap	250
Maximum column	225

Table 2.2: Surface acoustic wave-based electronic nose method settings for juice and wine analysis in 2008.

Components	Shoots/meter	Clusters/side	Fruit weight (kg)	Cluster weight (kg)	Berry weight (g)
East	20.60±1.02a	16.40±3.55a	3.12±1.09a	0.18±0.04ab	1.77±0.07ab
West	17.40±1.02a	17.40±3.55a	2.32±1.09a	0.13±0.04b	1.63±0.07b
North	18.80±1.02a	23.00±3.55a	5.50±1.09a	0.27±0.04a	1.97±0.07a
South	17.20±1.02a	22.80±3.55a	5.23±1.09a	0.23±0.04ab	1.89±0.07a
p - Value	0.11	0.43	0.15	0.07	0.03

Table 2.3: Differences detected between canopy sides using harvest components of yield, for Cabernet franc grapes in 2008 growing season. Columns with different letters and $p<0.05$/highlighted values indicate 95% significant difference between treatments.

A

Sampling date	2007			Sampling date	2008		
	Chemistry	CP ENose	SAW ENose		Chemistry	CP ENose	SAW ENose
week 12	80%	100%	N/A	week 14	80%	100%	100%
week 14	90%	100%	N/A	week 15	85%	100%	100%
week 15	95%	100%	N/A	week 17	95%	100%	100%

B

Canopy Side		Identified			
		East	North	South	West
Classified	East	3	0	0	2
	North	0	4	1	0
	South	0	0	5	0
	West	1	0	0	4

Table 2.4: (A) Canonical discriminant score percentage of Cabernet franc grapes: physico-chemistry (chemistry), conducting polymer-based (CP ENose) and surface acoustic wave-based (SAW ENose) electronic noses for years 2007 and 2008. (B) Canonical discriminant score table for grape physico-chemistry data of 2007: week 12 is provided for example. Percentages of correct classifications have been indicated by darker through lighter colors indicating higher through lower values.

Physico-chemistry indices	2007			2008		
	week 12	week 14	week 15	week 14	week 15	week 17
Brix	0.81	0.76	0.76	0.04	0.03	0.00
Berry weight (g)	0.00	0.00	0.00	0.03	0.02	0.03
pH	0.03	0.06	0.28	0.19	0.17	0.00
Titratable acidity (g/L)	0.00	0.18	0.00	0.39	0.03	0.52
Color intensity (A420+A520)	0.01	0.01	0.03	0.94	0.71	0.62
Hue (A420/A520)	0.04	0.47	0.14	0.35	0.00	0.50
Total phenols (A280)	0.20	0.57	0.05	0.16	0.16	0.52
PFGG (µm)	0.02	0.32	0.01	N/A	N/A	0.11
TGG (µm)	0.24	0.00	0.01	N/A	N/A	0.00

Table 2.5: p-value indicating the significance of canopy side differences detected by physico-chemistry analyses during 2007 and 2008 on three sampling dates (week 12, week 14 and week 15 – 2007 and week 14, week 15 and week 17 – 2008 post-bloom) using ANOVA, for Cabernet franc juice. Highlighted boxes indicate significant difference ($p < 0.05$).

A

2007	Canopy side			
	East	West	North	South
Brix	19.3±0.46a	19.3±0.46a	19.2±0.46a	18.8±0.46a
	21.0±0.30a	21.0±0.30a	21.1±0.30a	21.4±0.30a
	21.1±0.44a	21.5±0.44a	20.9±0.44a	21.4±0.44a
Berry weight (g)	1.34±0.09b	1.25±0.09b	1.62±0.09a	1.81±0.09a
	1.22±0.10b	1.43±0.10b	1.91±0.10a	1.81±0.10a
	1.31±0.08b	1.37±0.08b	1.78±0.08a	1.63±0.08a
pH	3.33±0.13bc	3.15±0.13c	3.75±0.13a	3.57±0.13ab
	3.76±0.07b	3.72±0.07b	3.93±0.07ab	3.99±0.07a
	3.67±0.05a	3.73±0.05a	3.82±0.05a	3.72±0.05a
Titratable acidity (g/L)	2.98±0.19b	3.07±0.19b	4.58±0.19a	4.57±0.19a
	2.94±0.17a	2.62±0.17a	3.07±0.17a	3.09±0.17a
	2.72±0.12bc	2.52±0.12c	3.03±0.12ab	3.38±0.12a
Color intensity (A520+A420)	0.25±0.02bc	0.23±0.02c	0.30±0.02ab	0.33±0.02a
	0.19±0.02a	0.20±0.02a	0.12±0.02b	0.13±0.02b
	0.17±0.01a	0.18±0.01a	0.13±0.01b	0.15±0.01ab
Hue (A420/A520)	1.46±0.21a	1.24±0.21ab	1.65±0.21a	0.75±0.21b
	1.75±0.14a	1.55±0.14a	1.65±0.14a	1.44±0.14a
	1.42±0.09ab	1.32±0.09b	1.63±0.09a	1.47±0.09ab
Total phenols (A280)	1.12±0.07a	1.10±0.07a	1.26±0.07a	1.26±0.07a
	1.00±0.06a	1.06±0.06a	0.93±0.06a	0.99±0.06a
	1.07±0.07ab	1.24±0.07a	0.94±0.07b	1.02±0.07b
PFGG (µm)	156.02±25.3c	174.03±25.3bc	263.35±25.3a	245.24±25.3ab
	375.67±32.0a	298.43±32.0a	371.45±32.0a	336.38±32.0a
	396.75±18.5ab	451.09±18.5a	354.20±18.5b	358.80±18.5b
TGG (µm)	2708.13±236.1a	2303.13±236.1ab	2420.00±236.1ab	1998.75±236.1b
	3908.13±267.2a	3130.00±267.2ab	2644.13±267.2b	2365.38±267.2b
	3273.50±224.6a	2837.88±224.6ab	2488.75±224.6bc	1948.75±224.6c

B

2008	Canopy side			
	East	West	North	South
Brix	20.5±0.5ab	19.5±0.5b	21.8±0.5a	21.1±0.5a
	20.1±0.5bc	19.7±0.5c	21.8±0.5a	21.3±0.5ab
	20.4±0.5b	20.8±0.5b	24.2±0.5a	23.7±0.5a
Berry weight (g)	1.70±0.07ab	1.57±0.07b	1.78±0.07ab	1.92±0.07a
	1.49±0.07b	1.70±0.07ab	1.83±0.07a	1.81±0.07a
	1.77±0.07ab	1.63±0.07b	1.97±0.07a	1.89±0.07a
pH	3.59±0.04a	3.60±0.04a	3.71±0.04a	3.70±0.04a
	3.64±0.06b	3.67±0.06ab	3.77±0.06ab	3.83±0.06a
	3.98±0.04bc	4.01±0.04b	4.14±0.04a	3.87±0.04c
Titratable acidity (g/L)	4.08±0.2a	4.06±0.2a	4.41±0.2a	4.45±0.2a
	3.62±0.20ab	3.23±0.20b	4.03±0.20a	4.05±0.20a
	3.74±0.13a	3.67±0.13a	3.93±0.13a	3.70±0.13a
Color intensity (A520+A420)	0.31±0.02a	0.29±0.02a	0.30±0.02a	0.29±0.02a
	0.22±0.02a	0.19±0.02a	0.21±0.02a	0.20±0.02a
	0.27±0.02a	0.28±0.02a	0.25±0.02a	0.30±0.02a
Hue (A420/A520)	1.11±0.13a	0.80±0.13a	0.82±0.13a	0.82±0.13a
	1.76±0.13a	1.82±0.13a	1.24±0.13b	1.09±0.13b
	1.22±0.09a	1.17±0.09a	1.16±0.09a	1.03±0.09a
Total phenols (A280)	1.56±0.07a	1.44±0.07ab	1.36±0.07ab	1.32±0.07b
	1.20±0.06a	1.04±0.06ab	1.00±0.06b	1.08±0.06ab
	1.42±0.10a	1.45±0.10a	1.27±0.10a	1.45±0.10a
PFGG (µm)	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A
	500.54±78.89a	197.25±78.89b	313.66±78.89ab	351.04±78.89ab
TGG (µm)	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A
	2051.88±88.59a	1542.50±88.59b	1398.75±88.59b	1636.25±88.59b

Table 2.6: Pair wise comparison of canopy sides using least significant difference test for Cabernet franc juice physico-chemistry data on three sampling dates for years (A) 2007 (week 12, week 14, week 15 post-bloom) and (B) 2008 (week 14, week 15 and week 17 post-bloom). Different cells in each column marked by different letters indicate 95% significant difference between them.

Sampling date	2007			Sampling date	2008			Principal component
	Chemistry	CP ENose	SAW ENose		Chemistry	CP ENose	SAW ENose	
Week 12	81.1	99.7	N/A	Week 14	66.5	92.6	72.6	PC1
	18.1	0.2	N/A		26	4.8	23.5	PC2
	0.8	0.1	N/A		7.5	2.6	3.9	PC3
Week 14	72.4	97.6	N/A	Week 15	69.5	58.8	54.9	PC1
	20.3	2.1	N/A		27.2	39.8	30.1	PC2
	7.3	0.3	N/A		3.3	1.4	15	PC3
Week 15	76.9	99.7	N/A	Week 17	49.3	95.6	42.9	PC1
	12.7	0.1	N/A		42.1	3.5	36.9	PC2
	10.3	0.1	N/A		8.6	0.9	20.2	PC3

Table 2.7: Principal component values of Cabernet franc juice, showing the difference between canopy sides detected by physicochemical analyses (chemistry), conducting polymer-based electronic nose (CP ENose) and surface acoustic wave-based electronic nose (SAW ENose) in the years 2007 and 2008. Values indicate the % of variation explained by each principal component. Key for color codes provided.

Treatment	Color Intensity (A420+A520)	Hue (A420/A520)	Total phenols (A280)	PFGG (µm)	TGG (µm)
East	0.717±0.03b	0.469±0.01c	2.673±0.03c	62.7±5.30a	1388±36.30b
West	0.520±0.03c	0.560±0.01b	2.454±0.03d	28.2±5.30bc	1323±36.30b
North	0.886±0.03a	0.605±0.01b	3.344±0.04a	36.8±5.30b	1608±36.30a
South	0.811±0.03a	0.663±0.01a	3.192±0.04b	15.2±5.30c	1598±36.30a

Table 2.8: Pairwise comparison data of Cabernet franc wine chemistry indices. Different cells in each column marked by different letters indicate 95% significant difference between them.

Compound	Concentration (µg/L)				p - Value
	East	West	North	South	
Ethyl acetate	24.88±0.45a	24.13±0.45a	25.53±0.45a	25.17±0.45a	0.2326
2-Methyl propanol	23.07±0.81a	22.44±0.81a	22.92±0.81a	24.03b±0.81a	0.5932
Isoamyl acetate	3004.95±95.28c	2781.96±95.28c	4069.96±95.28a	3702.57±95.28b	<0.0001
n-Butanol	9.12±0.02ab	9.13±0.02a	9.09±0.02ab	9.08±0.02b	0.1128
3-Methyl butanol	75.26±2.13ab	71.77±2.13b	81.58±2.13a	82.18±2.13a	0.0232
Ethyl hexanoate	216.73±5.98b	194.05±5.98c	240.05±5.98a	236.82±5.98a	0.0021
Hexyl acetate	4.17±0.08a	1.47±0.08d	3.54±0.08b	2.61±0.08c	<0.0001
Ethyl heptanoate	5.01±0.11b	5.05±0.11b	5.14±0.11b	6.27±0.11a	0.0001
n-Hexanol	1.54±0.03b	1.69±0.03a	1.04±0.03c	0.99±0.03c	<0.0001
Ethyl octanoate	109.93±2.54b	109.39±2.54b	144.98±2.54a	144.42±2.54a	<0.0001
2-Ethyl-1-hexanol	6.60±0.24b	8.14±0.24a	6.22±0.24b	5.36±0.24c	0.0002
Ethyl nonanoate	24.13±0.03c	24.29±0.03ab	24.32±0.03a	24.22±0.03b	0.0043
1-Octanol	106.82±2.51b	255.91±2.51a	68.72±2.51c	58.05±2.51d	<0.0001
Terpinene-4-ol	13.17±0.35c	41.86±0.35a	17.22±0.35b	9.13±0.35d	<0.0001
Ethyl decanoate	59.04±1.53d	82.00±1.53c	116.22±1.53a	109.95±1.53b	<0.0001
Isoamyl octanoate	36.21±0.05d	36.57±0.05c	38.04±0.05a	37.66±0.05b	<0.0001
Nonanol	7.92±0.14b	12.01±0.14a	6.82±0.14c	5.58±0.14d	<0.0001
Isovaleric acid	2.23±0.06bc	2.05±0.06c	2.40±0.06ab	2.57±0.06a	0.0014
Diethyl succinate	341.11±9.19a	275.90±9.19b	345.39±9.19a	338.10±9.19a	0.0020
Methionol	1.56±0.04a	1.57±0.04a	1.48±0.04a	1.57±0.04a	0.4287
Citronellol	10.17±1.20c	19.82±1.20a	15.34±1.20b	18.65±1.20ab	0.0019
Phenethyl acetate	93.22±2.44ab	88.60±2.44b	100.95±2.44a	100.90±2.44a	0.0175
Beta-Damascenone	24.91±0.41b	26.85±0.41a	14.96±0.41c	14.99±0.41c	<0.0001
Hexanoic acid	1.04±0.08a	0.98±0.08a	1.05±0.08a	1.13±0.08a	0.6676
Ethyl dodecanoate	26.42±0.41b	27.09±0.41b	40.47±0.41a	41.43±0.41a	<0.0001
Benzyl alcohol	137.62±4.60b	150.74±4.60b	166.67±4.60a	172.38±4.60a	0.0027
Phenethyl alcohol	21.12±0.85b	20.96±0.85b	22.71±0.85ab	24.71±0.85a	0.0450
gamma-Nonalactone	0.08±0.00b	0.09±0.00a	0.07±0.00c	0.07±0.00c	<0.0001
Ethyl myristate	41.18±0.16a	40.02±0.16b	39.74±0.16b	40.04±0.16b	0.0010
Octanoic acid	1.63±0.21a	1.63±0.21a	1.62±0.21a	1.65±0.21a	0.9998
Ethyl palmitate	89.01±0.66bc	87.61±0.66c	90.06±0.66b	96.95±0.66a	<0.0001

Table 2.9: Results of pair wise comparisons and ANOVA for volatile compounds analyzed by SPME GC-MS and reported concentrations for East, West, North and South Cabernet franc wines. Different cells in each row marked by different letters, and highlighted p-values in the last column indicate 95% significant difference between them.

Treatment	Correct responses	Total responses	Significant difference between treatments
East vs. West	17	32	Yes
North vs. South	13	32	No

Table 2.10: Cabernet franc wine aroma (sample temperature 19°C) triangle difference sensory results (n=32) of East vs. West and North vs. South treatments for the year 2008 (α = 0.05, β = 0.10, ρ_{max} = 40%, 16 or more correct responses corresponds to a significant difference).

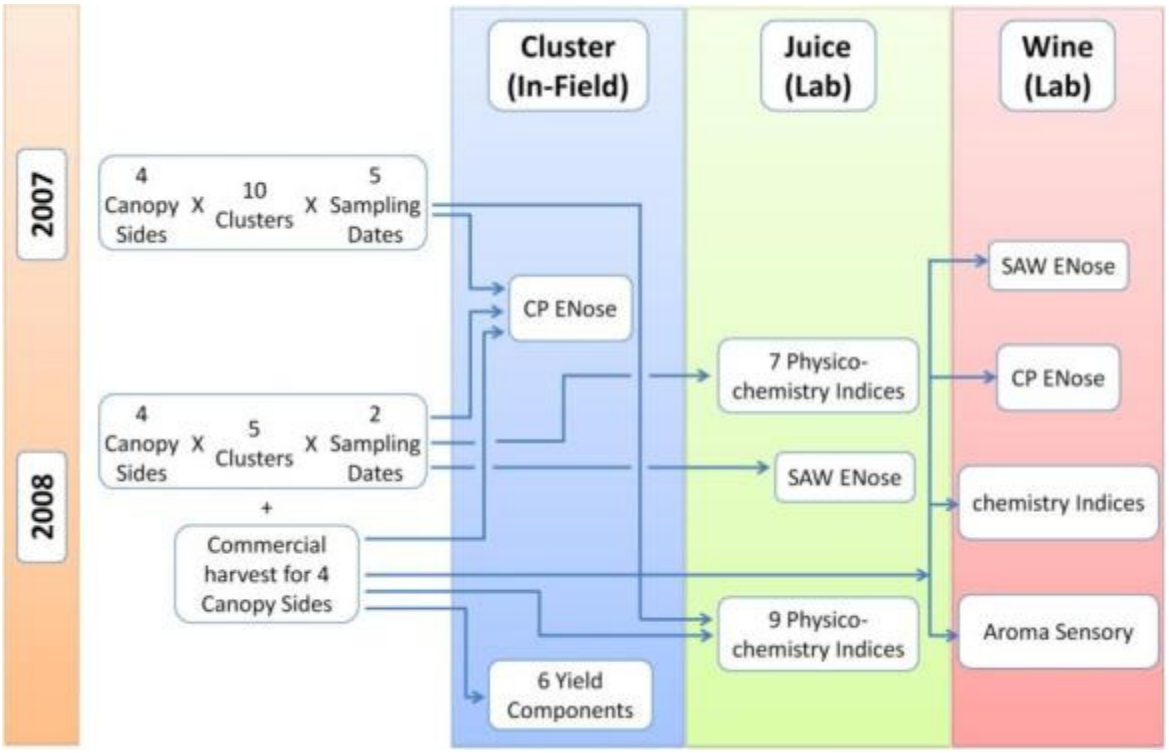

Figure 2.1: Research design for canopy side study.

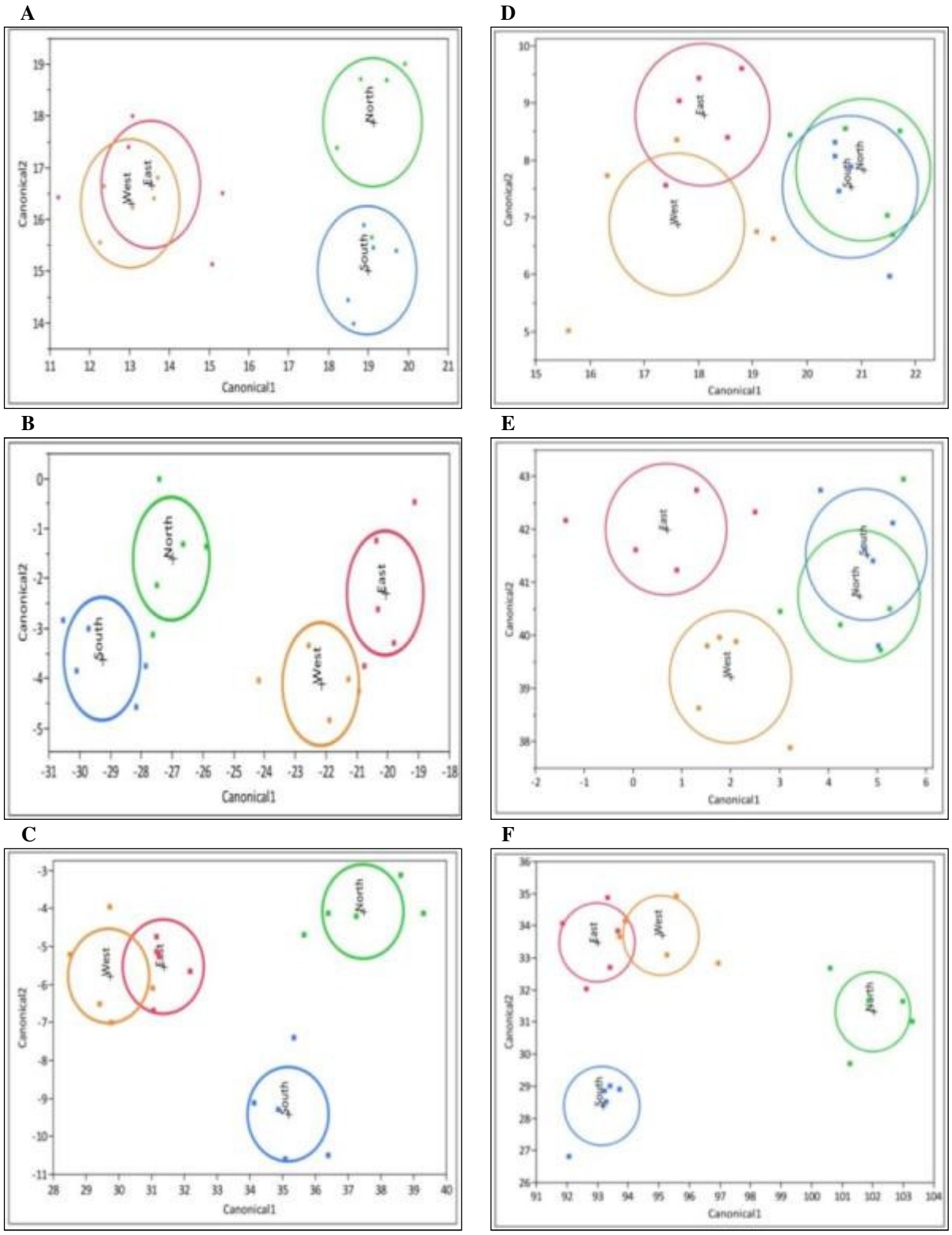

Figure 2.2: Canonical distribution of differences detected by physico-chemistry analyses on three sampling dates during 2007 (A) week 12, (B) week 14 and (C) week 15 post-bloom, and 2008 (D) week 14, (E) week 15 and (F) week 17 post-bloom, for Cabernet franc juice (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

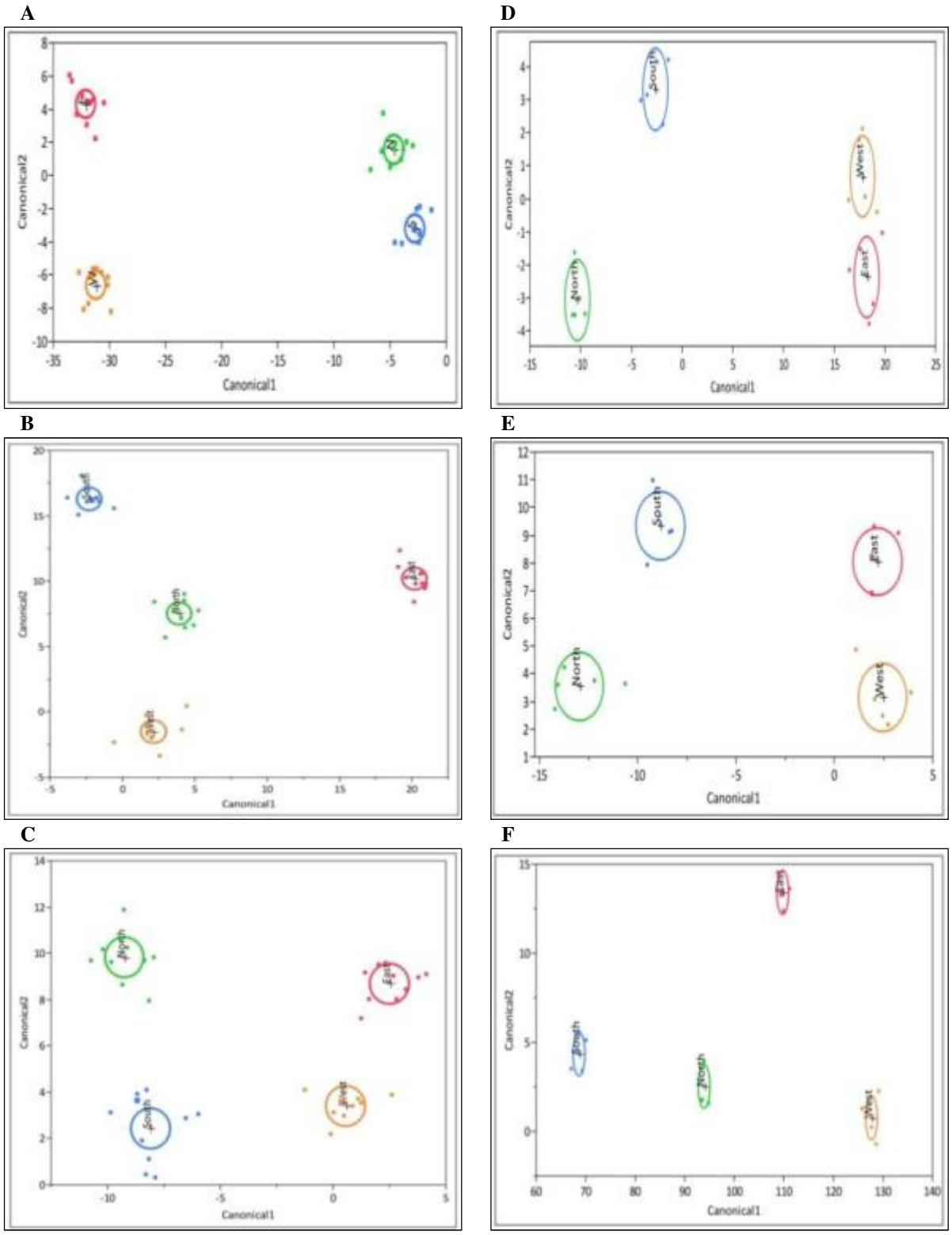

Figure 2.3: Canonical plots of canopy side differences for Cabernet franc grape berries, detected by conducting polymer-based electronic nose in field, in the years 2007 (A) week 12, (B) week 14 and (C) week 15 post-bloom and 2008 (D) week 14, (E) week 15 and (F) week 17 post-bloom (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

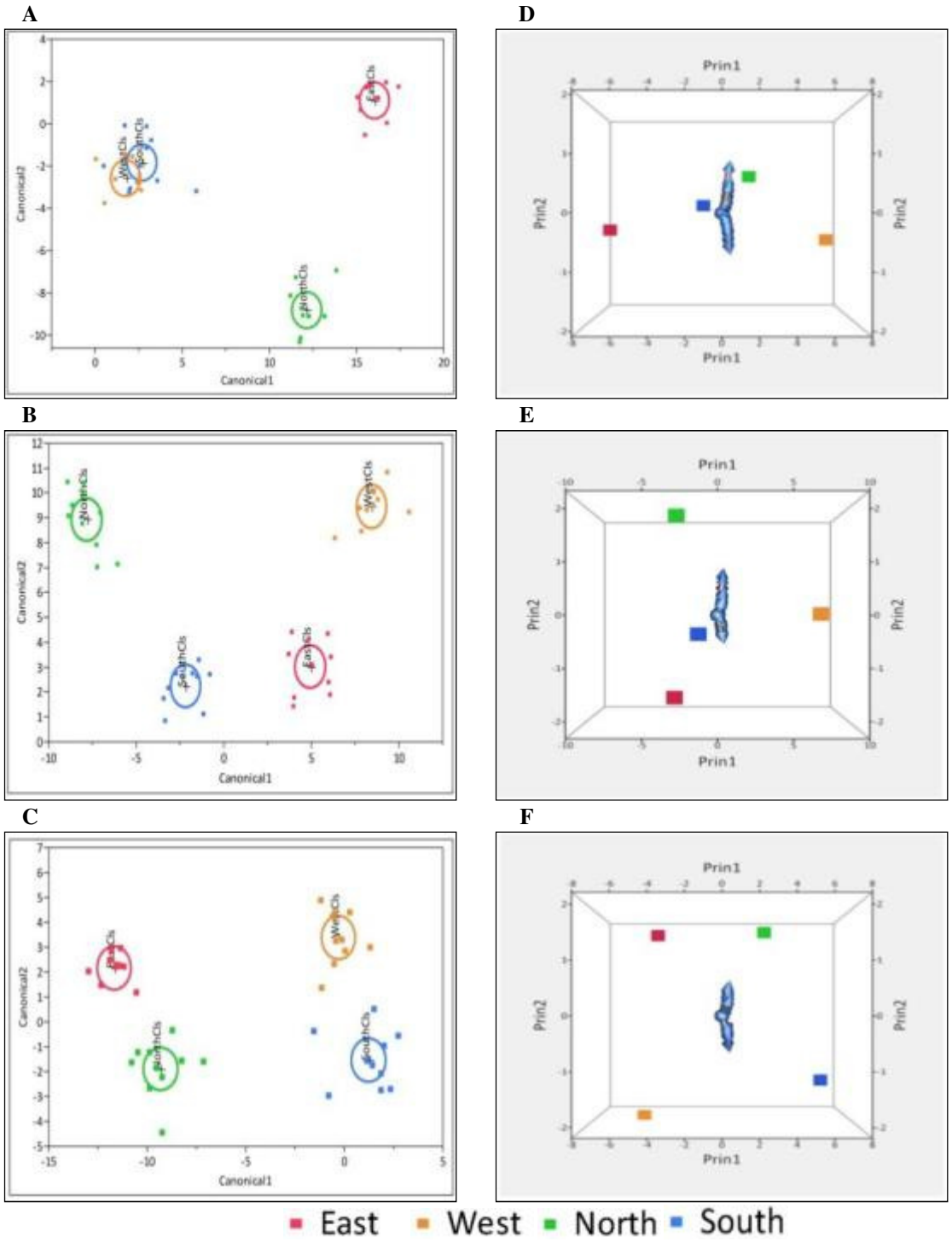

Figure 2.4: Canonical plots of canopy side differences for Cabernet franc grape clusters, detected by conducting polymer-based electronic nose in laboratory, in the year 2007 (A) week 12, (B) week 14 and (C) week 15 post-bloom and PCA (D) week 12, (E) week 14 and (F) week 15 post-bloom. (For canonical, significant differences at α = 0.05 level indicated by non-intersecting circles. Key for color code provided for PCA).

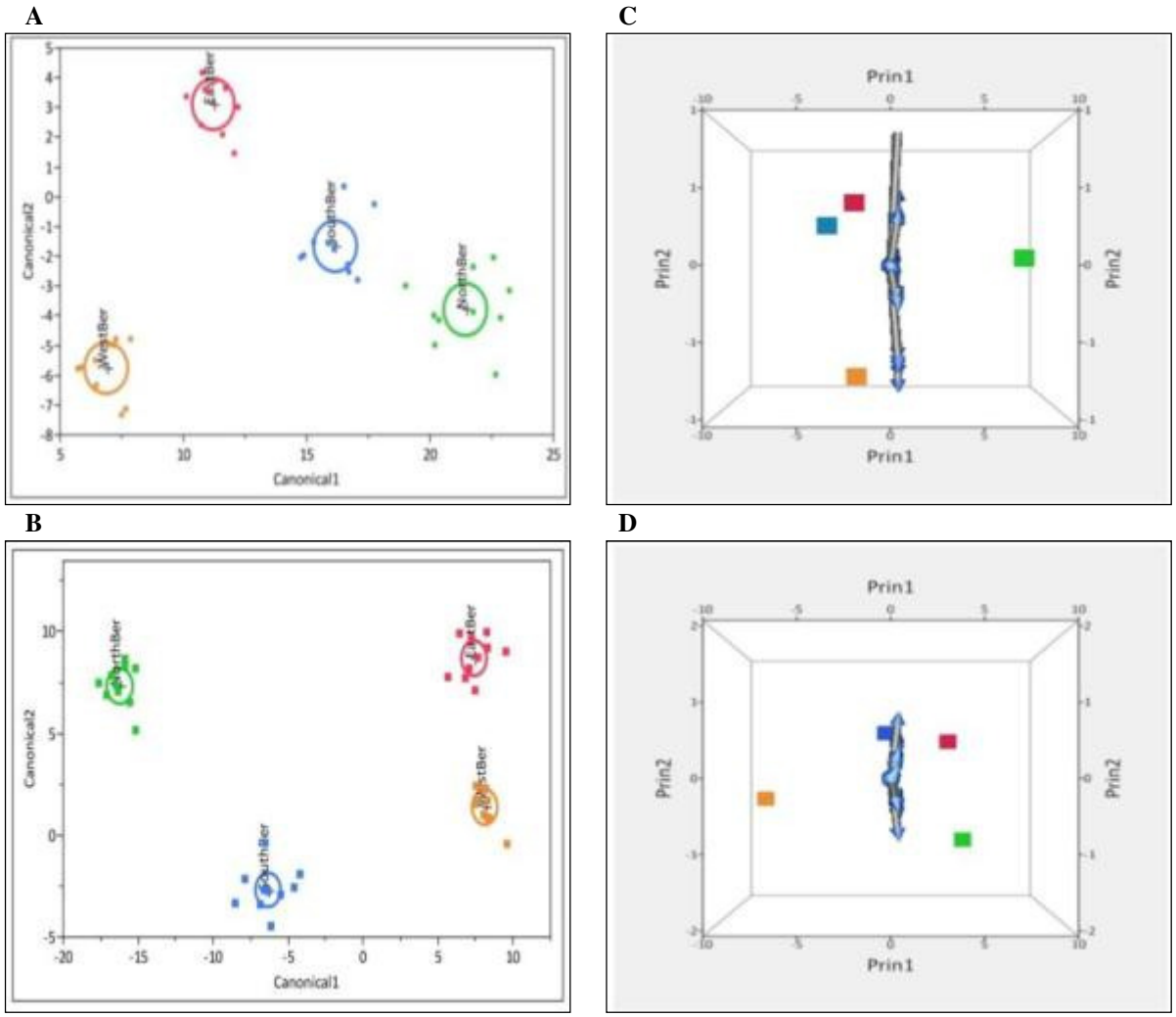

Figure 2.5: Canonical plots of canopy side differences for Cabernet franc grape berries, detected by conducting polymer-based electronic nose in laboratory, in the year 2007 (A) week 14 and (B) week 15 post-bloom and PCA (C) week 14, (D) week 15 post-bloom (For canonical, significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles. For PCA, key for color code provided in Figure 4).

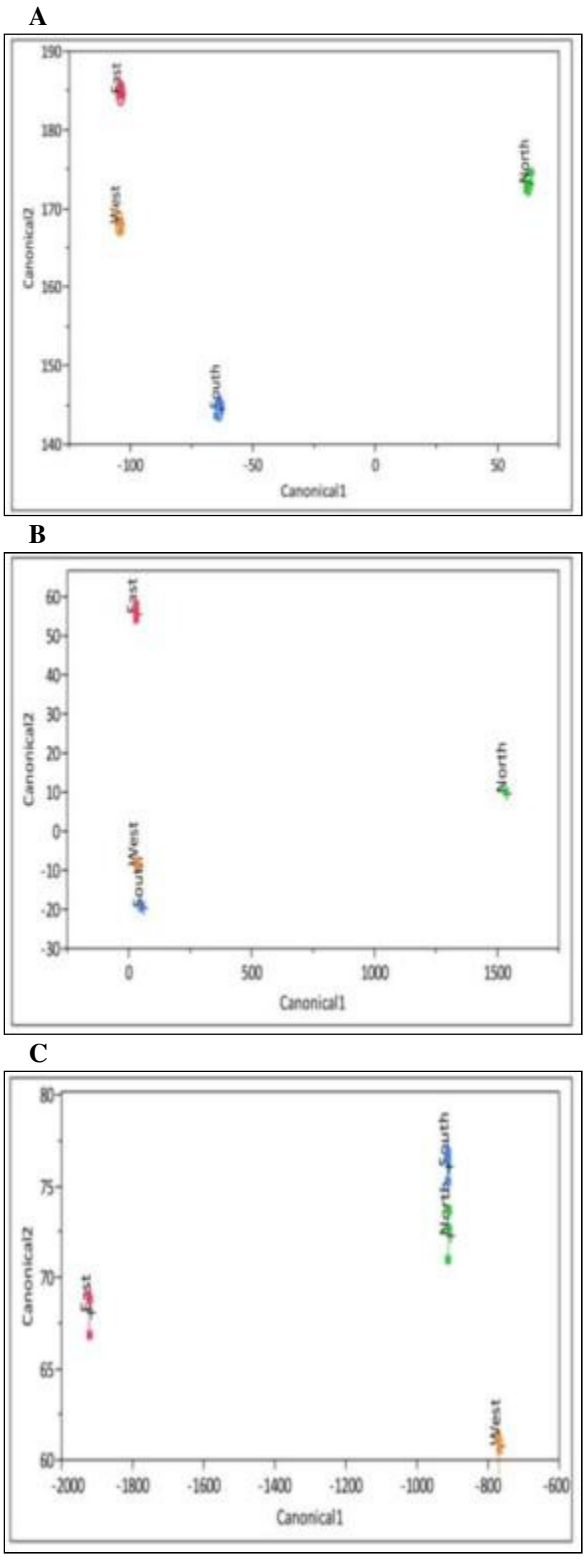

Figure 2.6: Canonical plots of canopy side differences for Cabernet franc grape juice, detected by surface acoustic wave-based electronic nose in laboratory, in the year 2008 (A) week 14, (B) week 15 and (C) week 17 post-bloom (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

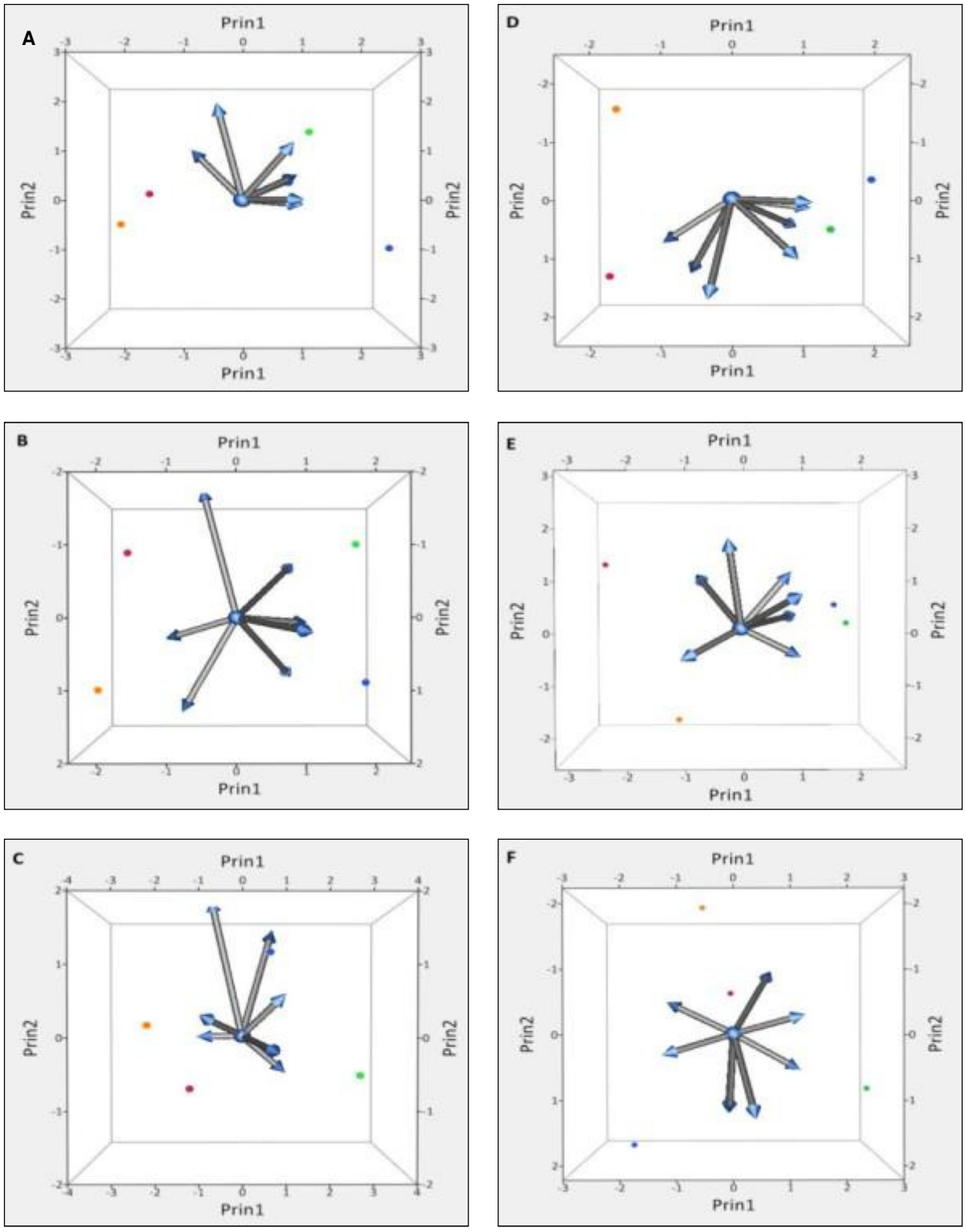

Figure 2.7: Principal component analysis of canopy side differences for Cabernet franc grape juice, detected by physico-chemistry analyses, in the years 2007 (A) week 12, (B) week 14 and (C) week 15 post-bloom and 2008 (A) week 14, (B) week 15 and (C) week 17 post-bloom (Points with different colors indicate different canopy sides, key for color codes provided in Figure 4).

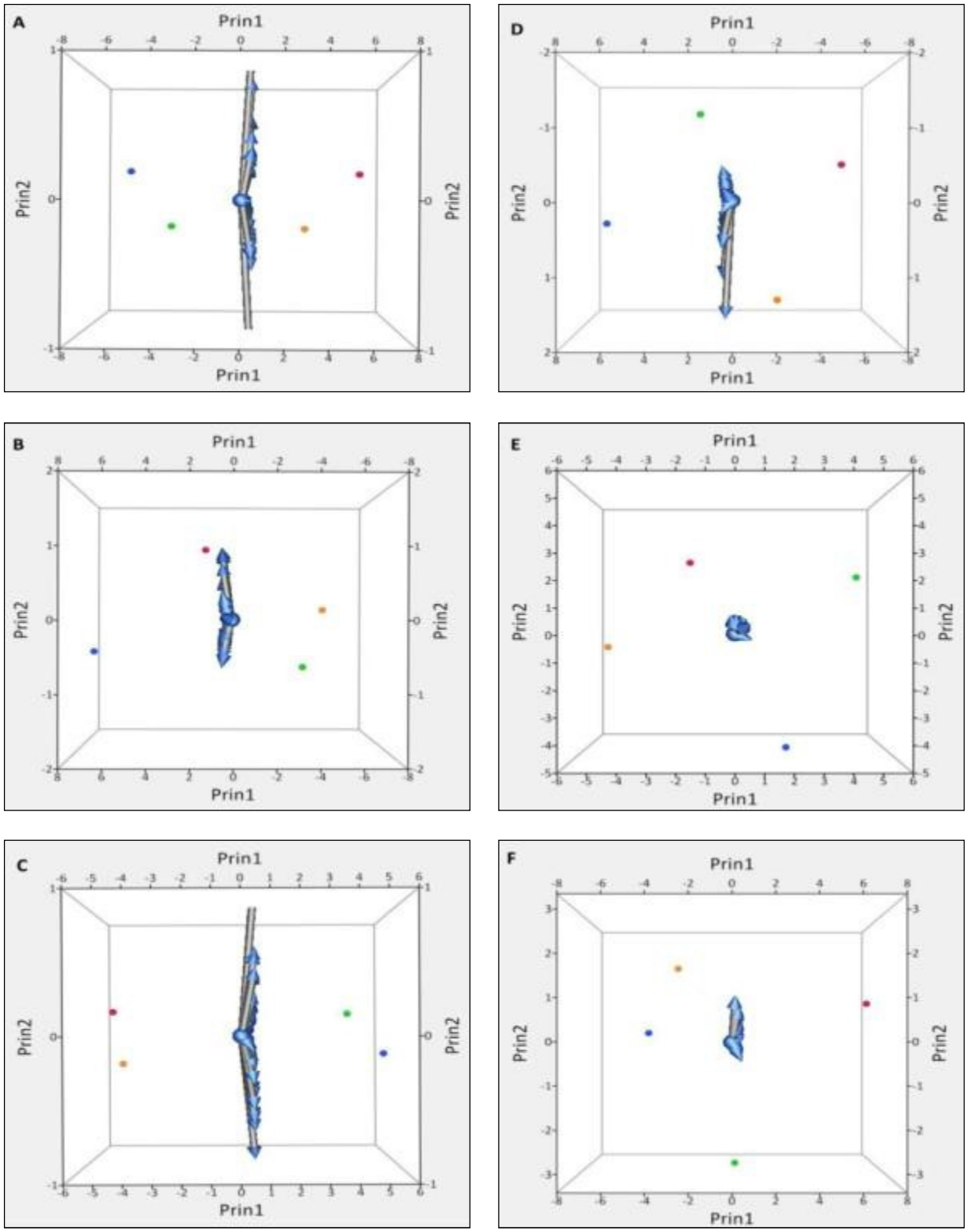

Figure 2.8: Principal component analysis of canopy side differences for Cabernet franc grape berry, detected by conducting polymer-based electronic nose in the years 2007 (A) week 12, (B) week 14 and (C) week 15 post-bloom and 2008 (A) week 14, (B) week 15 and (C) week 17 post-bloom (Points with different colors indicate different canopy sides, key for color codes provided in Figure 4).

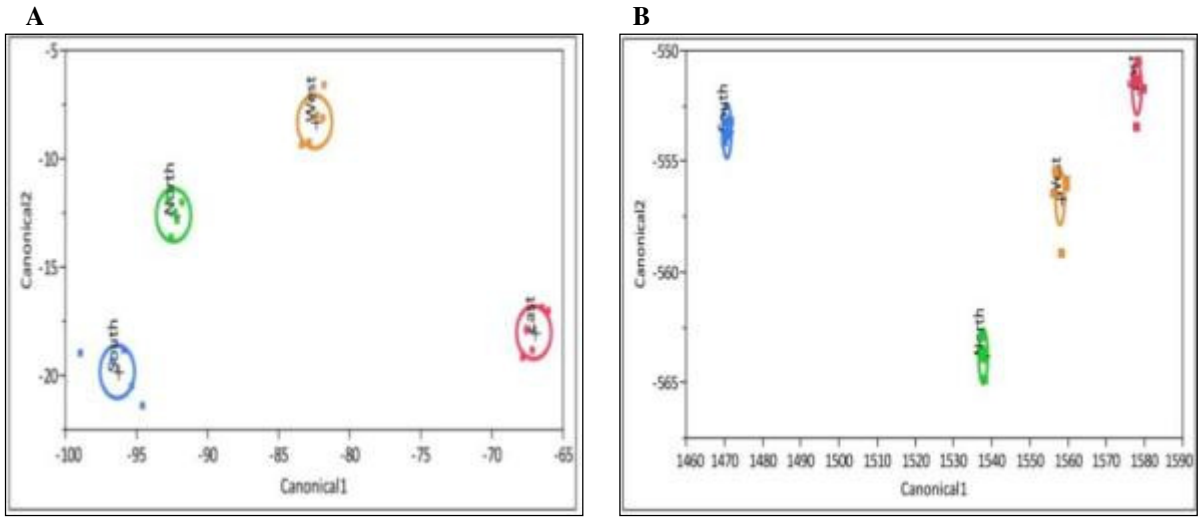

Figure 2.9: Canonical plots of canopy side differences for Cabernet franc wine, detected by conducting polymer-based electronic nose in laboratory, in the year 2008 (A) immediately post-fermentation, (B) six months Post-fermentation (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

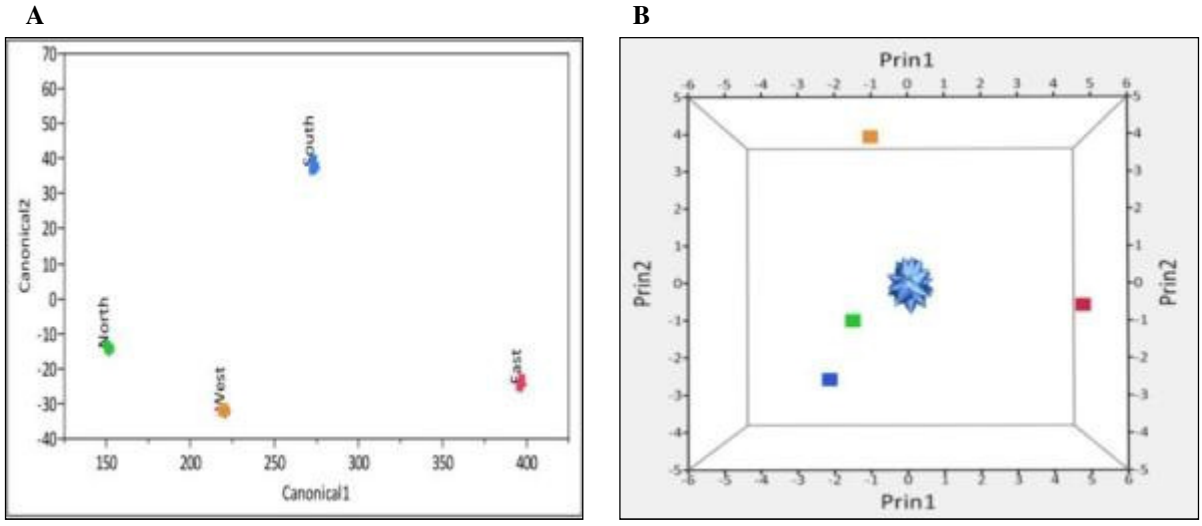

Figure 2.10: (A) Canonical distribution and (B) principal component analysis of Cabernet franc wine using surface acoustic wave-based electronic nose (Key for PCA color code provided in Figure 4).

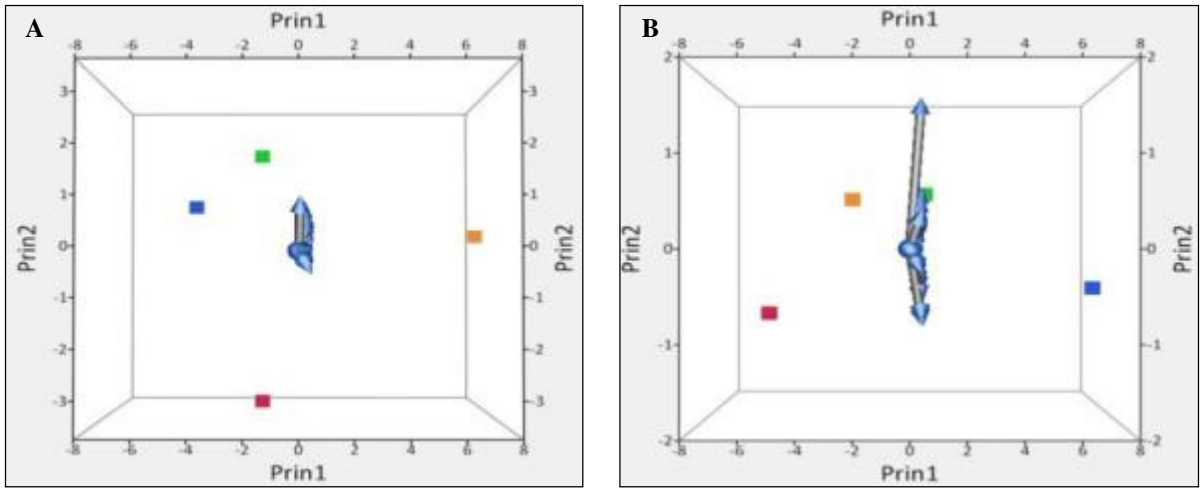

Figure 2.11: Principal component analysis of canopy side differences for Cabernet franc wine, detected by conducting polymer-based electronic nose in the years 2008 (A) immediately post-fermentation, (B) six months post-fermentation (Points with different colors indicate different canopy sides, key for color codes provided in Figure 4).

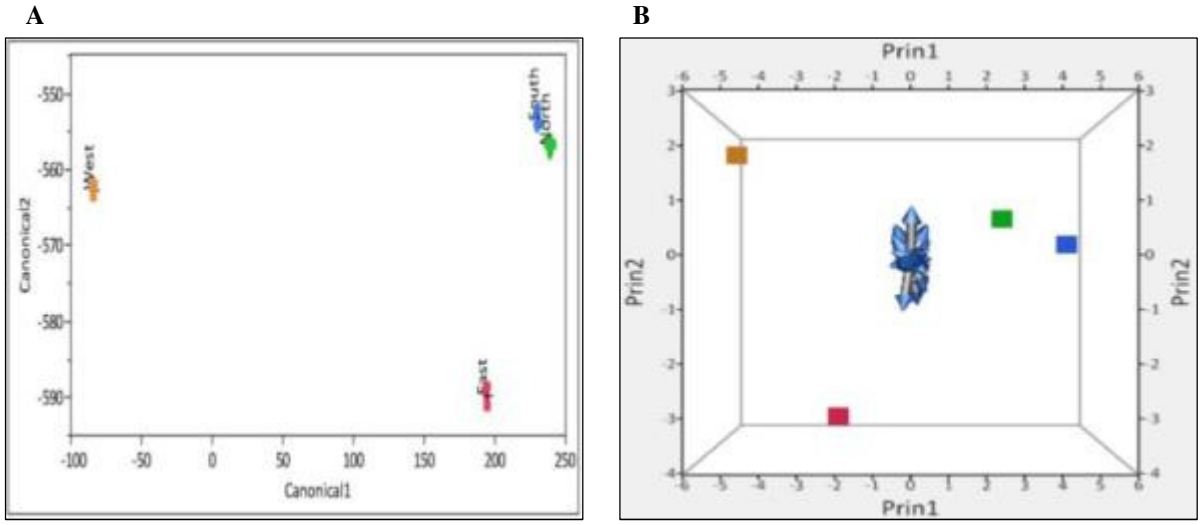

Figure 2.12: (A) Canonical distribution and (B) principal component analysis (PCA) of Cabernet franc wine from different canopy sides using GC/MS (Key for PCA color code provided in figure 4).

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Chapter III: Use of Electronic Nose Systems to Monitor the Effects of Cluster Thinning on *Vitis vinifera* L. cv. Merlot Grape and Wine Volatiles

Abstract

Cluster thinning is a common vineyard management practice used to increase fruit maturity rate and wine quality. In this study, grape and wine volatiles were analyzed on full cropped vines, 1 cluster/shoot and alternating 1&2 clusters/shoot treatments using a conducting polymer and surface acoustic wave-based electronic nose systems. Results from electronic noses were compared with physico-chemistry, GC/MS and wine aroma sensory evaluations. Using canonical discriminant analysis, physico-chemistry of grapes differentiated among all treatments only at harvest compared to differences found by both electronic nose systems on all sampling dates. Wine GC/MS analysis showed 100% discrimination using 32 volatile compounds identified from wines of all treatments out of which 25 compounds differentiated between treatments. Sensory analyses discriminated between the 1 cluster/shoot and 1&2 clusters/shoot treatments. Principal component analysis of grapes results indicated the conducting polymer-based system performed better in discriminating treatments (average of 81.9% using prin1) followed by physico-chemistry (64.7%) and surface acoustic wave-based system (61.1%). For wine both electronic noses were able to discriminate 100% of the replicates using canonical discriminant analysis, whereas with principal component analysis, CP-based system was able to explain 98% of the variation using prin1 compared to 56.9% by surface acoustic wave-based system. Both electronic nose systems performed better than other traditional methods used in this study.

Introduction

Cluster thinning (CT) is widely accepted as a beneficial vineyard management practice (Reynolds *et al.* 2007) though it involves increased labor and lower yield. It is essentially done to adjust the crop load in order to attain the highest sustainable yield, desired fruit maturation with varietal character and better wine quality (Keller *et al.* 2005, Reynolds *et al.* 2007). The level of cropping should be carefully decided, because over-cropping delays fruit maturation (Petrie and Clingeleffer 2006, Winkler 1954) and lowers the wine quality, color, pH and acidity (Weaver *et al.* 1957). Previous studies on CT show inconsistency in its effectiveness.

Dami and his group (2006) have shown that CT has an effect on the grape composition while Keller and his coworkers (Keller *et al.* 2005) have found in their study that the difference is negligible. Investigators have found that the cluster thinning increases the soluble solids and pH thus enhancing the juice composition (Dami *et al.* 2006). Increase in soluble solids is suggested to be because of the enhanced fruit maturity rather than the increased rate of sugar accumulation, which occurs as part of the change in berry growth phase (Petrie and Clingeleffer 2006). The treatment is also known to increase the number of berries per cluster and the cluster weight (Reynolds *et al.* 2007) compensating the yield loss to some extent.

The crop-level adjustment to low yields is known to increase the color of red wine (Bravdo *et al.* 1984). This finding is supported by the another study that shows an increase in anthocyanins concentration in cluster thinned vines (Petrie and Clingeleffer 2006). Wine made from these vines have a higher amount of acetyl ester and lower secondary alcohol giving it better aroma characteristics (Sinton *et al.* 1978). CT treatment enhances the monoterpene (the chemical class of compounds which are known to impart a distinctive wine aroma) levels in the fruit and this alteration also provides the desirable aroma and flavor attributes to the wine (Reynolds *et al.* 2007). There still exist other studies that show that there is little or no difference in the wine sensory properties due to CT despite the difference in grape composition observed (Bravdo *et al.* 1985, Ough and Nagaoka 1984, Reynolds *et al.* 2007). The current methods to analyze are time consuming, labor intensive and yet not adequate hence recent technologies need to be investigated.

The electronic nose (ENose) is an emerging technology used in areas like quality testing, process monitoring and storage in the food industry. Electronic nose systems are chemosensory-based and are classified based on their sensor type: metal oxide semiconductor (MOS), quartz microbalance (QMB), metal oxide semi-conductor field effect transistors (MOSFET), conducting polymer (CP) and surface acoustic wave (SAW) based systems. Mallikarjunan (2005) has reported that a major drawback of ENose systems are sensor drift and lack of consistency. According to him, developing optimum methodology and parameter settings specific for each product could partially help overcome these problems (Mallikarjunan 2005).

The CP system consists of 32 conducting polymer-based sensors formed electrochemically onto a carbon or silicon substrate. Polymers include polyaniline, polyprole, polythiophene, polyindole and polyacetylene of different oxidation and reduction states providing selectivity to different compounds (Mallikarjunan 2005, Pinheiro et al. 2002). Upon exposure to a chemical vapor, sensors expand causing a change in resistance, measured and stored as smellprint. The 'smellprint' is represented as bar graph, each bar demonstrating the resistance change of each sensor. This smellprint is stored in the database when a standard set of samples are introduced and unknown samples are identified by comparing the reading with the memory database (Cyrano Sciences™ Inc. 2000). The CP system provides better sensitivity and selectivity compared to other ENose systems and operates at moderate temperatures hence preventing the volatile breakdown due to increased heating of sensors (Mallikarjunan 2005).

SAW electronic nose system contains a single sensor, which simulates a virtual array as if consisting of hundred orthogonal sensors (Mallikarjunan 2005). This system adsorbs a chemical vapor and causes a change in sensor resonant frequency (Electronic Sensor Technology™ 2001). This system analyzes samples both quantitatively and qualitatively using both spectroscopic or chromatographic approaches (Lammertyn *et al.* 2004).

This study evaluated the impact of cluster thinning on grapes and wines using CP and SAW-based systems. The results from the electronic nose systems were compared with data from physico-chemistry, GC/MS and sensory analyses.

Materials and Methods

Field Design

This study was performed on Merlot grapes grown on a Ballerina training system in Charlottesville, Virginia, during 2008. Heat summation and average rainfall recorded in this growing season were 1154.4°C and 3 mm. Vines were planted in 1998 on a 20274.76 m^2 dyke silt loam soil type plot with 3.05X2.13m spacing. The grapes were of clone FPS 3 and grated on 3309C rootstock. Treatment consisted of full crop, 1 cluster/shoot and 1&2 clusters/shoot thinned at veraison. Three sampling dates were chosen (once per week) on weeks 12, 13 and 16 post-bloom. Five grape vines were selected using a randomized block design from each of the treatments.

Degrees brix using refractometer (AO Scientific instruments© 10430, New Hampshire, USA), %RH using digital hygrometer (Traceable™ 4187, Texas, USA) and temperatures using infrared thermometer (Extech Instruments© 42529, Massachusetts, USA) were measured within the vine canopy between 08:00 – 11:00 hrs. Five clusters were collected from the neighboring vines or from vines not used for ENose measurements every week for laboratory analysis. At the end growing season, approximately 84 kg of grapes were harvested and were frozen for processing. The components of yield determined include shoots/meter, clusters/vine, cluster weight, berry weight, fruit weight/vine and fruit weight/treatment.

Laboratory Analysis (Berry/Juice/Wine Chemistry)

Twenty-five berries per vine were randomly selected from frozen clusters. Berries were weighed, thawed and crushed in 30.97 cm^3 (15.24X20.32X0.10cm) volume plastic sampling bags (Minigrip®, Texas, USA), which approximately holds 200 grape berries. Juice was filtered through 0.45µm syringe filters (Whatman®, New Jersey, USA). The skins and the remaining juice were frozen for total glycosyl-glucose (TGG) and phenol-free glycosyl glucose (PFGG) analysis. Berry weight, Brix, pH and titratable acidity (TA) were determined as described by Zoecklein *et al.* (1999). Color intensity (A420 +A520), hue (A420/A520) and total phenols (A280) were determined using a Genesys™ 5 spectrophotometer (Spectronic, Leeds, UK). The TGG and PFGG analysis were performed according to methods described by Williams *et al.* (1995), as modified by Zoecklein *et al.* (2000). For wines, analyses of malic acid, fermentable

nitrogen, alcohol content (v/v), residual sugar and volatile acidity were also conducted. L-malic acid was determined enzymatically (R-Biopharm AG, Darmstadt, Germany). Fermentable nitrogen was also determined enzymatically (Megazyme©, Bray, Ireland), alcohol content by FTIR (Foss WineScan™ FT 120, Minnesota, USA) and residual sugar concentration estimated by Clinitest (Bayer, Indiana, USA).

Wine samples for GC-MS analysis were prepared using 4 mL sample with NaCl salt (1.0 g) in 10 mL clear glass vials sealed with a septa (MicroLiter® Analytical Supplies, Inc., Georgia, USA). Vials had a pre-incubation time of 30 seconds at 30°C with agitation at 250 rpm. A CAR/DVB/PDMS Grey SPME Fiber (Supelco Sigma-Adrich, St. Louis, Missouri, USA) was used to penetrate vials to a 32 mm depth. A GC-MS (Agilent Technologies, 6890N Network GC System, 5975B inert MSD) with injector temperature of 250°C, DB-Wax column (30x25x25), and helium carrier gas with a flow rate of 1 mL/min were used. Oven temperature was 40°C with a ramp rate of 6°C per minute to 230°C. Thirty-two standard compounds from each wine sample were manually integrated and quantified.

Processing and Fermentation

Harvested grapes (26.7kg) from each treatment, frozen at -20°C were thawed for at least 24 hours prior to processing. Clusters were crushed and destemmed using a Wottle (Anton, Poysdorf, Austria) destemmer crusher to about 50% berry breakage, estimated visually. Berries were distributed into six open-top 60LNalgene fermenting bins of equal height and volume. Each bin was treated with 250mg/L Velcorin® (Scott Laboratories, California, USA) dimethyl-dicarbonate (DMDC) to sterilize juice from internal yeast or bacteria. Bins were held for 24 hours at 7ºC cooler followed by additional 25 mg/L of potassium metabisulfite (KMBS) addition. Grapes were cold soaked for 5 days at 7ºC during which must and grapes were punched daily.

Must juice analysis was performed on the 2nd day of cold soak and °Brix, pH, titratable acidity (TA), and fermentable nitrogen adjustments made; pH and TA adjustments using tartaric acid, °Brix using sucrose, fermentable nitrogen using FermAid®K (Lallemand, Blagnac Cedex, France) and diammonium phosphate (DAP).

Go-Ferm® (Lallemand, Blagnac Cedex, France) yeast nutrients were prepared according to manufacturer directions and added during the yeast rehydration. *Saccharomyces cerevisiae* ICV-D254 (Lallemand, Blagnac Cedex, France) yeast of 20g/L was inoculated following the cold soak treatment. After inoculation, caps were punched 3 times daily. Fermentation was monitored by hydrometer and proceeded at approximately 23±2ºC until dryness (<1% residual sugar). Following fermentation, wines were pressed using a basket press. Free run and press run fractions were separated into sanitized, carbon-dioxide filled carboys. Wine was kept at 7ºC for 24 hours, racked and filled in 3.80Land 1.90L glass bottles.

Electronic Nose Analysis

Two electronic nose systems, a conducting polymer-based, Cyranose 320™ (Smiths Detection©, Pasadena, CA, USA; both in field and laboratory) and a surface acoustic wave-based, ZNose 7300 ™ (Electronic Sensor Technology, Newbury Park, CA, USA) were used to determine the volatile differences among grapes and wine.

The CP ENose settings optimization for grapes was that of Athamneh *et al.* (2008) (Table 1A). For field analysis, one cluster was chosen at random from each of five selected vines. Clusters were bagged with a HDPE bag and received 45 minutes equilibrium time and same clusters were used for the ENose measurements on all sampling dates. In-field analysis of grape clusters using Cyranose is illustrated in Figure 1. Additional 5 clusters from neighboring vines were collected and frozen until laboratory analysis.

Wine evaluation optimization method for CP ENose is given in Table 1B (Gardner 2009). Wine was analyzed twice, once immediately post-fermentation and again 6 months post-fermentation. Five replicates of 20mL wine samples were placed in 40mL GC clear glass vials sealed with Teflon/Silicone 3 mm septa top (MicroLiter® Analytical supplies, Inc., Suwanee, Georgia, USA). The samples were analyzed subsequently by Cyranose after placing it in a water bath at 30°C for 20 minutes.

For the SAW ENose, the default settings of DB-5 system were used, except for the sensor temperature (Table 2). The system was tuned with C6-C14 alkane standards each day. The same sampling technique for the CP ENose wine analysis was applied for both juice and wine.

Sensory Analysis

A triangle difference test was conducted 6 months post-fermentation on wine aroma as described by Meilgaard *et al.* (2007). The following treatments were compared in this study: Full crop vs. 1 Cluster/shoot, 1 Cluster/shoot vs. 1&2 Cluster/shoot and Full crop vs. 1&2 Cluster/shoot. Wines were pre-screened for sulfur-like off odors and the panelists were given 3 samples and asked to identify the odd sample. All wines were identified with a randomized 3-digit code. Standard ISO glasses were filled with 10mL of wine and covered with a plastic Petri dish. The wines of all treatments were presented to the panelists on the same day at approximately 19°C under a red light. A total of 32 panelists in the age range of 21-27 years were used (α=0.05, β=0.30, ρd=40%, 16 correct responses for significant difference). Male and female panelists in the ratio of 1:1 were chosen with a pre-requisite of wine consumption at least once a week. Analysis was performed by providing written instructions and each panelist smelling 2 sets of samples (Meilgaard *et al.*, 2007).

Data Analysis

The physico-chemistry, CP ENose and SAW ENose data of grapes and wines were analyzed and compared using univariate (one-way analysis of variance (ANOVA), least significant difference (LSD)) and multivariate (canonical discriminant analysis (CDA), principal component analysis (PCA)) statistical methods using SAS JMP Version 7 (SAS Institute, New Jersey, USA). The Znose data was analyzed using the regular gas chromatography (GC) approach as described by Lammertyn *et al.* (2004). For the sensory data, number of correct responses was counted.

Results and Discussion

Harvest components of yield were measured and did not differentiate among treatments (Table 3). According to the univariate statistical results, grape physico-chemistry data of 9 indices (°Brix, weight/berry, pH, titratable acidity (TA), color intensity, hue, total phenols, phenol-free glycosyl glucose (PFGG) and total glycosyl glucose (TGG)), did not differentiate among cluster thinning treatments for most sampling dates using ANOVA (Table 4). The exceptions were color intensity (week 12), total phenols (week 13) and PFGG (week 16).

Pairwise comparison results also provided details on indices showing difference between cluster thinning treatments (week 12, total phenols and color intensity and week 16, pH) (Table 5). Similar to the results obtained by Ough and Nagaoka (1984) on one-third and two-third cluster thinned treatments, we did not find many changes in grape composition for cluster thinned samples. Reynolds and Wardle (1989) suggested differences in cluster thinned vines resulted from differences in the rate of fruit maturity.

Based on the canonical plots and discriminant scores by canonical discriminant analysis (CDA) of 7 physico-chemistry indices (PFGG and TGG not included), the ability to identify treatment was highest at harvest (Figure 2). However, CDA did not classify as many pre-harvest treatments. PCA of physico-chemistry indices explained 100% of the variation in the data using prin1 and prin2 axes (Table 6). However, <75% of the variation was explained in the prin1 axis. The length of the biplot rays illustrated that out of 7 physico-chemistry indices utilized for PCA analysis; only 3 indices (total phenols, titratable acidity and °Brix) were associated with the discrimination of treatments on most sampling dates (Figure 3). Hence the physico-chemistry analysis may not be considered optimal for explaining the difference among these cluster thinning treatments.

The canonical distribution of grape data obtained using CP-based and SAW-based systems are provided in Figure 4 and 5. Both systems identified the treatment of all replicates, on all sampling dates. Additionally, PCA results showed that, both the systems explained 100% variation in data using prin1 and prin2 axes (Table 6). However, considering only the prin1 axis, the CP-based system better explained the difference among treatments most of the time (>80%) except at harvest (68.6%), compared to the SAW-based system <70%. The ability of SAW-based electronic nose in discriminating between the treatments using the linear statistical methods such as CDA, PCA etc., was not comparable to CP ENose, likely due to the non-linear nature of the SAW ENose data (Haugen and Kvaal 1998). In general, the variation percentage of both systems in discriminating the treatments, decreased with increase in maturity. The decline in efficiency of electronic noses could be due to the equalizing of volatiles across treatments, as harvest approached. Hence, the number of sensitive sensors of CP ENose (week 12: 16 (S1-S16), week 13: 5 (S6, S13, S14, S16 and S18), week 16: 2 (S3 and S24)-based on ANOVA), towards the difference in cluster thinning treatments also decreased with time. The length of PCA biplot rays illustrated that most of the sensors ranging were sensitive to volatile differences between cluster

thinning treatments except, S19, S21, S22, S24, S25, S26, S27, S30, S31 and S32 on most of the sampling dates (Figure 6). This sensor information also agrees with the other canopy side study by Devarajan (2009) where sensors S19, S24, S32 were insensitive towards grape volatile differences.

Wine chemistry data of five indices (color intensity, hue, total phenols, PFGG and TGG), demonstrated that color differed across treatments (Table 7). PFGG values showed no distinction among treatments. The other 5 chemistry indices shown in Figure 7 indicated little differences across treatments. CP-based system was able to indicate treatment differences (based on CDA (100%) and PCA (100% (prin1>98%)) immediately post-fermentation as well as six months post-fermentation (Figure 8). The biplot rays showed that sensors S22 and S31 were not sensitive to volatile differences due to cluster thinning (Figure 9). SAW-based system, explained 100% of the variation using CDA (Figure 10). PCA showed 56.9% of the variation from prin1, almost 30% lower than the CP-based system results.

GC/MS identified 25 compounds that differed among treatment wines (Table 8). The volatile concentration of more than half of the compounds was higher in the unthinned wine followed by 1 cluster/shoot and 1&2 clusters/shoot. CDA of GC/MS data correctly classified 100% of the samples (Figure 11) and PCA explained 96.9% of the variation (prin1 (75.1%) and prin2 (21.8%)). According to the length of PCA biplot rays methionol, diethyl succinate and gamma-nonalactone were the only compounds identified to be not associated with the thinning treatment differences out of the 32 compounds identified (Figure 12).This volatile concentration variations detected by GC/MS supports the differences detected by Enoses based on volatiles.

The aroma sensory evaluation results demonstrated that wines of 1 cluster/shoot and 1&2 clusters/shoot treatments were significantly different (Table 9). Since an untrained panel was used in order to represent a consumer group and since sensory evaluation involves variation across judges and over time (Meilgaard *et al.* 2007), lack of difference in treatments detected may not indicate that volatiles were similar. Although differences detected by ENoses resembles sensory evaluation in most cases (Mallikarjunan 2005), additional differences detected by CP-based and SAW-based systems might be possibly due to the sampling of both aroma and non-aroma volatiles and the ability to assess volatiles in a complex matrix such as wine.

Conclusions

This study evaluated the use of conducting polymer-based and surface acoustic wave-based electronic nose systems in differentiating cluster thinning treatments. The results from ENoses were compared with the physico-chemistry, GC/MS and sensory analyses. It was found that physico-chemistry was not able to differentiate among treatments adequately irrespective of the differences detected from several chemistry indices and volatile compounds from GC/MS. Sensory evaluation differentiated only between 1 cluster/shoot and 1&2 clusters/shoot. Both ENoses were able to differentiate treatments adequately both for grapes and wine using CDA. However, from PCA results it was found that CP-based system performed better than SAW-based system. To improve the percentage difference detected by SAW-based system in future, more research is needed to optimize the method settings for grapes and wine. The information about the number of specific CP ENose sensors could be used as reference when used for grape/wine specific ENose development and/or by the wineries in future. Also, non-linear data analysis methods, which have been found from earlier studies to provide better interpretation of ENose data than multivariate statistics (CDA, PCA) can be evaluated for processing the data from SAW-based system.

A) Grape method		
Parameter/Setting	**Value**	**Level**
Baseline purge	20 sec	Medium
Sample draw 1	30 sec	Medium
Sample draw 2	0 sec	Medium
Snout removal	0 sec	Low
1st sample gas purge	0 sec	High
1st air intake purge	10 sec	High
2nd sample gas purge	60 sec	high
2nd air intake purge	0 sec	High
Digital filtering	On	
Substrate heater	On	42
Training repeat count	1	
Identifying repeat count	1	
Algorithm	Canonical	
Preprocessing	Auto-scaling	
Normalization	Normalization 1	
Identification quality	Medium	

B) Wine method		
Parameter/Setting	**Value**	**Level**
Baseline purge	30 sec	Medium
Sample draw 1	20 sec	Medium
Sample draw 2	0 sec	Medium
Snout removal	0 sec	Low
1st sample gas purge	0 sec	High
1st air intake purge	10 sec	High
2nd sample gas purge	60 sec	High
2nd air intake purge	0 sec	High
Digital filtering	On	
Substrate heater	On	40°C
Training repeat count	1	
Identifying repeat count	1	
Algorithm	Canonical	
Preprocessing	Auto-scaling	
Normalization	Normalization 1	
Identification quality	Medium	

Table 3.1: Conducting polymer-based electronic nose analysis method settings used for (A) Grape (Athamneh *et al.* 2008) and (B) Wine (Gardner 2009).

Category	Temperature (°C)
Sensor	45
Db-5 column	40
Valve	165
Inlet	200
Trap	250
Maximum column	225

Table 3.2: Surface acoustic wave-based electronic nose method settings - wine and juice analysis in 2008.

Components	Shoots/meter	Clusters/vine	Fruit weight (kg)	Cluster weight (kg)	Berry weight (g)
Full crop	34.20±1.58a	36.20±2.76a	5.50±0.57a	0.15±0.01a	1.97±0.04a
1 cluster/shoot	36.40±1.58a	30.00±2.76a	5.83±0.57a	0.19±0.01a	1.95±0.04a
1&2 cluster/shoot	35.00±1.58a	32.40±2.76a	5.46±0.57a	0.17±0.01a	1.93±0.04a
p - Value	0.62	0.31	0.88	0.15	0.77

Table 3.3: Differences detected between three cluster thinning treatments using harvest components of yield of Merlot grapes. Columns with different letters and p-value <0.05 indicates 95% significant difference between treatments.

Physico-chemistry	week 12	week 13	week 16
Brix	0.41	0.20	0.96
Berry weight (g)	0.77	0.80	0.77
pH	0.67	0.21	0.12
Titratable acidity (g/L)	0.15	0.89	0.14
Color intensity (A420+A520)	0.03	0.38	0.05
Hue (A420/A520)	0.16	0.83	0.42
Total phenols (A280)	0.06	0.03	0.12
PFGG (µm)	N/A	N/A	0.04
TGG (µm)	N/A	N/A	0.31

N/A – No data point available

Table 3.4: p-value indicating the significance of cluster thinning treatment differences detected by physico-chemistry analyses on three sampling dates (week 12, week 13 and week 16 post-bloom) using ANOVA, for Merlot juice. Highlighted boxes indicate significant difference ($p < 0.05$).

Treatment	Full crop	1 Cluster/Shoot	1&2 Cluster/Shoot
Brix	22.3±0.32a	22.6±0.66a	23.3±0.31a
	22.6±0.32a	22.4±0.66a	23.4±0.31a
	22.0±0.32a	24±0.66a	23.3±0.31a
Berry weight (g)	1.66±0.10a	1.77±0.10a	1.97±0.05a
	1.76±0.10a	1.68±0.10a	1.95±0.05a
	1.71±0.10a	1.77±0.10a	1.92±0.05a
pH	3.52±0.06a	3.48±0.04a	3.55±0.03b
	3.56±0.06a	3.55±0.04a	3.59±0.03ab
	3.61±0.06a	3.6±0.04a	3.64±0.03a
Titratable acidity (g/L)	3.28±0.17a	3.45±0.10a	1.89±0.04a
	3.73±0.17a	3.48±0.10a	1.88±0.04a
	3.28±0.17a	3.42±0.10a	1.77±0.04a
Color intensity (A520+A420)	0.408±0.03a	0.354±0.04a	0.329±0.01b
	0.361±0.03ab	0.385±0.04a	0.377±0.01a
	0.268±0.03b	0.428±0.04a	0.329±0.01b
Hue (A420/A520)	0.519±0.03a	0.546±0.02a	0.551±0.02a
	0.533±0.03a	0.539±0.02a	0.538±0.02a
	0.613±0.03a	0.559±0.02a	0.584±0.02a
Total phenols (A280)	1.324±0.12a	1.22±0.07b	1.354±0.04a
	1.192±0.12ab	1.268±0.07b	1.478±0.04a
	0.869±0.12b	1.497±0.07a	1.371±0.04a
PFGG (µm)	N/A	N/A	317.97±10.87b
	N/A	N/A	363.97±10.87a
	N/A	N/A	345.29±10.87ab
TGG (µm)	N/A	N/A	2691.87±169.21a
	N/A	N/A	2426.25±169.21a
	N/A	N/A	2810.62±169.21a

N/A – No data point available

Table 3.5: Pair wise comparison of cluster thinning treatments using least significant difference test for Merlot juice physico-chemistry data on three sampling dates (week 12, week 13, week 16post-bloom). Different cells in each column marked by different letters indicate 95% significant difference between them.

Sampling date	Physico-chemistry	CP ENose	SAW ENose	Principal component
Week 12	58.1	90.5	66.3	PC1
	41.9	9.5	33.7	PC2
Week 13	72.2	86.5	65.3	PC1
	27.8	13.5	34.7	PC2
Week 16	63.8	68.6	51.6	PC1
	36.2	31.4	48.4	PC2

>80 >60 >40 >20

Table 3.6: Principal component values of Merlot juice, showing the difference between cluster thinning treatments detected by physicochemical analyses (chemistry), conducting polymer-based electronic nose (CP ENose) and surface acoustic wave-based electronic nose (SAW ENose). Values indicate the % of variation explained by each principal component. Key for color codes provided.

Treatment	Color intensity (A420+A520)	Hue (A420/A520)	Total phenols (A280)	PFGG (µm)	TGG (µm)
Full crop	1.41±0.01a	0.42±0.01b	3.71±0.01a	75.6±4.72b	1713±68.6b
1 cluster/shoot	1.3±0.01b	0.43±0.01b	3.64±0.01b	90.0±4.72ab	1963±68.6a
1&2 cluster/shoot	1.29 ±0.01b	0.45±0.01a	3.66±0.01b	92.9±4.72a	1828±68.6ab

Table 3.7: Pairwise comparison data of Merlot wine chemistry indices. Different cells in each column marked by different letters indicate 95% significant difference between them.

Compound	Concentration (µg/L)			p-Value
	Full Crop	1 Cluster/shoot	1&2 Cluster/shoot	
1-Octanol	29.35±0.77a	24.52±0.77b	21.27±0.77c	0.00
1-Propanol	40.40±0.52a	39.14±0.52a	35.42±0.52b	0.00
2-Ethyl-1-hexanol	5.94±0.3a	5.98±0.3a	4.3±0.3b	0.01
2-Methyl propanol	24.75±0.63a	21.04±0.63b	16.66±0.63c	0.00
3-Methyl butanol	98.90±3.26a	85.83±3.26b	70±3.26c	0.00
Benzaldehyde	17.76±0.09a	17.01±0.09b	16.81±0.09b	0.00
Benzyl alcohol	152.83±3.58b	176.51±3.58a	168.15±3.58a	0.01
Beta-Damascenone	5.31±0.21b	6.39±0.21a	7.04±0.21a	0.00
Citronellol	9.88±0.79a	3.81±0.79b	3.47±0.79b	0.00
Diethyl succinate	244.75±5.14b	264.11±5.14a	248.51±5.14ab	0.08
Ethyl acetate	31.27±0.83a	28.88±0.83a	25±0.83b	0.00
Ethyl decanoate	119.25±4.07a	121.6±4.07a	130.62±4.07a	0.19
Ethyl dodecanoate	45.36±0.96c	54.89±0.96b	60.81±0.96a	0.00
Ethyl heptanoate	8.98±0.43a	7.79±0.43ab	6.68±0.43b	0.03
Ethyl hexanoate	353.75±17.02a	342.71±17.02ab	287.47±17.02b	0.07
Ethyl myristate	43.40±0.35a	41.84±0.35b	41.25±0.35b	0.01
Ethyl nonanoate	25.54±0.18a	24.8±0.18b	24.72±0.18b	0.03
Ethyl octanoate	182.28±6.32a	168.08±6.32a	163.96±6.32a	0.18
Ethyl palmitate	119.72±2.81a	104.78±2.81b	99.69±2.81b	0.01
gamma-Nonalactone	0.06±0.002a	0.06±0.002a	0.06±0.002a	0.42
Hexyl acetate	5.78±0.28c	10.25±0.28a	7.03±0.28b	0.00
Isoamyl acetate	4963.28±241.93ab	5571.85±241.93a	4201.17±241.93b	0.02
Isoamyl octanoate	38.40±0.18b	38.71±0.18b	39.36±0.18a	0.03
Isovaleric acid	2.26±0.04a	2.05±0.04b	1.865±0.04c	0.00
n-Hexanol	1.03±0.04a	1.13±0.04a	0.84±0.04b	0.01
Methionol	1.81±0.08ab	2.05±0.08a	1.75±0.08b	0.09
n-Butanol	9.33±0.02a	9.30±0.02a	9.14±0.02b	0.00
Nonanol	10.95±0.3a	8.34±0.3b	7.82±0.3b	0.00
Octanoic acid	1.38±0.15b	2.08±0.15a	2.28±0.15a	0.01
Phenethyl acetate	118.22±3.66b	147.30±3.66a	146.42±3.66a	0.00
Phenethyl alcohol	25±0.66a	24±0.66a	23.51±0.66a	0.34
Terpinene-4-ol	2.16±0.03a	2.11±0.03a	1.42±0.03b	0.00

Table 3.8: Results of pair wise comparisons and ANOVA for volatile compounds analyzed by SPME GC-MS and reported concentrations for full crop, 1 cluster/shoot and 1&2 clusters/shoot Merlot wines. Different cells in each row marked by different letters, and highlighted p-values in the last column indicate 95% significant difference between them.

Treatment	Correct responses	Total responses	Significant difference between treatments
Full crop vs.1 cluster/shoot	14	32	No
1 cluster/shoot vs. 1&2 clusters/shoot	18	32	Yes
1&2 clusters/shoot vs. full crop	15	32	No

Table 3.9: Merlot wine aroma (sample temperature 19°C) triangle difference sensory results (n=32) of cluster thinned and unthinned treatments for the year 2008 ($\alpha = 0.05$, $\beta = 0.10$, $\rho_{max} = 40\%$, 16 or more correct responses corresponds to a significant difference).

Figure 3.1: In-field analysis of different cluster thinning treatments, of Merlot grape clusters using conducting polymer-based electronic nose (photograph taken by Yamuna Devarajan, September 2008)

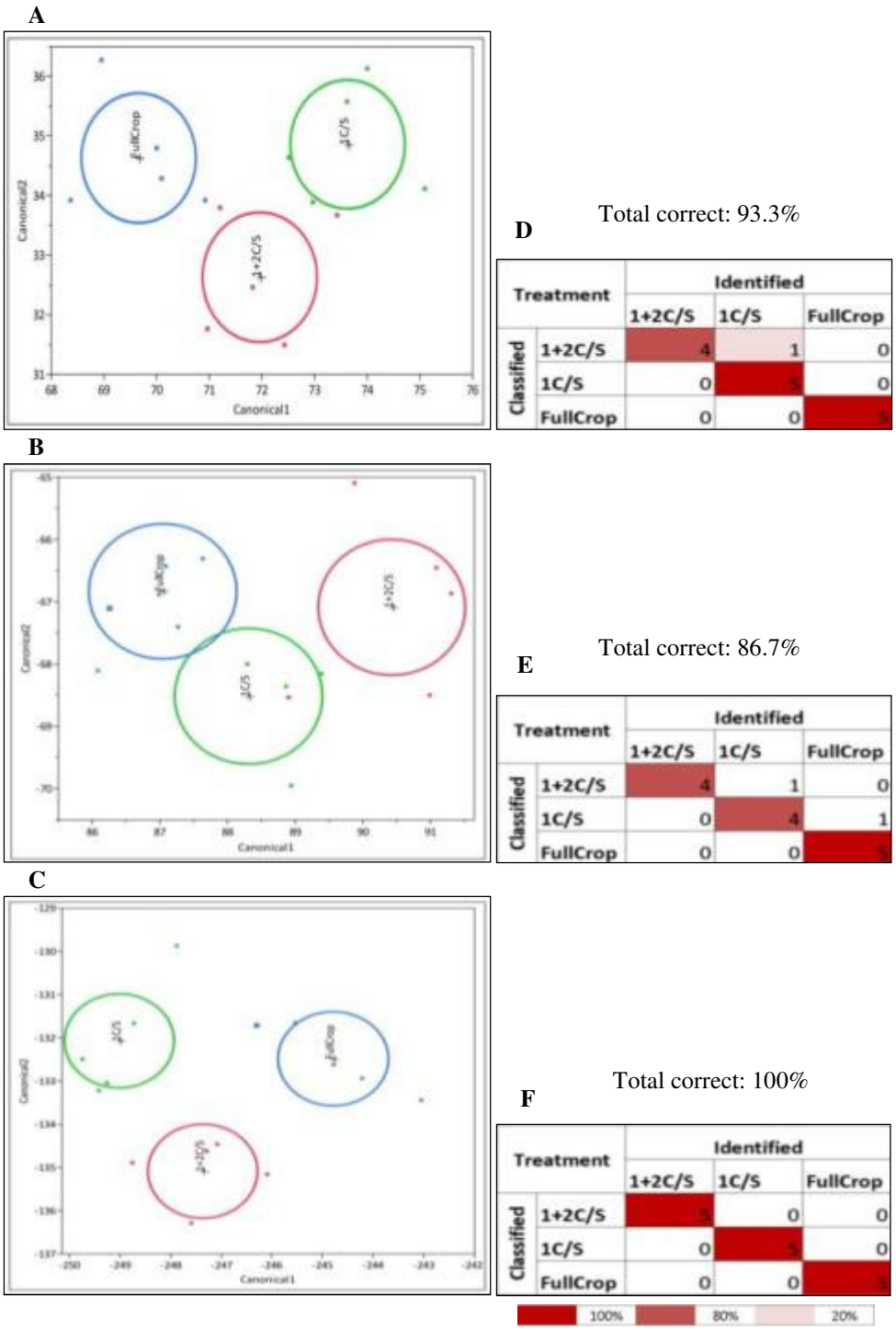


Figure 3.2: Canonical plots of cluster thinning treatments for Merlot grape juice, detected by physico-chemistry analysis (A) week 12, (B) week 13, (C) week 16 post-bloom and discriminant scores (D) week 12, (E) week 13, (F) 16 weeks post-bloom (non-intersecting circles indicate significant difference (α=0.05). Key for color code of discriminant scores provided).

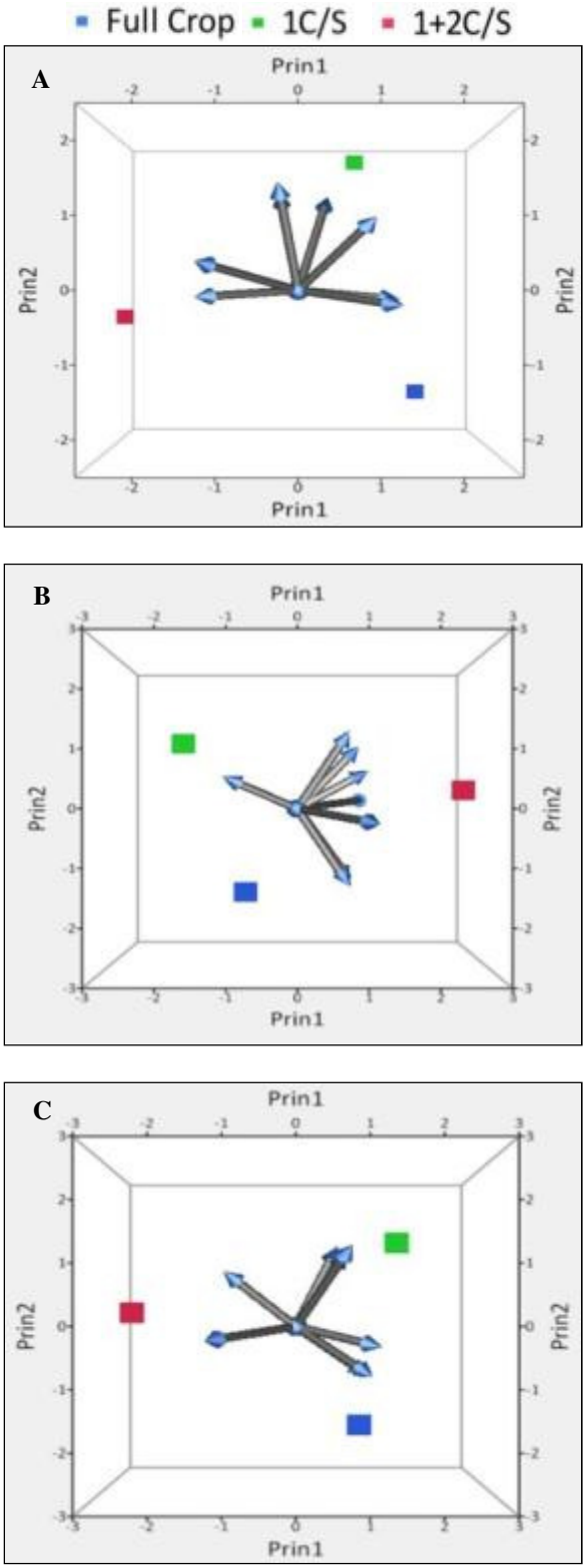
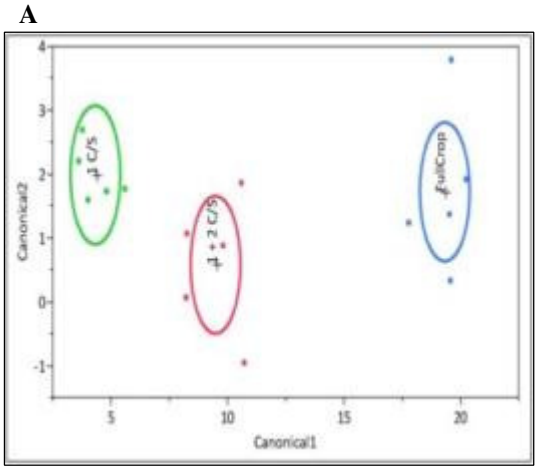
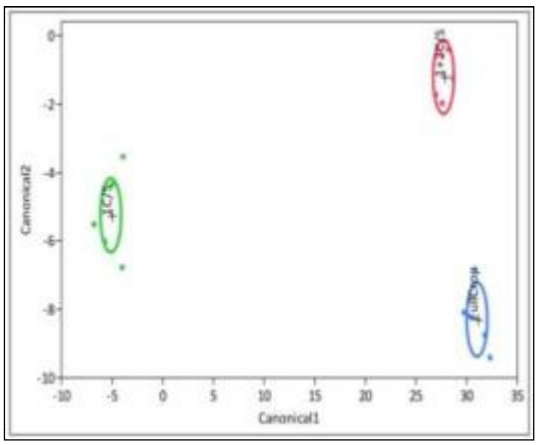


Figure 3.3: Principal component analysis of different cluster thinning treatments for Merlot grape juice at different sampling dates, detected by physico-chemistry analysis (A) week 12, (B) week 13 and (C) week 16 post-bloom (different colors indicate different cluster thinning treatments, key for color code provided).



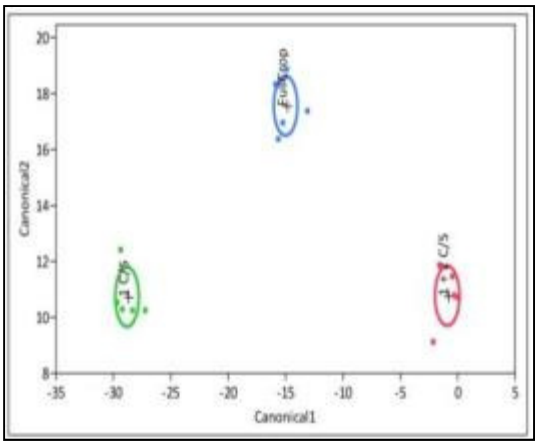
D Total: Correct 100%

Treatment		Identified		
		1+2C/S	1C/S	FullCrop
Classified	1+2C/S	5	0	0
	1C/S	0	5	0
	FullCrop	0	0	5



E Total: Correct 100%

Treatment		Identified		
		1+2C/S	1C/S	FullCrop
Classified	1+2C/S	5	0	0
	1C/S	0	5	0
	FullCrop	0	0	5



F Total: Correct 100%

Treatment		Identified		
		1+2C/S	1C/S	FullCrop
Classified	1+2C/S	5	0	0
	1C/S	0	5	0
	FullCrop	0	0	5

Figure 3.4: Canonical plots of different cluster thinning treatments for Merlot grape berries, detected by conducting polymer-based electronic nose (A) 12 weeks post-bloom, (B) 13 weeks post-bloom, (C) 16 weeks post-bloom and discriminant scores (D) 12 weeks post-bloom, (E) 13 weeks post-bloom, (F) 16 weeks post-bloom (non-intersecting circles indicate significant difference (α=0.05). Key for color code of discriminant scores provided in Figure 2).

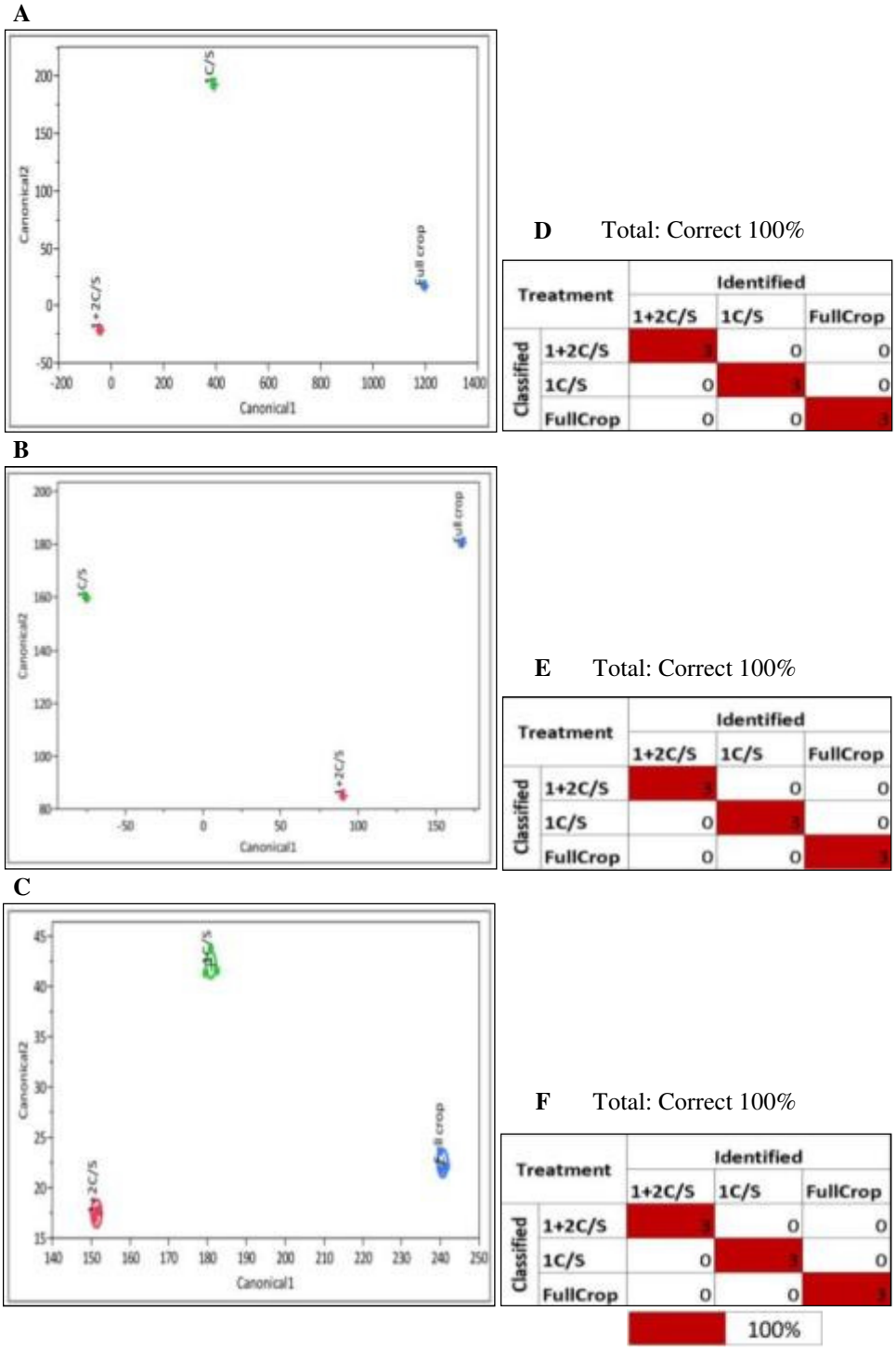


Figure 3.5: Canonical plots of different cluster thinning treatments for Merlot juice, detected by surface acoustic wave-based electronic nose (A) 12 weeks post-bloom, (B) 13 weeks post-bloom, (C) 16 weeks post-bloom and discriminant scores (D) 12 weeks post-bloom, (E) 13 weeks post-bloom, (F) 16 weeks post-bloom (non-intersecting circles indicate significant difference (α=0.05). Key for color code of discriminant scores provided).

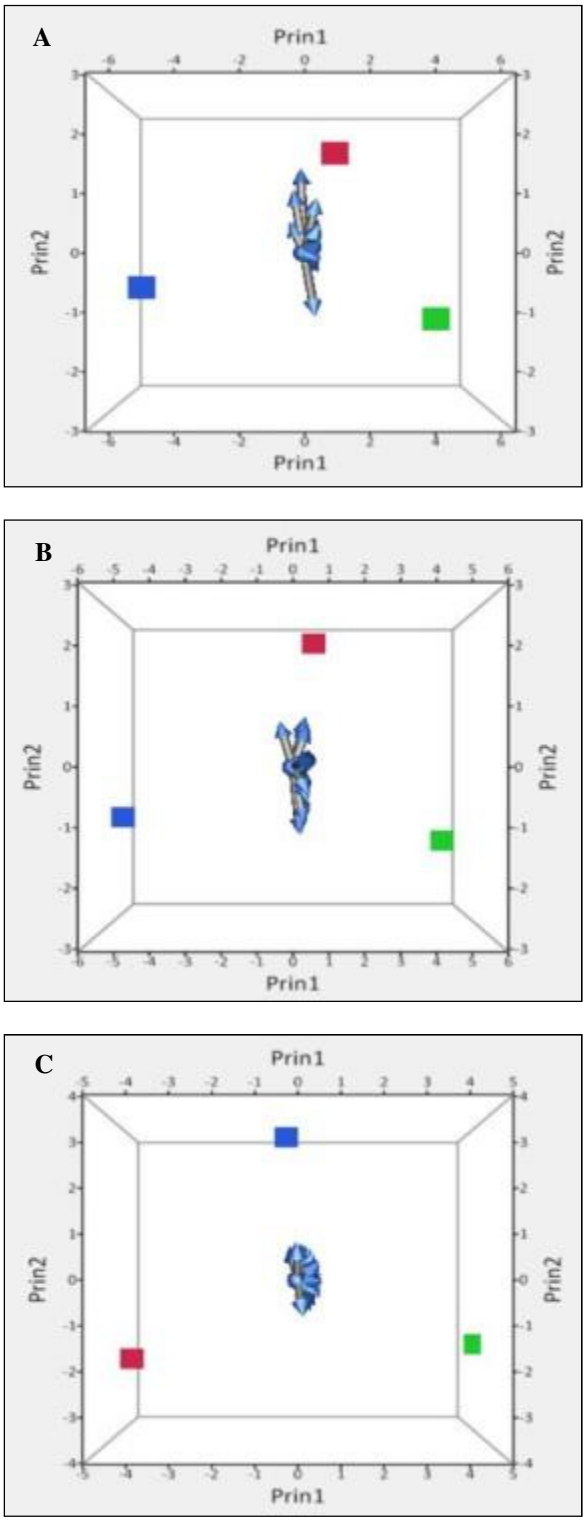


Figure 3.6: Principal component analysis of different cluster thinning treatments for Merlot grape berry, detected by conducting polymer-based electronic nose (A) week 12, (B) week 13 and (C) week 16 post-bloom (different colors indicate different cluster thinning treatments. Key for color code provided in Figure 3).

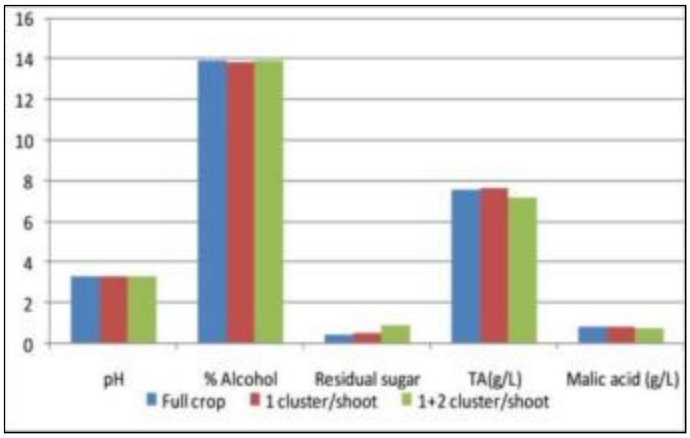


Figure 3.7: Comparison of Merlot wine from different cluster thinning treatments using 5 chemistry indices (key for color code provided).

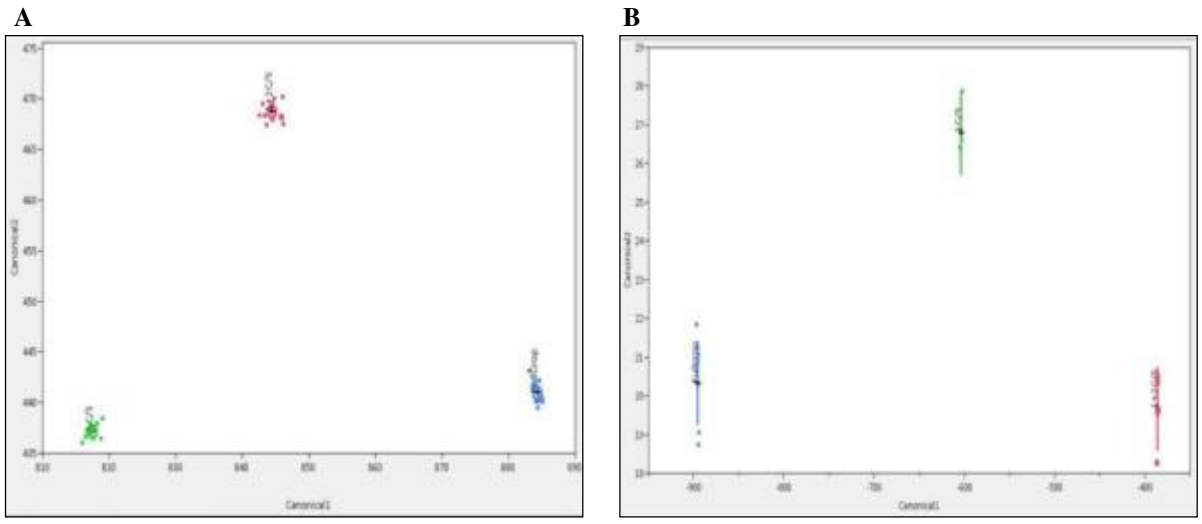


Figure 3.8: Canonical distribution of different cluster thinning treatments for Merlot wine detected by conducting polymer-based electronic nose (A) immediately post-fermentation, (B) six months post-fermentation (non-intersecting circles indicate significant difference (α=0.05)).

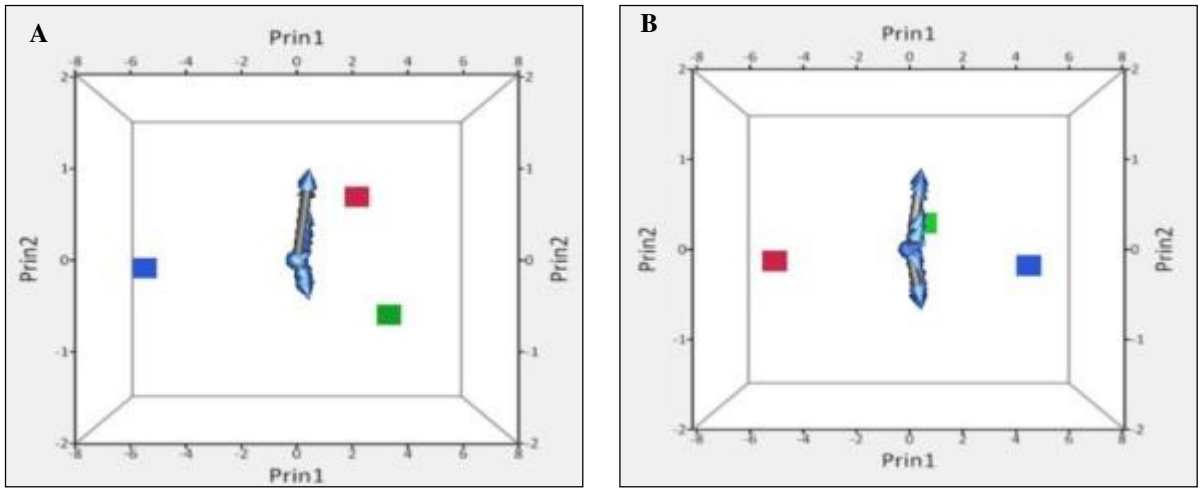


Figure 3.9: Principal component analysis of different cluster thinning treatments from Merlot wine detected by conducting polymer-based electronic nose (A) immediately post-fermentation, (B) six months post-fermentation (different colors indicate different cluster thinning treatments, key for color code provided in Figure 3).

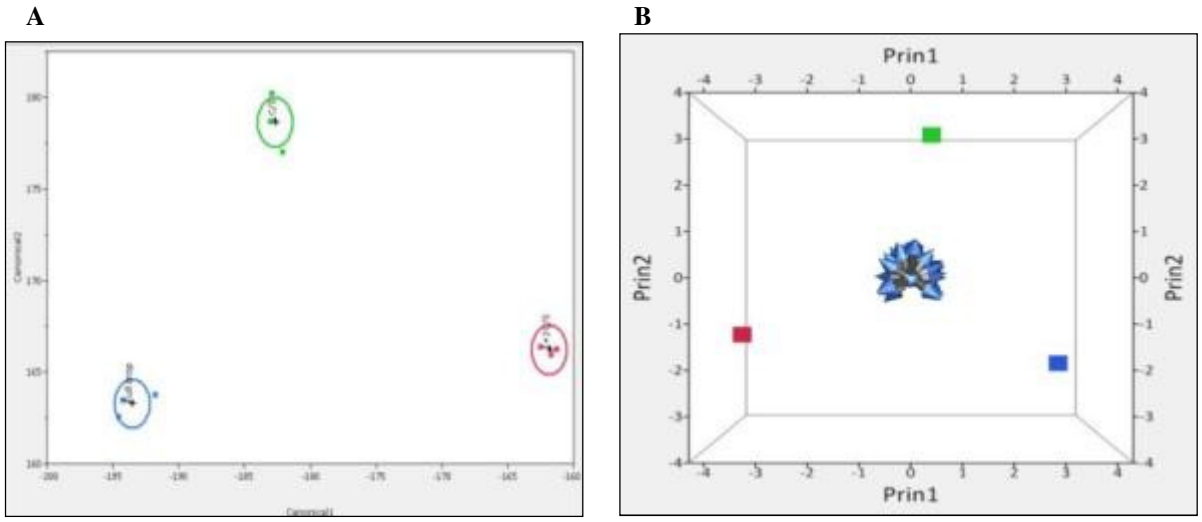
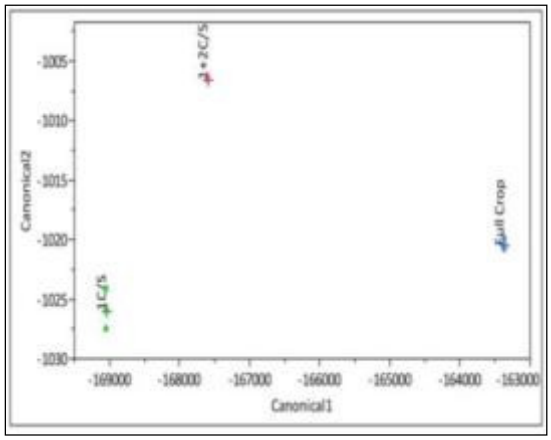


Figure 3.10: Merlot wine (A) canonical distribution and (B) principal component analysis (PCA) of surface acoustic wave-based electronic nose data (different colors indicate different cluster thinning treatments, key for color code of PCA provided in figure 3).

A



Total: Correct 100%

B

Treatment		Identified		
		1+2C/S	1C/S	FullCrop
Classified	1+2C/S	34	0	0
	1C/S	0	34	0
	FullCrop	0	0	34

Figure 3.11: Canonical distribution of Merlot wine for GC/MS data (A) canonical plot and (B) discriminant scores (non-intersecting circles indicate significant difference, key for color code provided in Figure 5).

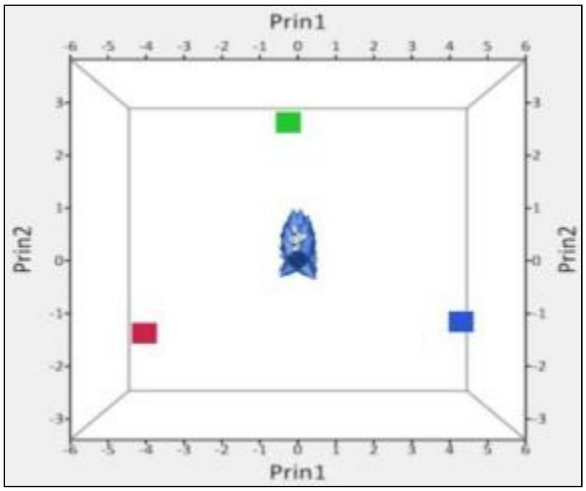


Figure 3.12: Principal component analysis of Merlot wine for GC/MS data (key for color code provided in Figure 3).

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Chapter IV: Effects of Ethanol Spray on Cabernet franc and Merlot Grape and Wine Volatiles using Electronic Nose Systems

Abstract

This study evaluated two electronic nose systems (conducting polymer-based and a surface acoustic wave-based) to differentiate grape and wine volatiles affected by ethanol spray treatment (5% v/v) at veraison on Cabernet franc and Merlot grapes. Electronic nose results were compared with physico-chemistry and wine aroma sensory data. Canonical discriminant and principal component analysis found that both the electronic noses and physico-chemistry measures (Brix, TA, pH, color intensity, hue, total phenols and berry weight) were able to show 100% discrimination between ethanol-treated and untreated grapes and wines for both cultivars. Grape physico-chemistry treatment differences were mainly due to variation in color hue, phenolic-free glycosides and total phenols. While differences were detected using the both physico-chemistry and chemosensory methods, the aroma sensory evaluation of wine was not able to differentiate between ethanol treatments and control for Cabernet franc. With only one measurement electronic noses demonstrated greater potential to discriminate between ethanol-treated and untreated grapes and wines.

Introduction

Ethylene is a plant hormone and its production at the onset of ripening is required to attain desired maturity for most fruits (Barry and Giovannoni 2007). Grapes are non-climacteric fruits (Coombe and Hale 1973). The level of endogenous ethylene in grapes declines (Shulman *et al.* 1985) from bloom to ripening (Weaver and Singh 1978).

Investigations have shown that ethanol (EtOH) applied to grapes can retard or hasten the ripening process, depending on the concentration and timing (Beaulieu and Saltveit 1997). Ethephon (2-chloroethyl-phosphonic acid), formally a commercially available growth-regulating hormone, delayed maturation when applied before veraison (Hale *et al.* 1970). Spraying ethanol between 8-13 weeks post-bloom was found to enhance fruit and wine anthocyanin concentration, and decrease acidity (Chervin and El-kereamy 2001, El-Kereamy *et al.* 2002). Martin *et al.* (2008) found no significant difference in wine taste and aroma characteristics from ethanol-treated grapes.

Electronic nose (ENose) is an emerging technology that has been used in a variety of food applications. These system consists of an array of chemistry sensors, providing various levels of sensitivity and selectivity (Mallikarjunan 2005). The major types of ENoses, classified based on the type of sensor are, conducting polymer (CP), metal oxide semiconductor (MOS), quartz micro balance (QMB) and surface acoustic wave systems (SAW).

The conducting polymer units consist of an array of polymer sensors which on exposure to chemistry vapor expand, causing a change in resistance. Each sensors are made of different conductive material such as polypyrole, polythiophene, polyaniline, polyacetylene and poyindole, with a range of properties to allow discrimination among different types of vapors where cross-selectivity sometimes does occur (Mallikarjunan 2005, Pinheiro et al. 2002). Responses of sensors are stored as 'smell prints' in the memory database and compared with the present readings during identification (Cyrano Sciences^{TM} Inc. 2000). Surface acoustic wave systems use a single uncoated sensor to analyze the sample volatiles quantitatively. Sample are adsorbed in a trap; the trap is heated to vaporize the sample; and the vapor is passed through a column where, the volatile is identified and quantified (Electronic Sensor Technology^{TM} 2001). The objective of this study was to use a conducting polymer-based and surface acoustic wave based electronic nose systems to evaluate the effect of ethanol spray on grape and wine volatiles.

Materials and Methods

Field Design

The study was performed using two grape varieties; Cabernet franc and Merlot, grown on a Ballerina training system in Charlottesville, Virginia during 2008. Heat summation of 1154.4°C and average rainfall of 3 mm were recorded in this growing season. Clone 4 Cabernet franc grapes were planted in 2004 on a 4046.86 m^2 plot spaced 2.13X3.05m apart, on 101-14 rootstock. Clone FPS 3 Merlot vines were planted in 1998 on a 20274.76 m^2 plot with 3.05X2.13m spacing, grated on 3309C rootstock.

Ethanol (EtOH) water treatment (5% v/v) at veraison was carried out on 12 vines in the East/West oriented plot for Cabernet franc and 9 vines in the one cluster/shoot plot for Merlot. Aqueous ethanol was sprayed directly on clusters to saturation using a back-pack sprayer. Three sampling dates were chosen (once per week) on weeks 14, 15 and 17 for Cabernet franc and week 12, 13 and 16 post-bloom for Merlot.

Degrees Brix using refractometer (AO Scientific instruments© 10430, New Hampshire, USA), %Relative humidity using digital hygrometer (Traceable™ 4187, Texas, USA), and temperatures using an infrared thermometer (Extech instruments© 42529, Massachusetts, USA) were measured within the vine canopy of control and ethanol-treated vines between 08:00 – 11:00 hrs. Five clusters were collected from the neighboring vines or from vines not used for ENose measurements every week for laboratory analysis. At the end of growing season, approximately 56.7kg of Cabernet franc and 71.7kg Merlot grapes were harvested. The components of yield determined included shoots/meter, clusters/vine, cluster weight, berry weight, fruit weight/vine and fruit weight/treatment.

Lab Analysis (Berry/Juice/Wine Chemistry)

Twenty-five berries per vine were randomly selected from frozen clusters. Berries were weighed, thawed and crushed in a 30.97 cm^3 (15.24X20.32X0.10cm) volume plastic sampling bags (Minigrip®, Texas, USA), which approximately holds 200 grape berries. Juice was filtered through 0.45µm syringe filters (Whatman®, New Jersey, USA). The skins and juice were frozen for total glycosyl-glucose (TGG) and phenol-free glycosyl glucose (PFGG) analysis. Berry weight, Brix, pH and titratable acidity (TA) were determined as described by Zoecklein *et al.* (1999). Color intensity (A420 +A520), hue (A420/A520) and total phenols (A280) were

determined using a Genesys™ 5 spectrophotometer (Spectronic, Leeds, UK). The TGG and PFGG analysis were performed according to methods described by Williams *et al.* (1995), as modified by Zoecklein *et al.*(2000). For wines, analyses of malic acid, yeast assimilable nitrogen (YAN), alcohol content (v/v), residual sugar and volatile acidity were also conducted. L-malic acid was determined enzymatically (R-Biopharm AG, Darmstadt, Germany). Fermentable nitrogen was also determined enzymatically (Megazyme©, Bray, Ireland), alcohol content by FTIR (Foss WineScan™ FT 120, Minnesota, USA) and residual sugar concentration estimated by Clinitest (Bayer, Indiana, USA).

Processing and Fermentation

Cabernet franc (11.5kg) and Merlot (27kg) grapes, frozen at –20°C were thawed for 24 hours prior to processing. Clusters were crushed and destemmed using a Wottle (Anton, Poysdorf, Austria) destemmer-crusher to about 50% berry breakage, estimated visually. Berries were distributed into four open-top 60L Nalgene fermenting bins of equal height and volume for each variety. Each bin was treated with 250mg/L Velcorin® (Scott Laboratories, California, USA) dimethyl-dicarbonate (DMDC) followed by addition of 25mg/L of potassium metabisulfite (KMBS). Grapes were cold soaked for 6 days at 7ºC during which must caps were punched daily.

Must juice analysis was performed on the 4th day of cold soak for Cabernet franc and 2nd day for Merlot. Brix, pH, titratable acidity (TA), and yeast assimilable nitrogen (YAN) adjustments were made; pH and TA adjustments using tartaric acid, Brix using sucrose, fermentable nitrogen using FermAid®K (Lallemand, Blagnac Cedex, France) and diammonium phosphate (DAP).

Go-Ferm® (Lallemand, Blagnac Cedex, France) yeast nutrient were prepared according to manufacturer recommendations and added during rehydration. *Saccharomyces cerevisiae* ICV-D254 (Lallemand, Blagnac Cedex, France) yeast (20g/L) was inoculated following cold soak. Caps were punched 3 times daily. Fermentation was monitored by hydrometer and carried out at approximately 23±2ºC until dryness (<1% residual sugar).Following fermentation, wines were pressed using a basket press. Free-run and press-run fractions were separated into carboys. Wine was stored at 7ºC for 24 hrs, racked and filled in 3.80L and 1.9L glass bottles.

Electronic Nose Analysis

Two electronic nose systems, a conducting polymer-based (CP), Cyranose 320™ (Smiths Detection©, Pasadena, CA, USA) and a surface acoustic wave-based (SAW), ZNose 7300™ (Electronic Sensor Technology, Newbury Park, CA, USA) were used.

The CP ENose method optimization for grapes used was that reported by Athamneh *et al.* (2008) (Table 1A).For field analysis, 5 ethanol sprayed vines were randomly selected and used for measurements. Two clusters for Cabernet franc (1 for each canopy side) and 1 cluster for Merlot were chosen at random from each of selected vine. Clusters were bagged with a HDPE bag and received 45 minutes equilibrium time. Same clusters were used for the ENose measurements on all sampling dates.

The wine evaluation method optimization for the CP ENose was used from an earlier study for Cabernet Sauvignon is provided in Table 1B (Gardner 2009). Wine was analyzed twice, once immediately post-fermentation and again 6 months post-fermentation. Five replicates of 20mL wine samples were placed in 40mL GC clear glass vials sealed with Teflon/Silicone 3 mm septa top (MicroLiter® Analytical supplies, Inc., Suwanee, Georgia, USA). The samples were analyzed after placing in a water bath at 30°C for 20 minutes.

For the SAW ENose, the default settings of DB-5 system were used, except for the sensor temperature (Table 2). The system was tuned with C6-C14 alkane standards each day. The same sampling technique for the CP ENose wine analysis was applied for both juice and wine.

Sensory Analysis

A triangle difference test was conducted 6 months post-fermentation on wine aroma as described by Meilgaard *et al.* (2007). The control and ethanol treatments of Cabernet franc and Merlot varietals were compared. Wines were pre-screened for sulfur-like off odors. All wines were identified with a randomized 3-digit code. Standard ISO glasses were filled with 10 mL of wine and covered with a plastic petridish. The wines were presented to the panelists at approximately 19°C under a red light. A total of 32 panelists in the age range of 21-27 years were used (α=0.05, β=0.30, ρd=40%, 16 correct responses for significant difference). Male and female panelists in the ratio of 1:1 were chosen with a pre-requisite of wine consumption at least once a week. Analysis was performed by providing written instructions and each panelist smelling 2 sets of samples (Meilgaard *et al.*, 2007).

Data Analysis

The physico-chemistry, CP ENose and SAW ENose data of grapes and wines were analyzed and compared using univariate (one-way Analysis of Variance (ANOVA), least significant difference (LSD) and multivariate (canonical discriminant analysis (CDA), principal component analysis (PCA)) statistical methods using SAS JMP Version 7 (SAS Institute, New Jersey, USA). The SAW ENose data was analyzed using the regular GC chromatographic approach as described by Lammertyn *et al.* (2004). For the sensory data, number of correct responses was counted.

Results and Discussion

Harvest components of yield measured did not show any differences between the control and ethanol treatment for both grape varieties (Table 3). This result was similar to findings by Chervin and El-kereamy (2001), where differences in berry weight was not observed as a result of ethanol treatment. Differences between treatments as detected by each of the physico-chemistry indices for grapes of both varieties are provided in Table 4 (ANOVA) and Table 5 (pair-wise comparison), with significance highlighted. Among all indices, color hue was the only Cabernet franc grape index that showed difference between ethanol-treated and control samples on 5 out of 6 sampling dates. The color intensity values of ethanol-treated Cabernet franc were higher than that of the controls at harvest. This agrees with the results of previous studies (El-Kereamy *et al.* 2002). Phenolic-free glycosides, in part, aroma and flavor precursors, illustrated treatment differences for both Merlot and Cabernet franc grapes at harvest.

Canonical discriminant analysis (CDA) and principal component analysis (PCA) of the 7 physico-chemistry indices (available for single sampling date), explained 100% of variation on all sampling dates, for both varieties (Figure 1 and 2). The canonical discriminant score that validates the grouping of the classes also indicated that all replicates of a treatment were identified correctly (data not shown). Based on the PCA biplot rays for Merlot, hue and titratable acidity were the parameters most associated with treatment differences (Figure 2) and hue, Brix, color intensity and total phenols were the ones correlated with differences between control and ethanol treatment (Figure 3) for Cabernet franc.

Both conducting polymer (CP) and surface acoustic wave (SAW) based systems were able to explain 100% variation with one qualitative/quantitative measurement (Figure 4, 5, 6 and 7). From the CP-based system results of grapes and wine, S31 was the only sensor (identified by PCA biplot rays) that did not explain the difference between control and ethanol treatment in both Cabernet franc and Merlot (Figure 7, 8, 9 and 10). Wine canonical plots for both CP-based and SAW-based systems demonstrated 100% difference between treatments similar to the grape volatile results (Figure 11, 12, 13 and 14). For the additional parameters provided in Table 6, all indices except total glycosides indicated significant difference in Cabernet franc. In addition to total glycosides, color intensity and phenolic-free glycosides did not differentiate between the treatments in Merlot wine.

The GC/MS results using ANOVA and pairwise-comparison indicated differences in volatile concentrations between ethanol treatment and control for both grape varietals (Table 7 and 8). This supports the ENose results where differences were detected across treatments based on volatiles. Also using CDA, GC/MS was able to 100% discriminate the ethanol treatment from control (Figure 15). Using PCA 96.9 % variation was explained using prin1 for Merlot compared to only 60.7% for Cabernet franc. The PCA biplot rays indicated that out of 32 compounds detected from wines of both grapes octanoic acid for Merlot and hexyl acetate, 1-octanol, n-hexanol and nonanol for Cabernet franc were the compounds mainly associated with the treatment differences.

Differences in volatile concentration detected from GC/MS for Merlot were also reflected in aroma sensory evaluations. Panelists were able to differentiate the ethanol-treated and control wines. However, panelists were not able to correctly differentiate Cabernet franc wines (Table 9). The volatile concentration variation detected by GC/MS was not as great in Cabernet franc compared to Merlot. Additionally, the ethanol concentration (Williams and Rosser 1981) may also have altered the panelists perception of volatile compounds, since the alcohol content appeared to be slightly higher for ethanol-treated Cabernet franc wines (Table 10). The fact that ENose takes both aroma and non-aroma volatiles into consideration while evaluating (Haugen and Kvaal 1998) could have provided it the ability to show discrimination that is otherwise not detected by sensory evaluations.

Conclusions

Changes in grape and wine volatiles caused by ethanol treatment on both Cabernet franc and Merlot varieties were evaluated using conducting polymer-based and surface acoustic wave-based electronic nose systems. Both the ENoses were able to differentiate ethanol-treated grapes and wine from controls. Among the traditional methods used in this study (physico-chemistry and sensory), aroma sensory evaluation differentiated between the treatments for Merlot only. Individual physico-chemistry indices did not show differences between treatments for both varieties. However, when 7 indices (except TGG and PFGG) measured for physico-chemistry was analyzed together (multivariate), ethanol treatment was 100% distinguished from the control. Although, multivariate statistical results indicate some usefulness of grape physico-chemistry composition, the general inconsistency in detecting differences and the amount of time required to make these measurements may suggest the inadequacy of these methods. Electronic nose analysis is a rapid and non-destructive technology that may supplement or replace the existing methods for evaluating selected vineyard management practices.

A) Grape method		
Parameter/Setting	**Value**	**Level**
Baseline purge	20 sec	Medium
Sample draw 1	30 sec	Medium
Sample draw 2	0 sec	Medium
Snout removal	0 sec	Low
1st sample gas purge	0 sec	High
1st air intake purge	10 sec	High
2nd sample gas purge	60 sec	high
2nd air intake purge	0 sec	High
Digital filtering	On	
Substrate heater	On	42
Training repeat count	1	
Identifying repeat count	1	
Algorithm	Canonical	
Preprocessing	Auto-scaling	
Normalization	Normalization 1	
Identification quality	Medium	

B) Wine method		
Parameter/Setting	**Value**	**Level**
Baseline purge	30 sec	Medium
Sample draw 1	20 sec	Medium
Sample draw 2	0 sec	Medium
Snout removal	0 sec	Low
1st sample gas purge	0 sec	High
1st air intake purge	10 sec	High
2nd sample gas purge	60 sec	High
2nd air intake purge	0 sec	High
Digital filtering	On	
Substrate heater	On	40°C
Training repeat count	1	
Identifying repeat count	1	
Algorithm	Canonical	
Preprocessing	Auto-scaling	
Normalization	Normalization 1	
Identification quality	Medium	

Table 4.1: Conducting polymer-based electronic nose analysis method settings used for (A) Grape (Athamneh *et al.* 2008) and (B) Wine (Gardner 2009).

Category	Temperature (°C)
Sensor	45
Db-5 column	40
Valve	165
Inlet	200
Trap	250
Maximum column	225

Table 4.2: Surface acoustic wave-based electronic nose method settings - wine and juice analysis in 2008.

Components	Shoots/meter	Clusters/vine	Fruit weight (kg)	Cluster weight (kg)	Berry weight (g)
Merlot					
Control	30.00±2.34a	30.00±2.34a	5.83±0.56a	0.19±0.01a	1.95±0.04a
EtOH	27.22±1.75a	27.22±1.75a	4.71±0.42a	0.17±0.01a	1.90±0.03a
p - Value	0.36	0.36	0.13	0.11	0.38
Cabernet franc					
Control-1	38.00±1.20a	33.80±4.25a	3.12±0.60a	0.15±0.01a	1.77±0.07a
EtOH-1	36.00±1.20a	38.20±4.25a	3.31±0.60a	0.16±0.01a	1.82±0.07a
Control-2	N/A	N/A	2.32±0.60a	N/A	1.63±0.07a
EtOH-2	N/A	N/A	2.62±0.60a	N/A	1.8±0.07a
p - Value	0.27	0.49	0.65	0.81	0.27

N/A - indicates that no data points are available for those samples

Table 4.3: Differences detected between control and Ethanol treatment, from the harvest components of yield of Merlot and Cabernet franc grapes. Columns with different letters and p-value <0.05 indicate 95% significant difference between treatments.

Physico-chemistry indices	Cabernet franc-1			Cabernet franc-2			Merlot		
	Week 14	Week 15	Week 17	Week 14	Week 15	Week 17	Week 12	Week 13	Week 16
Brix	0.79	0.81	0.22	0.97	0.69	0.27	0.72	0.86	0.19
Berry weight (g)	0.24	0.31	0.94	0.71	0.10	0.08	0.71	0.95	0.63
pH	0.96	0.62	0.00	0.82	0.61	0.01	0.80	0.93	0.44
Titratable acidity (g/L)	0.20	0.14	0.12	0.26	0.48	0.41	0.27	0.78	0.70
Color intensity (A420+A520)	0.05	0.15	0.38	0.05	0.15	0.11	0.66	0.79	0.05
Hue (A420/A520)	0.67	0.00	0.03	0.04	0.00	0.01	0.88	0.77	0.28
Total phenols (A280)	0.00	0.08	0.71	0.03	0.04	0.34	0.07	0.21	0.01
PFGG (µm)	N/A	N/A	0.34	N/A	N/A	0.00	N/A	N/A	0.03
TGG (µm)	N/A	N/A	0.09	N/A	N/A	0.85	N/A	N/A	0.94

N/A - indicates that no data points are available for those samples

Table 4.4: p-value indicating the significance of differences between ethanol treatment and control detected by physico-chemistry analyses on three sampling dates using ANOVA, for Cabernet franc (week 14, week 15 and week 17 post-bloom) and Merlot (week 12, week 13 and week 16 post-bloom) juice. Highlighted boxes indicate significant difference ($p < 0.05$).

A

Physico-chemistry indices	Control - 1	EtOH - 1	Control - 2	EtOH - 2
Brix	20.5±0.76a	20.2±0.76a	19.5±0.76a	19.5±0.76a
	20.1±0.63a	20.3±0.63a	19.7±0.48a	19.9±0.48a
	20.4±0.85a	22.0±0.85a	20.8±0.80a	22.1±0.80a
Berry weight (g)	1.70±0.08a	1.85±0.08a	1.57±0.09a	1.62±0.09a
	1.49±0.11a	1.67±0.11a	1.70±0.05a	1.57±0.05a
	1.80±0.07a	1.81±0.07a	1.61±0.08a	1.83±0.08a
pH	3.59±0.08a	3.60±0.08a	3.60±0.07a	3.63±0.07a
	3.64±0.07a	3.59±0.07a	3.67±0.08a	3.74±0.08a
	3.98±0.05a	3.70±0.05b	4.01±0.05a	3.73±0.05b
Titratable acidity (g/L)	4.08±0.23a	3.62±0.23a	4.06±0.20a	3.71±0.20a
	3.62±0.11a	3.88±0.11a	3.23±0.14a	3.38±0.14a
	3.74±0.10a	3.49±0.10a	3.67±0.08a	3.56±0.08a
Color Intensity (A420 + A520)	0.306±0.03a	0.212±0.03a	0.290±0.02a	0.220±0.02b
	0.220±0.02a	0.179±0.02a	0.186±0.01a	0.218±0.01a
	0.269±0.02a	0.301±0.02a	0.278±0.02a	0.336±0.02a
Hue (A420/A520)	1.106±0.15a	1.010±0.15a	0.804±0.06b	1.020±0.06a
	1.756±0.10a	1.118±0.10b	1.819±0.06a	1.225±0.06b
	1.217±0.07a	0.935±0.07b	1.172±0.07a	0.861±0.07b
Total phenols (A280)	1.558±0.09a	1.080±0.09b	1.437±0.09a	1.105±0.09b
	1.203±0.12a	0.878±0.12a	1.044±0.05b	1.217±0.05a
	1.424±0.11a	1.485±0.11a	1.453±0.08a	1.571±0.08a
PFGG (µm)	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A
	500.54±117.52a	326.6±117.52a	197.25±10.50b	300.72±10.50a
TGG (µm)	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A
	2051.88±101.00a	1767.5±101.00a	1542.5±90.28a	1567.5±90.28a

N/A indicates that no points are available

B

Physico-chemistry indices	Control	Ethanol
Brix	22.7±0.32a	22.5±0.32a
	22.6±0.46a	22.7±0.46a
	23.5±0.30a	22.9±0.30a
Berry weight (g)	1.78±0.12a	1.71±0.12a
	1.63±0.11a	1.64±0.11a
	1.93±0.04a	1.96±0.04a
pH	3.54±0.09a	3.57±0.09a
	3.56±0.06a	3.57±0.06a
	3.57±0.05a	3.63±0.05a
Titratable acidity (g/L)	3.82±0.24a	3.41±0.24a
	3.49±0.16a	3.43±0.16a
	1.90±0.06a	1.86±0.06a
Color intensity (A420 + A520)	0.367±0.04a	0.397±0.04a
	0.411±0.05a	0.431±0.05a
	0.389±0.02a	0.331±0.02a
Hue (A420/A520)	0.526±0.05a	0.541±0.05a
	0.540±0.03a	0.556±0.05a
	0.534±0.03a	0.582±0.03a
Total phenols (A280)	1.186±0.04a	1.308±0.04a
	1.341±0.07a	1.478±0.07a
	1.505±0.03a	1.338±0.03b
PFGG (µm)	N/A	N/A
	N/A	N/A
	363.98±12.37a	315.10±12.37b
TGG (µm)	N/A	N/A
	N/A	N/A
	2426.25±144.06a	2410.63±144.06a

N/A indicates that no points are available

Table 4.5: Pair wise comparison of ethanol treatment and control detected by physico-chemistry analyses on three sampling dates, for (A) Cabernet franc (week 14, week 15 and week 17 post-bloom) and (B) Merlot (week 12, week 13 and week 16 post-bloom) juice. Different cells in each column marked by different letters and $p<0.05$/highlighted values indicate 95% significant difference between them.

Variety	Treatment	Color Intensity (A420+A520)	Hue (A420/A520)	Total phenols (A280)	PFGG (µm)	TGG (µm)
Cabernet franc	Control - 1	0.72±0.01b	0.47±0.01b	2.67±0.02b	62.68±3.71a	1388±32.91a
	EtOH - 1	0.78±0.01a	0.50±0.01a	2.87±0.02a	28.18±3.71b	1498±32.91a
	Control - 2	0.52±0.02b	0.56±0.01a	2.45±0.03b	28.18±2.03b	1323±37.75a
	EtOH - 2	0.72±0.02a	0.57±0.01a	3.02±0.03a	65.55±2.03a	1373±37.75a
Merlot	Control	1.31±0.02a	0.43±0.01b	3.65±0.01b	89.99±4.62a	1963±53.77a
	EtOH	1.29±0.02a	0.47±0.01a	3.81±0.01a	78.49±4.62a	1883±53.77a

Table 4.6: Pairwise comparison data of Cabernet franc and Merlot wine chemistry indices. Different cells in each column marked by different letters indicate 95% significant difference between them.

Compound	Concentration (µg/L)		p-Value
	Control	EtOH	
Ethyl acetate	28.88±0.66a	30.28±0.66a	0.21
1-Propanol	39.14±0.80a	38.53±0.80a	0.62
2-Methyl propanol	21.04±0.68a	20.83±0.68a	0.84
Isoamyl acetate	5571.85±179.78a	5157.57±179.78a	0.18
n-Butanol	9.30±0.02a	9.34±0.02a	0.24
3-Methyl butanol	85.83±2.81a	86.62±2.81a	0.85
Ethyl hexanoate	342.71±10.09a	351.06±10.09a	0.59
Hexyl acetate	10.25±0.21a	6.82±0.21b	0.00
Ethyl heptanoate	7.79±0.19a	8.38±0.19a	0.09
n-Hexanol	1.13±0.04a	1.02±0.04a	0.09
Ethyl octanoate	168.08±3.07a	175.48±3.07a	0.16
2-Ethyl-1-hexanol	5.98±0.22b	7.48±0.22a	0.01
Benzaldehyde	17.01±0.08b	18.73±0.08a	0.00
Ethyl nonanoate	24.80±0.07b	25.41±0.07a	0.00
1-Octanol	24.52±0.84a	26.11±0.84a	0.25
Terpinene-4-ol	2.11±0.05b	2.91±0.05a	0.00
Ethyl decanoate	121.60±1.85a	113.14±1.85b	0.03
Isoamyl octanoate	38.71±0.05a	37.46±0.05b	0.00
Nonanol	8.34±0.24b	9.31±0.24a	0.05
Isovaleric acid	2.05±0.05a	2.12±0.05a	0.41
Diethyl succinate	264.11±7.97a	278.10±7.97a	0.28
Methionol	2.05±0.12a	2.11±0.12a	0.78
Citronellol	3.81±1.25a	2.81±1.25a	0.60
Phenethyl acetate	147.30±4.19a	119.46±4.19b	0.01
Beta-Damascenone	6.39±0.20a	6.27±0.20a	0.71
Ethyl dodecanoate	54.89±0.76a	46.28±0.76b	0.00
Benzyl alcohol	176.51±5.27a	175.22±5.27a	0.87
Phenethyl alcohol	24.00±0.88a	25.37±0.88a	0.33
gamma-Nonalactone	0.06±0.00a	0.06±0.00a	0.37
Ethyl myristate	41.84±0.29a	41.22±0.29a	0.20
Octanoic acid	2.08±0.16a	1.06±0.16b	0.01
Ethyl palmitate	104.78±3.56a	111.72±3.56a	0.24

Table 4.7: Results of pair wise comparisons and ANOVA for volatile compounds analyzed by SPME GC-MS and reported concentrations for ethanol treatment and control Merlot wines. Different cells in each row marked by different letters, and highlighted p-values (<0.05) in the last column indicate 95% significant difference between them.

Compound	Concentration (µg/L)				p - Value
	Control -1	EtOH -1	Control -2	EtOH-2	
Ethyl acetate	24.88±0.34bc	25.71±0.34a	24.13±0.34c	29.13±0.34a	0.00
2-Methyl propanol	23.07±0.65bc	25.09±0.65b	22.44±0.65c	27.33±0.65a	0.00
Isoamyl acetate	3004.95±65.3b	3557.83±65.3a	2781.96±65.3c	3393.67±65.3a	0.00
n-Butanol	9.12±0.01c	9.18±0.01b	9.13±0.01c	9.24±0.01a	0.00
3-Methyl butanol	75.26±1.76bc	80.12±1.76b	71.77±1.76c	90.37±1.76a	0.00
Ethyl hexanoate	216.73±4.29b	213.06±4.29b	194.05±4.29c	246.09±4.29a	0.00
Hexyl acetate	4.17±0.07a	3.78±0.07b	1.47±0.07d	2.48±0.07c	0.00
Ethyl heptanoate	5.01±0.10c	9.66±0.10a	5.05±0.10c	5.81±0.10b	0.00
n-Hexanol	1.54±0.04b	1.49±0.04b	1.69±0.04a	1.71±0.04a	0.01
Ethyl octanoate	109.93±2.06c	117.37±2.06b	109.39±2.06c	155.68±2.06a	0.00
2-Ethyl-1-hexanol	6.60±0.23c	9.60±0.23a	8.14±0.23b	9.59±0.23a	0.00
Ethyl nonanoate	24.13±0.04c	24.79±0.04a	24.29±0.04b	24.69±0.04a	0.00
1-Octanol	106.82±3.01d	129.47±3.01c	255.91±3.01a	220.85±3.01b	0.00
Terpinene-4-ol	13.17±0.76d	27.37±0.76c	41.86±0.76b	50.93±0.76a	0.00
Ethyl decanoate	59.04±1.70c	80.74±1.70b	82.00±1.70b	130.36±1.70a	0.00
Isoamyl octanoate	36.21±0.05d	36.80±0.05b	36.57±0.05c	37.46±0.05a	0.00
Nonanol	7.92±0.25b	8.55b±0.25b	12.01±0.25a	8.69±0.25b	0.00
Isovaleric acid	2.23±0.05b	2.27±0.05b	2.05±0.05c	2.51±0.05a	0.00
Diethyl succinate	341.11±11.13b	369.07±11.13b	275.90±11.13c	515.46±11.13a	0.00
Methionol	1.56±0.04a	1.60±0.04a	1.57±0.04a	1.63±0.04a	0.59
Citronellol	10.17±1.16b	10.28±1.16b	19.82±1.16a	22.74±1.16a	0.00
Phenethyl acetate	93.22±2.42ab	98.47±2.42a	88.60±2.42b	89.15±2.42b	0.07
Beta-Damascenone	24.91±0.47b	22.61±0.47c	26.85±0.47a	17.84±0.47d	0.00
Hexanoic acid	1.04±0.05a	1.00±0.05a	0.98±0.05a	1.14±0.05a	0.22
Ethyl dodecanoate	26.42±0.64b	27.87±0.64b	27.09±0.64b	39.78±0.64a	0.00
Benzyl alcohol	137.62±2.87c	149.97±2.87b	150.74±2.87b	166.07±2.87a	0.00
Phenethyl alcohol	21.12±0.63b	21.86±0.63b	20.96±0.63b	26.31±0.63a	0.00
gamma-Nonalactone	0.08±0.00b	0.08±0.00ab	0.09±0.00a	0.09±0.00a	0.01
Ethyl myristate	41.18±0.16a	39.70±0.16b	40.02±0.16b	39.66±0.16b	0.00
Octanoic acid	1.63±0.14a	1.32±0.14a	1.63±0.14a	1.22±0.14a	0.16
Ethyl palmitate	89.01±0.53a	87.12±0.53b	87.61±0.53ab	89.05±0.53a	0.07

Table 4.8: Results of pair wise comparisons and ANOVA for volatile compounds analyzed by SPME GC-MS and reported concentrations for ethanol treatment and control Cabernet franc wines. Different cells in each row marked by different letters, and highlighted p-values in the last column indicate 95% significant difference between them.

Variety	Treatment	Correct responses	Total responses	Significant difference between treatments
cabernet franc	Control - 1 vs. EtOH - 1	9	32	No
	Control - 2 vs. EtOH - 2	14	32	No
Merlot	Control vs. EtOH	19	32	Yes

Table 4.9: Cabernet franc and Merlot wine aroma (sample temperature 19°C) triangle difference sensory results (n=32) of control and ethanol treatments ($\alpha = 0.05$, $\beta = 0.10$, $\rho_{max} = 40\%$, 16 or more correct responses corresponds to a significant difference).

Variety	Treatment	pH	% Alcohol	Residual sugar	TA (g/L)	Malic acid (g/L)
Cabernet franc	Control - 1	3.24	12.9	<1	7.29	0.893
	EtOH - 1	3.31	13.2	<1	7.11	0.964
	Control - 2	3.34	12.6	<1	6.67	0.978
	EtOH - 2	3.34	13.3	<1	7.12	0.95
Merlot	Control	3.27	13.83	0.5	7.65	0.832
	EtOH	3.29	13.79	1.2	7.21	0.775

Table 4.10: Comparison of Cabernet franc and Merlot control and ethanol treatments using wine chemistry values (pH, % Alcohol, residual sugar, TA and Malic acid).

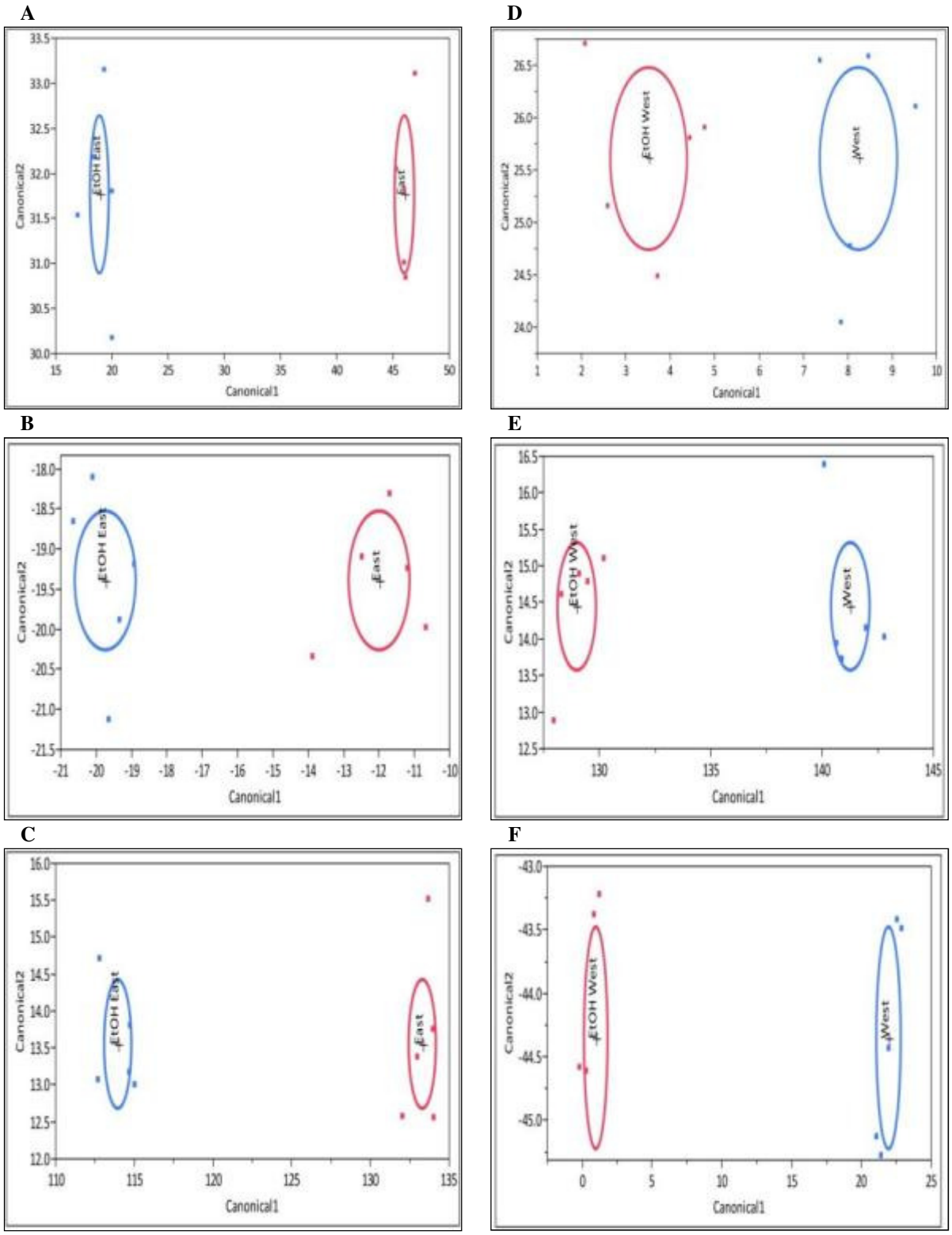

Figure 4.1: Canonical distribution of ethanol treatment differences detected by physico-chemistry analyses for Cabernet franc juice on three sampling dates (A) week 14, (B) week 15 and (C) week 17 post-bloom for ethanol-treated and control – 1 and (D) week 14, (E) week 15 and (F) week 17 post-bloom for ethanol-treated and control – 2 (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

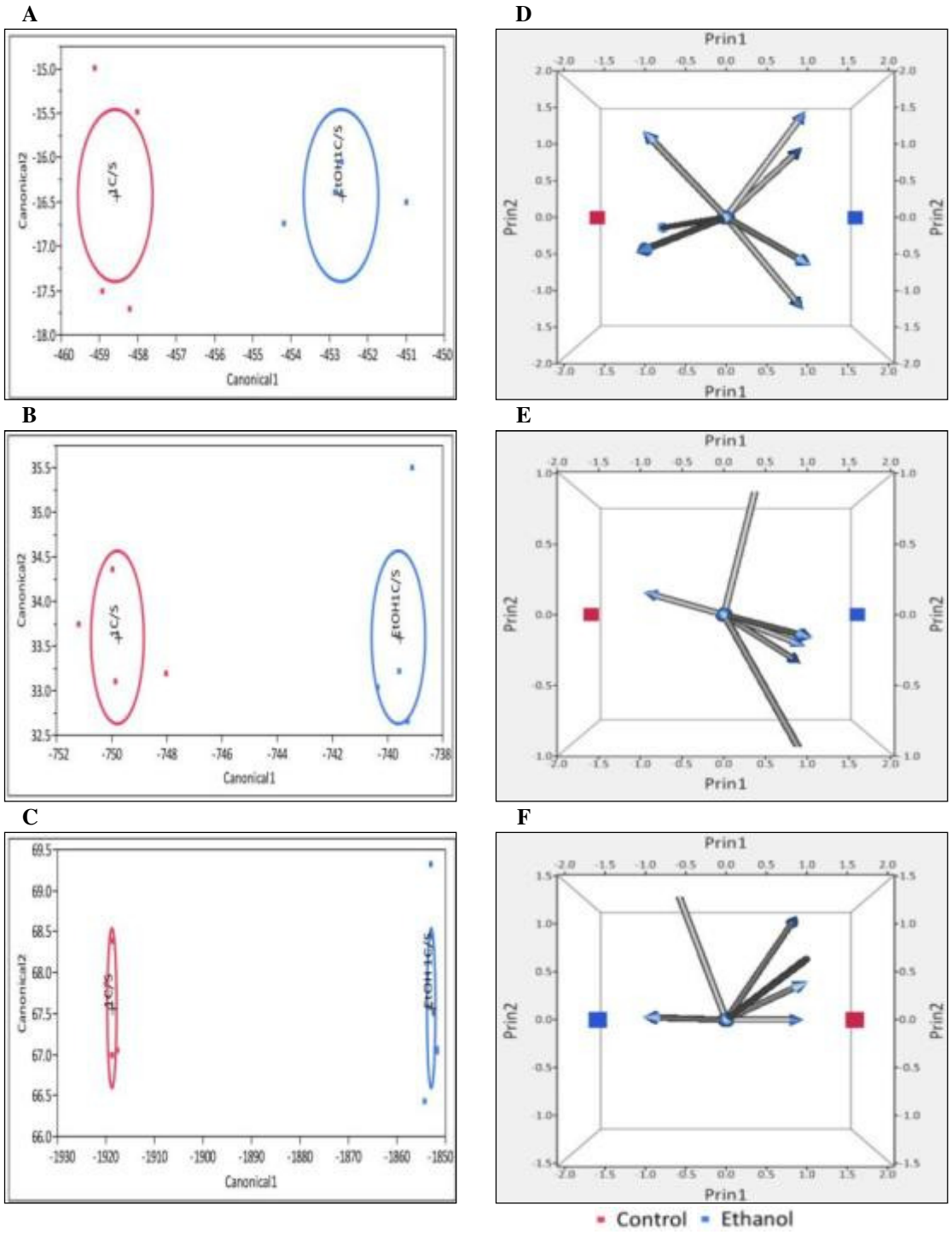

Figure 4.2: Canonical distribution (A) week 12, (B) week 13 and (C) week 16 post-bloom and principal component analysis (D) week 12, (E) week 13 and (F) week 16 post-bloom, of differences between ethanol treatment and control, detected by physico-chemistry analyses for Merlot juice on three sampling dates (For canonical, significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles. Key for PCA color code provided).

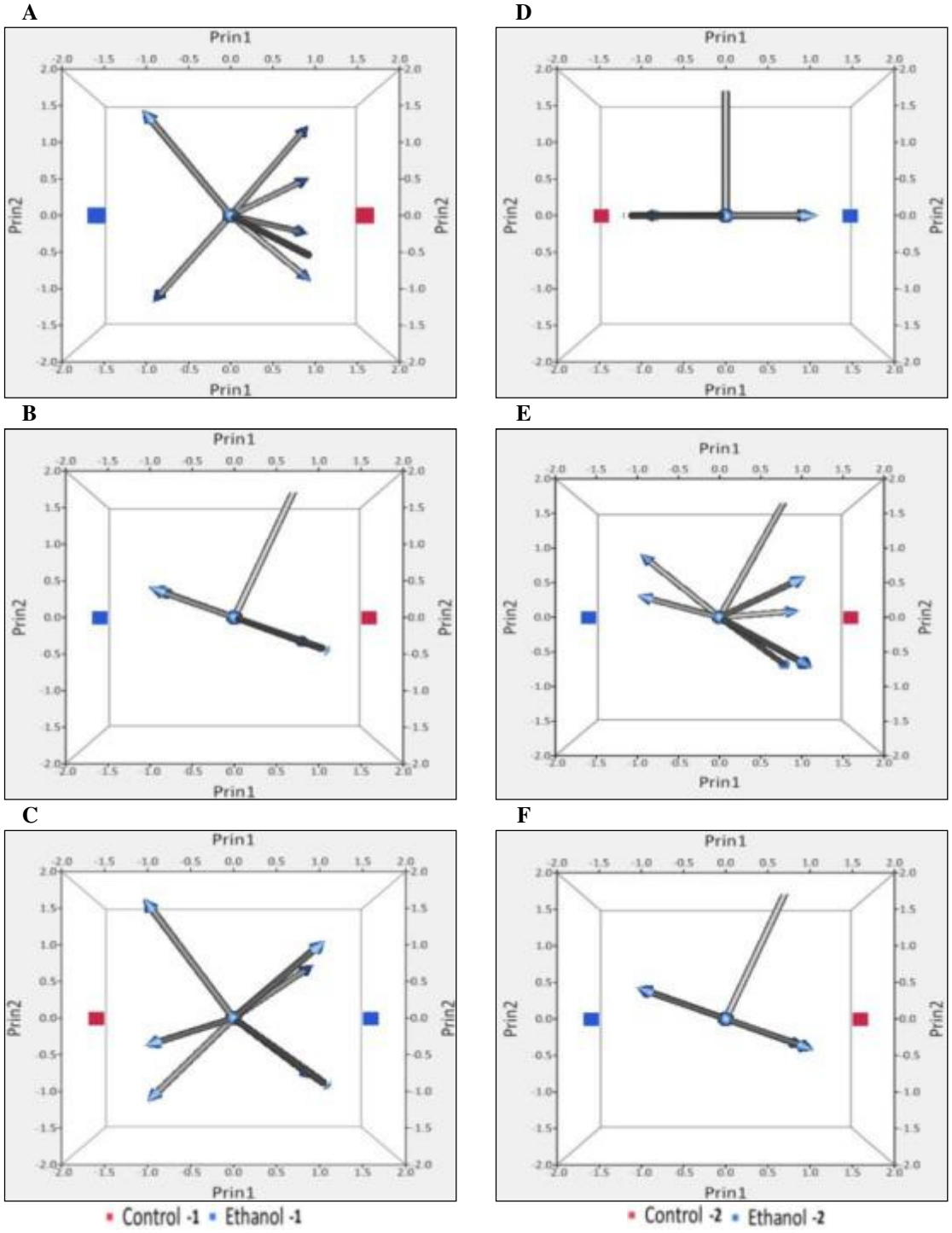

Figure 4.3: Principal component analysis of ethanol treatment differences detected by physico-chemistry analyses for Cabernet franc juice on three sampling dates (A) week 14, (B) week 15 and (C) week 17 post-bloom for ethanol-treated and control – 1 and (D) week 14, (E) week 15 and (F) week 17 post-bloom for ethanol-treated and control – 2 (Key for PCA color code provided).

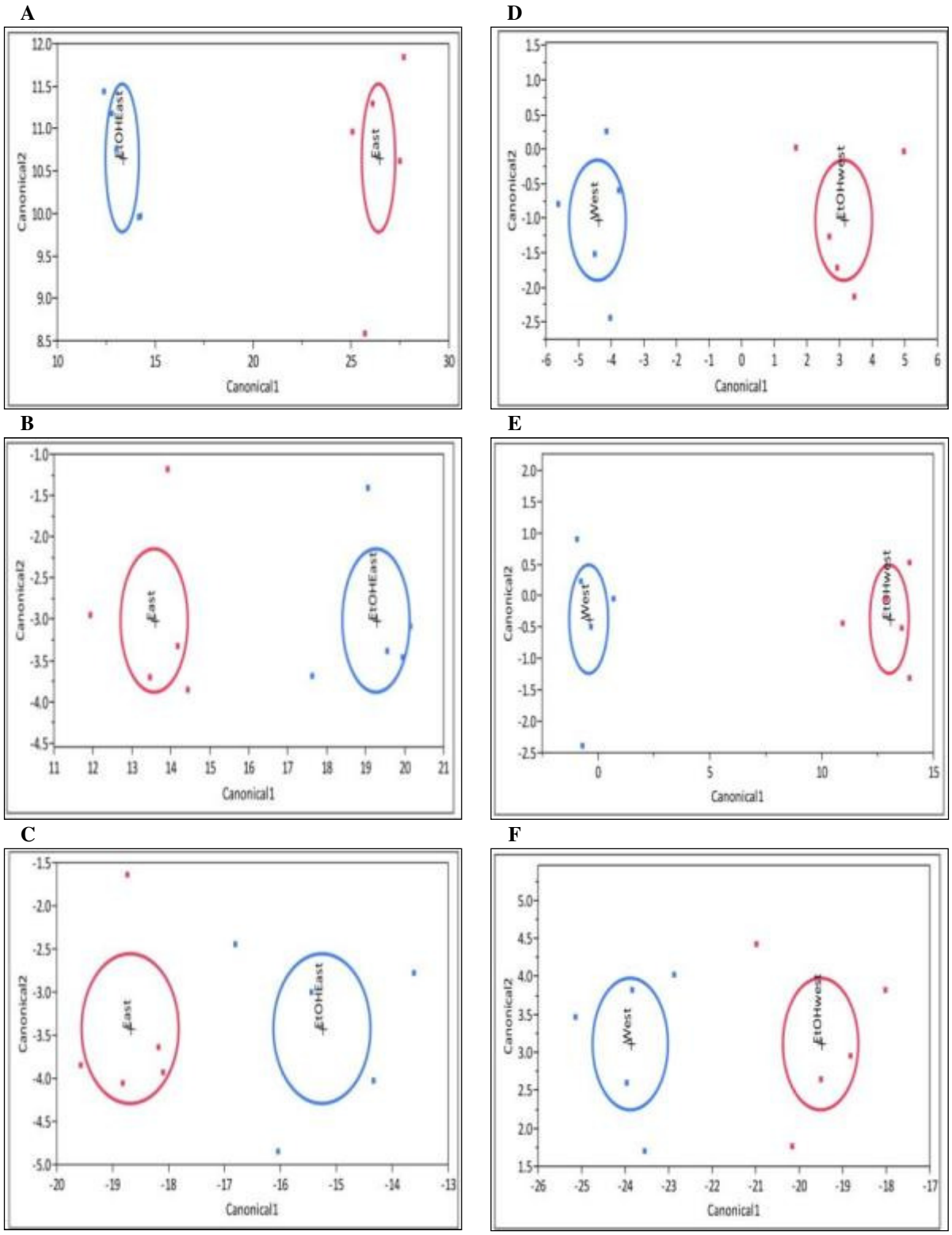

Figure 4.4: Canonical distribution of ethanol treatment differences detected by conducting polymer-based electronic nose for Cabernet franc berry on three sampling dates (A) week 14, (B) week 15 and (C) week 17 post-bloom for ethanol-treated and control – 1 and (D) week 14, (E) week 15 and (F) week 17 post-bloom for ethanol-treated and control – 2 (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

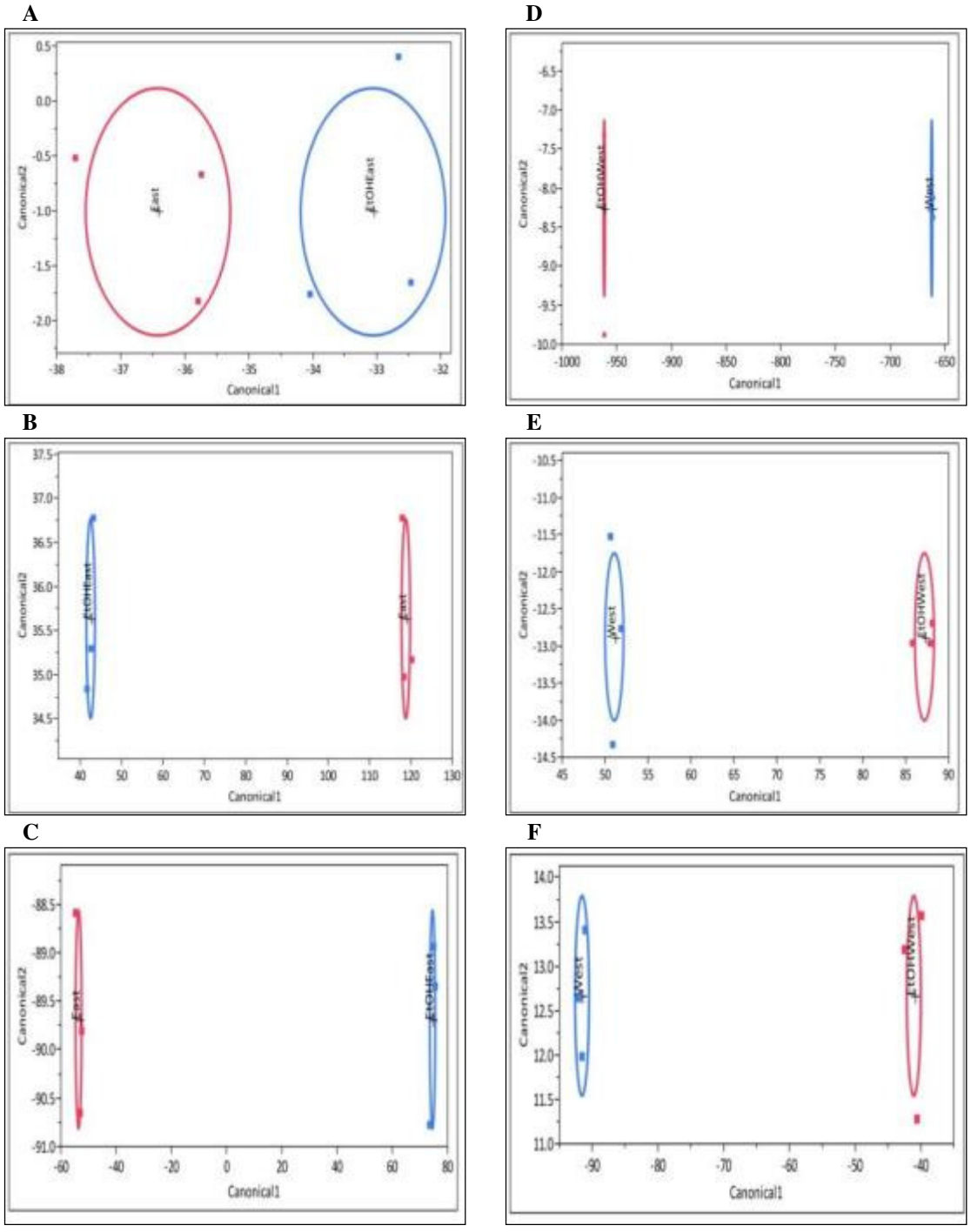

Figure 4.5: Canonical distribution of ethanol treatment differences detected by surface acoustic wave-based electronic nose for Cabernet franc berry on three sampling dates (A) week 14, (B) week 15 and (C) week 17 post-bloom for ethanol-treated and control – 1 and (D) week 14, (E) week 15 and (F) week 17 post-bloom for ethanol-treated and control – 2 (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

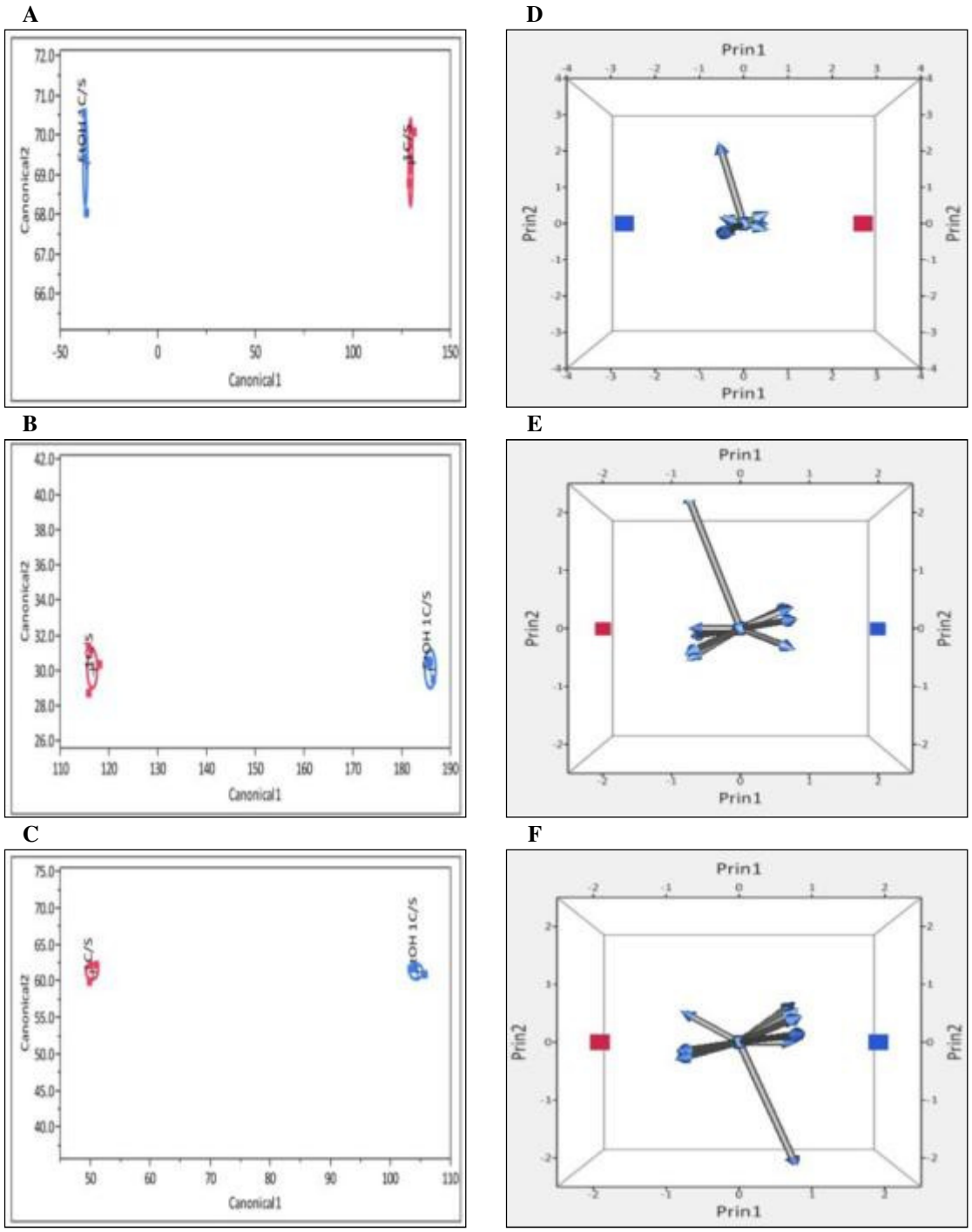

Figure 4.6: Canonical distribution (A) week 12, (B) week 13 and (C) week 16 post-bloom and principal component analysis (D) week 12, (E) week 13 and (F) week 16 post-bloom, of differences between ethanol treatment and control, detected by surface acoustic wave-based electronic nose for Merlot juice on three sampling dates (For canonical, significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles. Key for PCA color code provided in Figure 2).

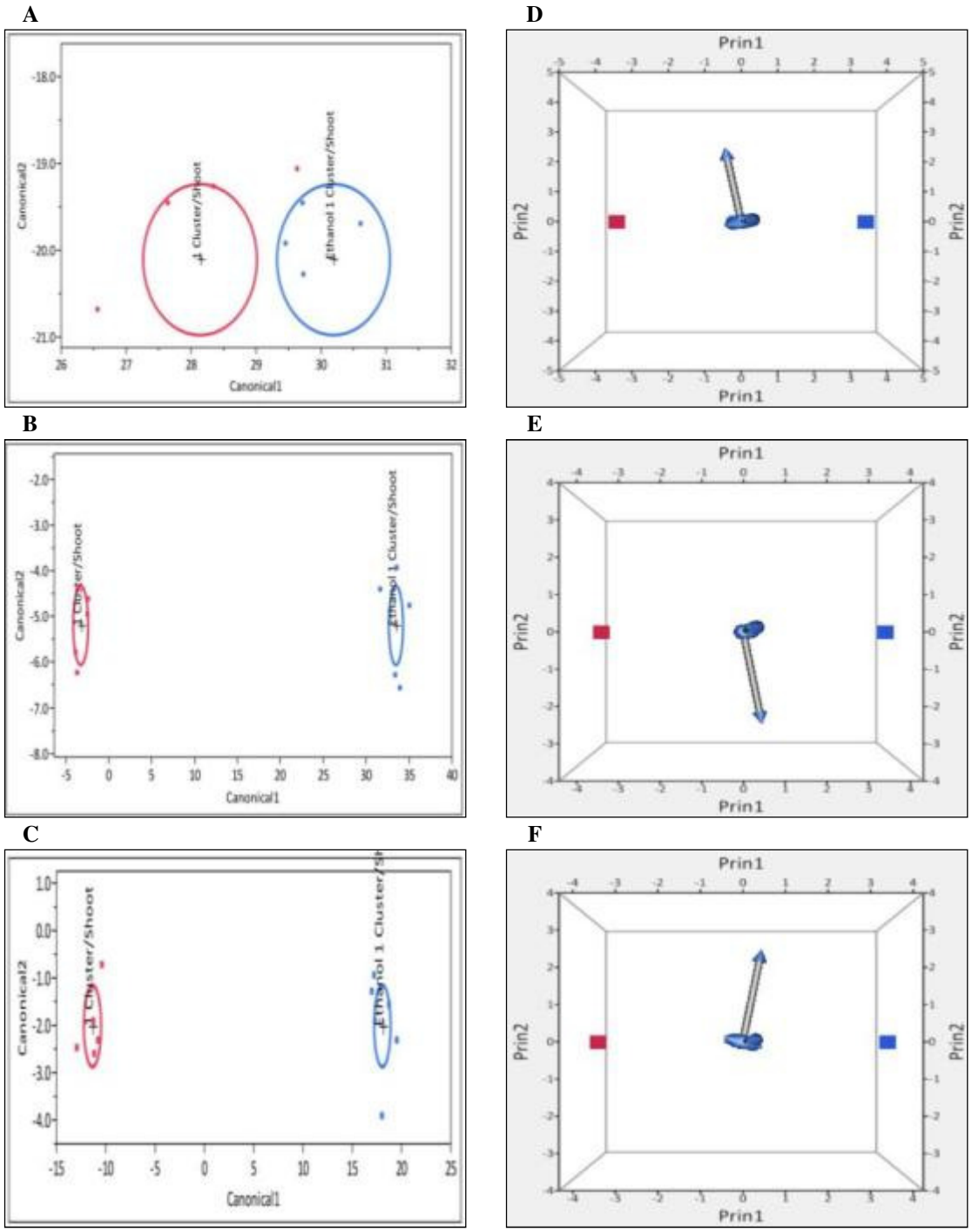

Figure 4.7: Canonical distribution (A) week 12, (B) week 13 and (C) week 16 post-bloom and principal component analysis (D) week 12, (E) week 13 and (F) week 16 post-bloom, of differences between ethanol treatment and control, detected by conducting polymer-based electronic nose for Merlot juice on three sampling dates (For canonical, significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles. Key for PCA color code provided in Figure 2).

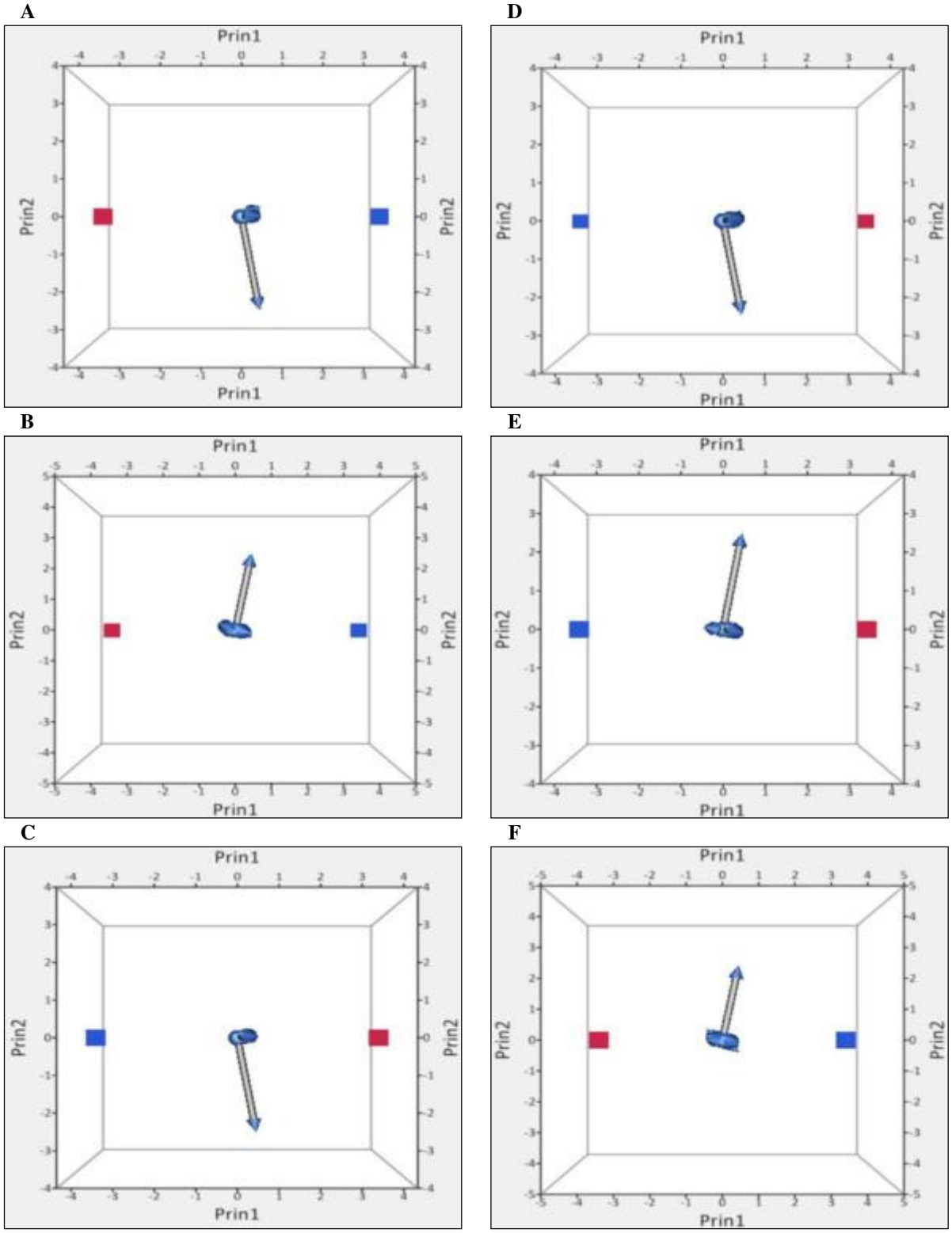

Figure 4.8: Principal component analysis of ethanol treatment differences detected by conducting polymer-based electronic nose for Cabernet franc juice on three sampling dates (A) week 14, (B) week 15 and (C) week 17 post-bloom for ethanol-treated and control – 1 and (D) week 14, (E) week 15 and (F) week 17 post-bloom for ethanol-treated and control – 2 (Key for PCA color code provided in Figure 3).

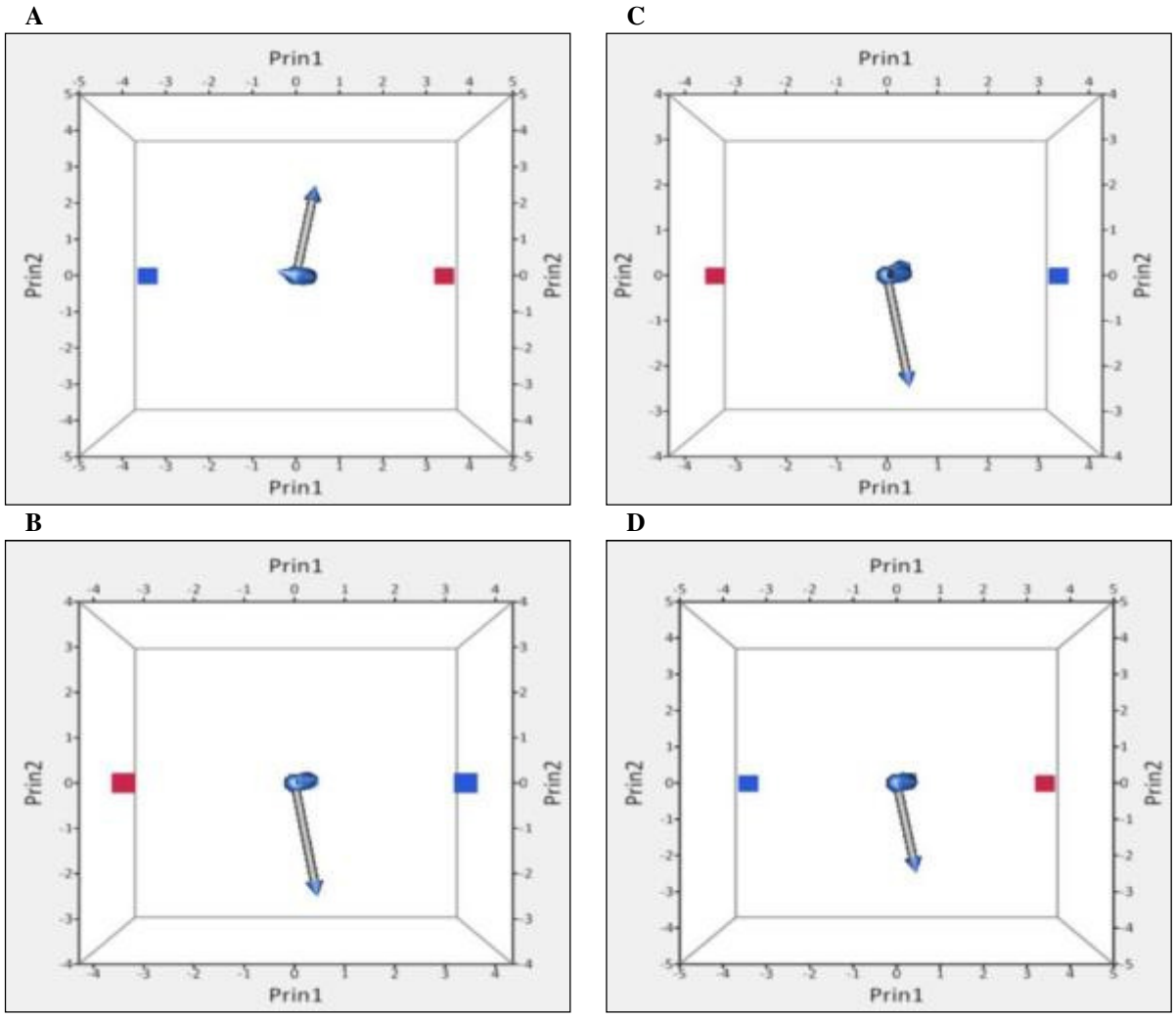

Figure 4.9: Principal component analysis of ethanol treatment differences detected by conducting polymer-based electronic nose for Cabernet franc wine (A) immediately post-fermentation and (C) six months post-fermentation for ethanol-treated and control – 1 (B) immediately post-fermentation and (D) six months post-fermentation for ethanol-treated and control – 2 (Key for PCA color code provided in Figure 3).

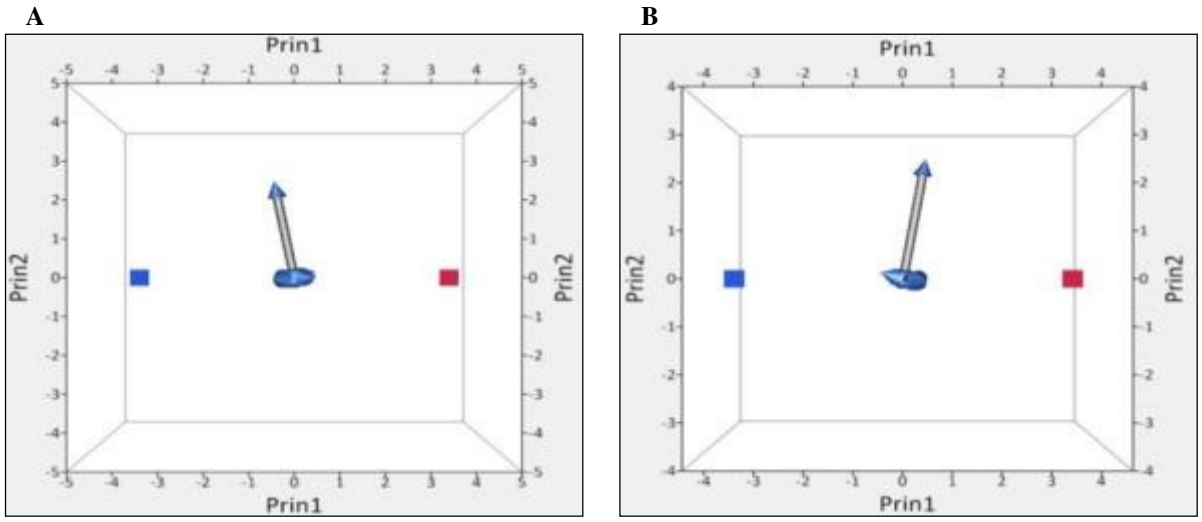

Figure 4.10: Principal component analysis of ethanol treatment differences detected by conducting polymer-based electronic nose for Merlot wine (A) immediately post-fermentation and (B) six months post-fermentation (key for color code provided in Figure 2).

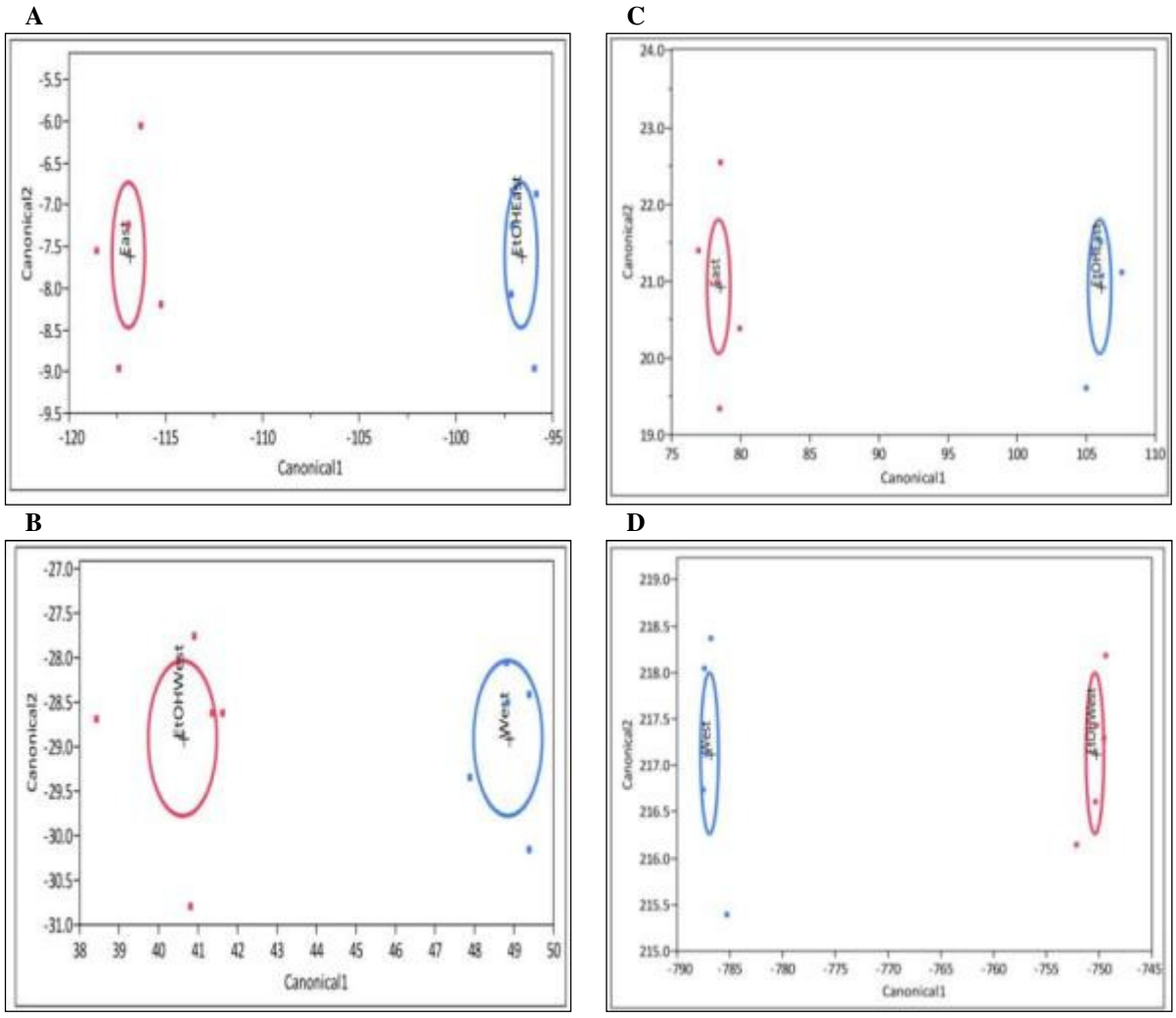

Figure 4.11: Canonical distribution of ethanol treatment differences detected by conducting polymer-based electronic nose for Cabernet franc wine (A) immediately post-fermentation and (C) six months post-fermentation for ethanol-treated and control – 1 (B) immediately post-fermentation and (D) six months post-fermentation for ethanol-treated and control – 2 (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

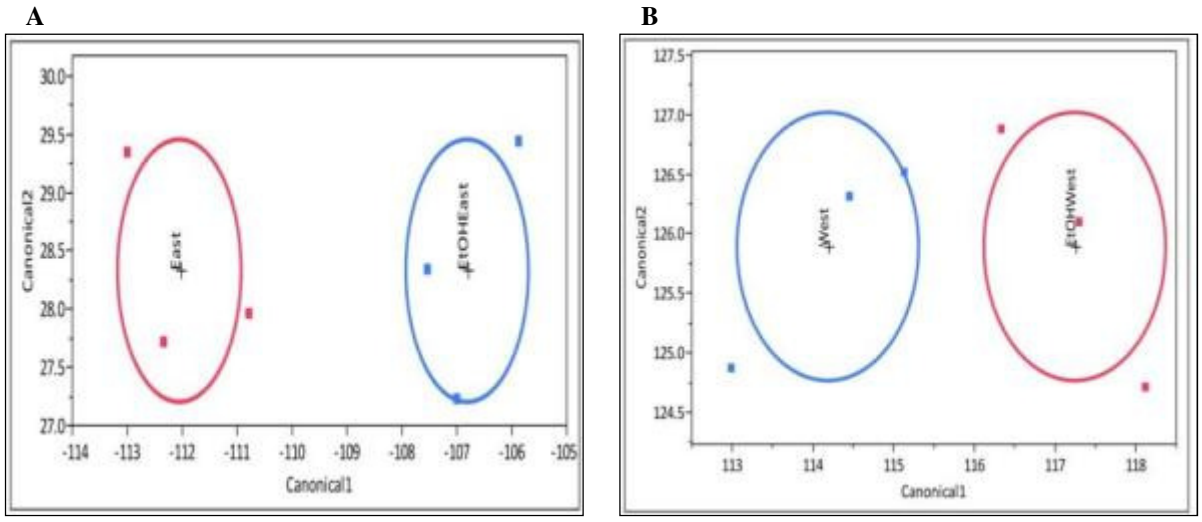

Figure 4.12: Canonical distribution of ethanol treatment differences detected by surface acoustic wave-based electronic nose for Cabernet franc wine immediately post-fermentation (A) ethanol-treated and control – 1 (B) ethanol-treated and control – 2 (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

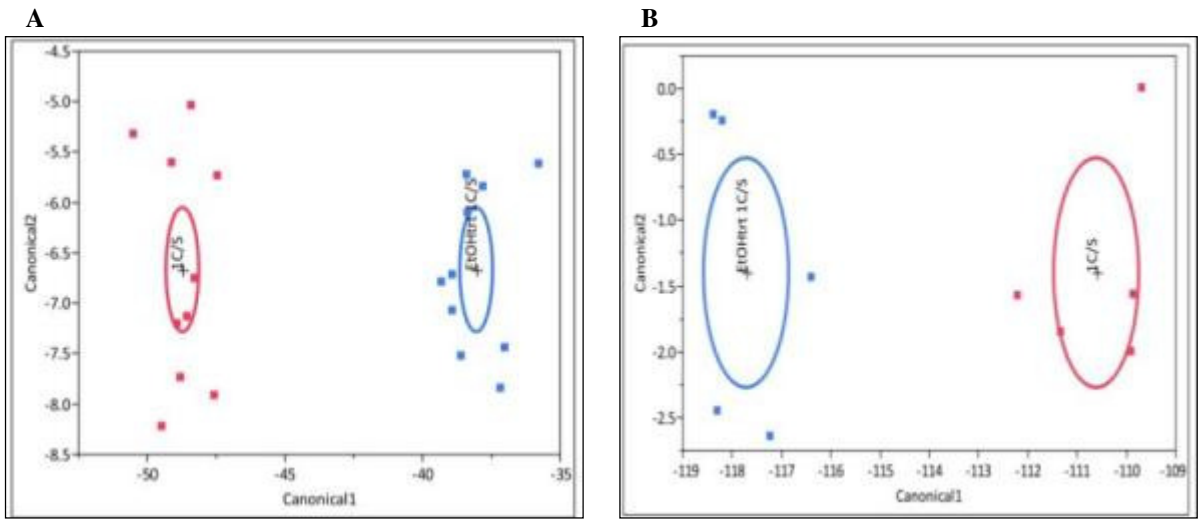

Figure 4.13: Canonical distribution of differences between ethanol treatment and control, detected by conducting polymer-based electronic nose, for Merlot wine (A) immediately post-fermentation and (B) six months post-fermentation (significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles).

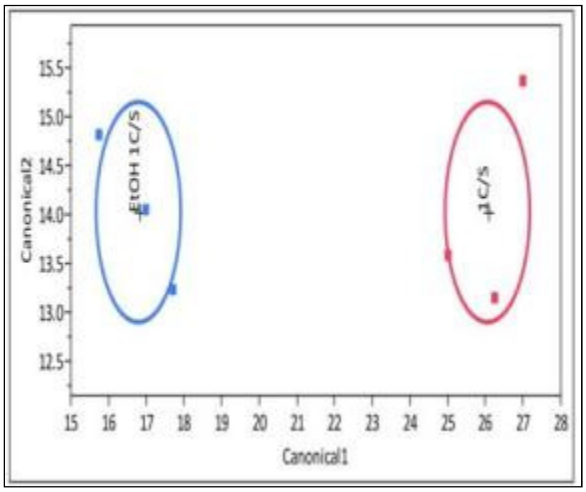

Figure 4.14: Canonical distribution of differences between ethanol treatment and control, detected by surface acoustic wave-based electronic nose, for Merlot wine immediately post-fermentation (significant differences at α = 0.05 level indicated by non-intersecting circles).

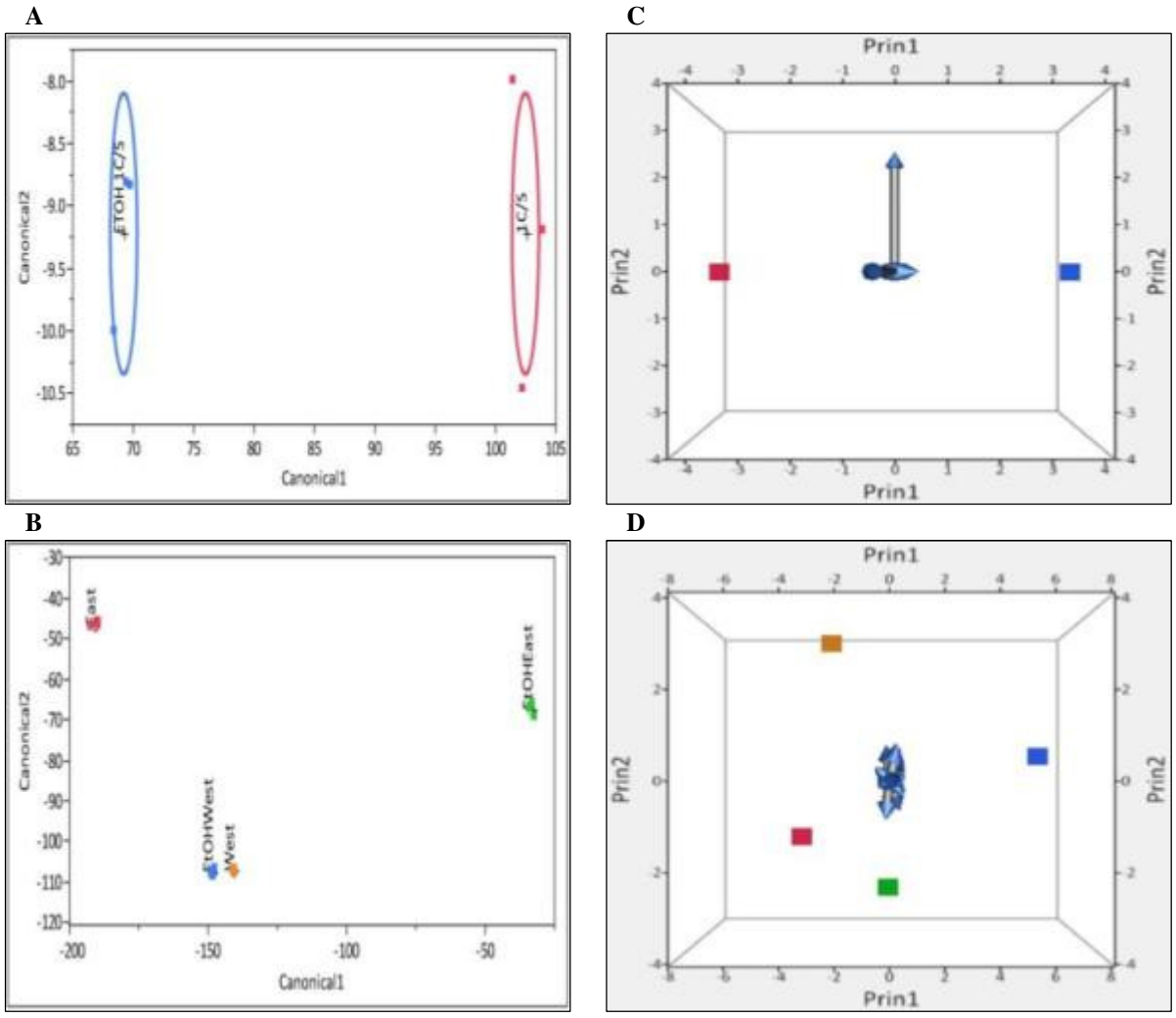

Figure 4.15: Canonical distribution (A) Merlot (B) Cabernet franc and principal component analysis (C) Merlot and (D) Cabernet franc, of differences between ethanol treatment and control, detected by GC/MS for wine six months-post fermentation (For canonical, significant differences at $\alpha = 0.05$ level indicated by non-intersecting circles. Key for PCA color code provided in Figure 2 & 3).

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Summary and Conclusions

This research on vineyard management practices was divided into three studies: 1. Effect of canopy side, 2. Effect of cluster thinning and 3. Effect of ethanol spray. In all three studies the ability of two electronic nose systems (CP ENose 320) and (SAW ENose 7300) to characterize the grape and wine volatiles based on their vineyard management practice was evaluated. The results from electronic nose systems were also compared with the traditional methods like physico-chemistry (grapes), chemistry (wine), sensory and GC-MS (only cluster thinning) using linear statistical analysis methods (CDA, PCA, ANOVA and LSD).

In the first study the effect of canopy side was studied on Cabernet franc grapes. The East/West and North/South facing fruits were evaluated over two growing seasons (2007 and 2008). The second study involved the comparison of unthinned, 1 cluster/shoot and 1&2 cluster/shoot in the year 2008 on Merlot grapes. The third project evaluated the differences between ethanol sprayed (5% v/v) and control grapes. In this study, the variation with ethanol spray in both Cabernet franc (East/West) and Merlot grapes were examined.

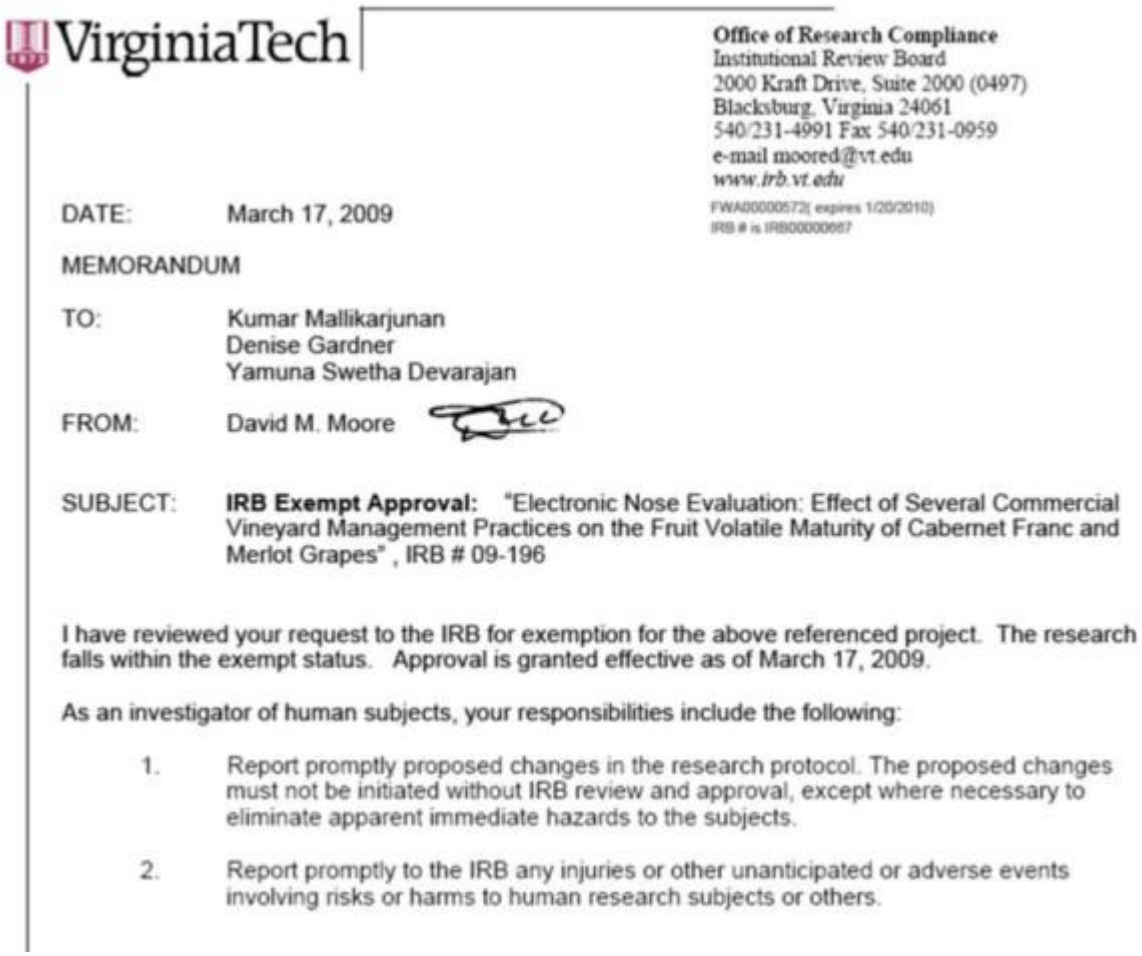
The results from all three studies indicated that the current methods available lacked consistency in identifying the differences between vineyard management practices. Both the electronic noses have been identified as effective monitoring and screening tool for vineyard management practices. Though both ENose systems were able to differentiate between the viticultural practices when, comparing the discrimination percentage obtained by CP ENose and SAW ENose the former was found to better of the two.

In future, the potential of different data analysis methods in the evaluation of SAW ENose data could be studied. Instead of linear statistical methods (CDA, PCA, etc.,), non-linear method such as neural networks, which has been identified in the past to provide better discrimination for ENose data can be assessed. Extra efforts are required to optimize the method settings of SAW ENose for wine and grapes. Other recommendations for research prospects would be that the use of CP ENose sensor information obtained from this study for the development of low-cost electronic nose systems specific for grapes and wines. Also, samples of several grape varieties ought to be collected from different vineyards in different locations as to

find the practical application of Enoses in wineries for discriminating between the vineyard management practices.

Appendix

A.1: IRB Approval Documentation



VirginiaTech

Office of Research Compliance
Institutional Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24061
540/231-4991 Fax 540/231-0959
e-mail moored@vt.edu
www.irb.vt.edu
FWA00000572(expires 1/20/2010)
IRB # is IRB00000667

DATE: March 17, 2009

MEMORANDUM

TO: Kumar Mallikarjunan
Denise Gardner
Yamuna Swetha Devarajan

FROM: David M. Moore

SUBJECT: **IRB Exempt Approval:** "Electronic Nose Evaluation: Effect of Several Commercial Vineyard Management Practices on the Fruit Volatile Maturity of Cabernet Franc and Merlot Grapes" , IRB # 09-196

I have reviewed your request to the IRB for exemption for the above referenced project. The research falls within the exempt status. Approval is granted effective as of March 17, 2009.

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

1. Report promptly proposed changes in the research protocol. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.

2. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

A.2: IRB Sensory Consent Form

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

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

Title of Project: Electronic nose evaluation: Effect of several commercial vineyard management practices on the fruit volatile maturity of Cabernet franc and Merlot grapes

Investigators: Yamuna Swetha Devarajan, Denise M. Gardner and Kumar Mallikarjunan, Ph.D.

I. Purpose of this Research/Project

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

II. Procedures

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

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

III. Risks

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

IV. Benefits

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

V. Extent of Anonymity and Confidentiality

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

VI. Compensation

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

VII. Freedom to Withdraw

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

VIII. Subject's Responsibilities

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

1) Smell red wine samples.

IX. Subject's Permission

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

_____ Date_____

Subject signature

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

Kumar Mallikarjunan, Faculty/Investigator (540) 231-7937; kumar@vt.edu

Yamuna S. Devarajan, Graduate Research Assistant, Investigator (540) 231-6509;

dyamuna@vt.edu

Denise M. Gardner, Graduate Research Assistant, Co-Investigator (540) 231-9843

dmg1214@vt.edu