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Discrimination of Wines Produced from Cabernet Sauvignon Grapes Treated with Aqueous Ethanol Post-Bloom Using an Electronic Nose

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ABSTRACT

Wine discrimination and analysis is typically done through chemical analysis and sensory evaluation by a trained panel. Both of these methods are proven to be successful in wine discrimination, but require extensive preparation, time and money. The electronic nose is an objective, rapid-analysis tool that has been used in the food industry for a number of applications. The purpose of this study was to determine if an electronic nose can accurately discriminate between Cabernet Sauvignon (*Vitis vinifera* L.) wines made from grapes that have received different pre-harvest but post-bloom spray treatments to enhance growth.

Aqueous ethanol, which has been shown to impact fruit maturity, was sprayed on the grape clusters at 13 weeks post bloom in different concentrations (control, 5% and 10% v/v). Chemical analysis was able to accurately discriminate between the wines produced from these grapes. Triangle difference testing by a consumer panel was not able to differentiate between the different treatments. The electronic nose data was able to accurately identify the control group and the 5% EtOH treatment 90% of the time. Placement of the 10% EtOH group was only 13% correct. The results show the promising potential for an electronic nose to discriminate between control and treated wine samples.

Keywords: electronic nose, wine discrimination, sensory evaluation, Cabernet Sauvignon

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INTRODUCTION

Research indicates that aqueous ethanol vapor and spray can increase the anthocyanin concentration and ripening in tomatoes and cranberries, respectively (Farag and others 1992; Beauleiu and Saltveit 1997). It has also been reported that aqueous ethanol sprayed at 8-13 weeks post bloom on Cabernet Sauvignon (*Vitis vinifera* L.) grape clusters increased the anthocyanin content in berry skins, juice, and wines (El Kereamy and others 2002). Anthocyanin synthesis is related to aroma and flavor production and the formation of glycosides, which suggests that factors effecting anthocyanin development may also affect aroma and flavor development. This is significant to grape and wine production, because aroma and flavor are identifying factors in grape maturity. Wine quality is a subjective judgment that depends on the degree to which the wine is satisfying and balanced and reflects the character of the grape. Therefore, grape maturity is an important wine quality parameter.

Discrimination and quality analysis of wine is completed to ensure that a value product is produced. Current methods for wine discrimination and quality analysis include chemical analysis and sensory evaluation from a trained sensory panel. Chemical analyses can accurately discriminate between wines, but can be very time intensive and expensive (Buratti and others 2007). The wine industry currently uses pH, alcohol, titratable acidity, ºBrix, tartaric and malic acids, total glycosides, phenol free glycosides, total phenols, color intensity and hue, total anthocyanins, pigment cofactors and polymers in order to analyze wine and determine the quality.

Sensory evaluation is another way to discriminate between wines and assess the quality of the wines. Unfortunately, sensory analysis often requires a specially trained sensory panel, requires a long preparation time and a specialist in sensory evaluation to analyze the data (Buratti and others 2007). Sensory panels are subjective, making it difficult to replicate data, and it can be difficult to correlate the data received from a sensory panel with chemical analysis data. Because of these drawbacks with current wine discrimination techniques, identifying an objective, rapid-analysis technique for wine discrimination would save the wine industry time and money (Garcia and others 2006).

The electronic nose is a relatively new technology that has gained popularity in the food industry for a number of different applications (Lozano and others 2005; Garcia and others 2006; Moens and others 2006) from food analysis (Natale and others 1997) to bioprocess monitoring (Bachinger and Mandenius 2001). It has a multisensor array that is used to measure aroma compounds much like the human olfactory system. The unique feature of the electronic nose system is that its response takes into account all the characteristic features (chemical and

physical) of a sample, but does not provide information about the composition of the complex mixture (Hai and Wang 2006). Because of this, the electronic nose system has been proposed as a method of wine discrimination, where volatile compounds are most important for discrimination and analysis.

Research has been done using an electronic nose with metal-oxide sensors to classify different types of wine with different denominations of origin (Buratti and others 2004). Garcia and others (2006) also used a metal-oxide electronic nose to classify four different wines of the same varietal which come from the same cellar. In fact, over the past few years, numerous attempts have been made to utilize these chemosensory systems for wine analysis and discrimination (Guadarrama and others 2001; Kallithraka and others 2001; Penza and Cassano 2004; Lozano and others 2005; Garcia and others 2006; Buratti and others 2007).

For this study, wines made from Cabernet Sauvignon grapes that received three different aqueous ethanol treatments during maturity were tested via chemical analysis, sensory evaluation, and electronic nose evaluation. The overall objective of the study was to determine if a quartz microbalance electronic nose system was capable of discriminating between these three treatments as accurately as a sensory panel. Sensory and chemical parameters were also compared. The hypothesis of the study was that the electronic nose will be able to discriminate between the three different treatments, similar to that of the chemical and sensory analysis.

MATERIALS AND METHODS

Experimental Design

Six vines per treatment were randomly selected within a Cabernet Sauvignon (*Vitis vinifera* L.) vineyard in northwestern Virginia at the Winchester Field Research Facility, Winchester, Virginia. Treatments consisted of control (water) and aqueous ethanol (5% or 10% v/v) sprayed on the clusters 13 weeks post bloom. Each treatment had 16 vines for a total of 48 Cabernet Sauvignon vines available for the experiment. Three fermentation replications were prepared for each treatment for a total of 9 experimental units. Wine chemical analyses utilized by the wine industry were completed for this study. Electronic nose sampling was conducted 5 months post fermentation for wine discrimination analysis and to give a basis of comparison for the wine that was presented to the sensory panel.

Winemaking

Approximately 87kg of fruit was harvested per treatment, with an average of 1.8kg of grapes per vine. Fruit was transported to the Virginia Tech Research Winery, Blacksburg, Virginia and was kept at 7°C until ready for processing. Discolored and soft fruit was removed from each lug in order to improve the

quality of fruit to be processed. The grapes were destemmed/crushed with 70% berry breakage using a Wottle type-A2 destemmer/crusher (Figure 1). Pectinase (ColorX, Scott Labs, Petaluma, CA) at a rate of 100mL/ton was added post-crush. Twenty-two kg of each treatment was transferred to separate cylindrical tanks and cold soaked at 7°C for four days with an addition of 250mg/L dimethyl dicarbonate (DMDC) (Velcorin™, Bayer Corporation, Pittsburgh, PA). Must was mixed once a day during the cold soaking. Post cold soak, each lot was inoculated with 0.24g/L *Saccharomyces cerevisiae* strain ICV-D254 (Scott Labs, Petaluma, CA). Fermentation was conducted in cylindrical tanks at 27°C with hand cap punching three times daily, five hours apart (Figure 2). At dryness (≤2.0g/L residual sugar), wines were dejuiced with an addition of 250mg/L dimethyl dicarbonate, cold settled at 7°C for 24 hours, and racked into 3gal glass carboys in an anaerobic environment. An addition of 40mg/L sulfur dioxide was made post-racking. The wines were racked into 1gal containers prior to sensory evaluation and electronic nose evaluation, and they were stored at a constant temperature of 7°C.

Figure 1. Wottle type-A2 destemmer crusher used for Cabernet Sauvignon berry destemming and crushing


Figure 2. Fermentation replications for Cabernet Sauvignon wine production

Wine Chemical Analysis

The commercial harvesting standards for Cabernet Sauvignon were utilized for this study. °Brix was determined using an American Optical model 10419 temperature-compensating refractometer and pH with a Fisher (Pittsburgh, PA) Accument® model 20 pH/conductivity meter. Titratable acidity was determined by titration with NaOH to an end-point of pH 8.2. Total glycoside concentration was determined as described by (Iland and others 1996), and modified by (Zoecklein and others 2000) with 200mg polymeric reverse-phase

extraction cartridges using Strata X™ HLB (Phenomenex, Torrance, CA). Phenol-free glycosides were analyzed as described by Zoecklein and others (2000) using Oasis™ HLB hydrophilic, lipophilic balance (Waters, Milford, MA). Total phenols (A_{280nm} – 4), color intensity (A_{520nm} + A_{420nm}), color hue (A_{520nm}/A_{420nm}), total anthocyanins (20 x A_{520nm}), and polymers were estimated spectrophotometrically (Genesys 5™, Spectronic Instruments Inc., Rochester, NY).

Electronic Nose Analysis

Five replications of each wine were evaluated by the electronic nose system as described by the QMB6 user's manual (Perkin-Elmer 1999). The HKR Sensorsystems QMB6 system equipped with six quartz crystal based sensors (Figure 3) used was connected to an automatic headspace sampler (Model HS-40, Perkin-Elmer LLC, Norwalk, CT). The system, whose sensors coated with varying degrees of affinity to polar compounds (polar to non-polar) and oscillated at 10MHz, used 21mL headspace vials and nitrogen as the carrier gas. During the electronic nose evaluation, a sample headspace was generated and passed through a sensor chamber by pressurizing the headspace with the nitrogen carrier gas. In order to obtain maximum sensor response, operating parameters for the electronic nose were optimized for the wine samples. Equilibrium time was set at 20 min, sensor temperature at 40°C, and sample temperature was set at 56°C.

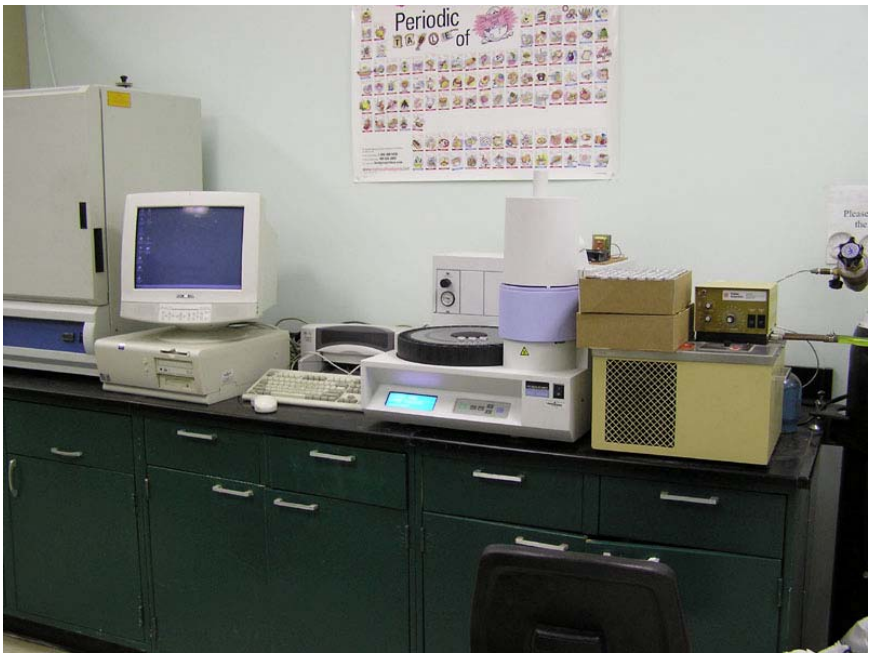

Figure 3. Electronic nose system used for Cabernet Sauvignon analysis

Sensory Evaluation

Wines were evaluated 5-months post fermentation at the Food Science and Technology Sensory Laboratory, Virginia Tech, Blacksburg, Virginia using triangle difference testing. Two tests were conducted per panelist per session concerning aroma and flavor. For each session, panelists were given 10 minutes to determine an aroma difference and 10 minutes to determine a flavor difference. Panelists were given oral instructions at the beginning and written instructions during each testing. In the triangle test, each panelist was given three samples of wine with random code numbers to analyze. Two of these samples were the same and one was different. The panelists were asked to identify the different wine based on aroma. Once those samples were taken away, each panelist was given three new samples with different random code numbers for the flavor testing. The panelists were once again asked to identify the wine that was different based on flavor. Samples were presented to the panelists under red light to ensure that color variations would not effect their decision.

Statistical Analysis

Multivariate discriminate analysis was performed on the data obtained from this study using the statistical software package PC-SAS (SAS Inc., Cary, NC). The GLM procedure was run to generate ANOVA tables, generate plots and run t-tests on the data. In addition STEPDISC, CANDISC and PRIN COMP were used to identify significant variables, canonical discriminate analysis and principle component analysis, respectively. The PROC STEPDISC in SAS was also used to rank sensor data based on the contribution to the discriminatory power of the system. In addition to SAS, the electronic nose data was evaluated using QMBSoft v. 1.22 (QMB6 software). Evaluation of the sensory data was completed using statistical tables located in Meilgaard and others (1999).

RESULTS AND DISCUSSION

Wine samples were assessed using chemical analyses, sensory evaluation, and electronic nose evaluation. The data obtained from this work was used to make a comparison between the electronic nose and the other methods of wine analysis in order to determine if the electronic nose can be used as a discriminatory tool for wine analysis. The volatiles tested were from Cabernet Sauvignon wines that received aqueous ethanol treatments (0%, 5% and 10% v/v) during grape maturation.

Wine Chemical Analysis

Each treatment of wine was evaluated using the same set of chemical analyses including pH, percent alcohol, titratable acidity, total and phenol-free glycoside concentration, color hue, color intensity and total anthocyanins. Table 1 provides a summary of the results that were obtained from the chemical analyses. Principle component analysis was completed in order to verify a cluster structure within the data. The plot in Figure 4 shows a distinct separation in the different treatment groups.

Table 1. Summary of wine chemical analysis results for each treatment

Trt	Lot	pH	TA (g/L)	Alcohol (%)	PFGG (µM)	TGG (µM)	Color Intensity	Hue	TAC
0	1	3.86	6.03	12.7	118.45	1616.88	4.36	0.62	20.87
0	2	3.88	6.18	12.7	122.28	1738.75	4.31	0.61	23.68
0	3	3.94	6.05	12.7	na[1]	1438.75	4.17	0.65	25.45
5	1	3.97	6.48	12.5	105.03	1588.75	4.52	0.63	26.05
5	2	3.97	6.16	12.4	77.24	1707.50	4.83	0.68	25.58
5	3	3.98	6.80	12.4	122.28	1738.75	4.22	0.66	23.01
10	1	3.96	6.46	12.3	104.08	1504.38	3.83	0.64	22.64
10	2	3.93	6.33	12.3	105.03	1532.50	4.09	0.66	22.59
10	3	3.94	7.16	12.2	84.91	1298.13	3.64	0.63	22.90

Trt Treatment
TA Titratable Acidity
PFGG Phenol-free Glycosides
TGG Total Glycosides
TAC Total Anthocyanins
[1] PFGG readings for control treatment lot three were not available

Figure 5 gives a visual representation of the data results shown on a relative scale. No statistical significance was found between the three treatments based on titratable acidity, total and phenol-free glycosides, hue or total anthocyanins. Results from pH displayed statistical significance between 0% EtOH and 5% EtOH. Percent alcohol was statistically significant for all three treatments, and color intensity resulted in a statistical difference between 5% and 10% EtOH respectively.

Because there were three variables that resulted in a significant difference between the treatments, the CANDISC procedure was run using SAS (SAS Inc., Cary, NC) to determine which two variables were the most significant in separating the treatments. Percent alcohol and pH were found to be the most significant variables in determining the separation of the treatments.

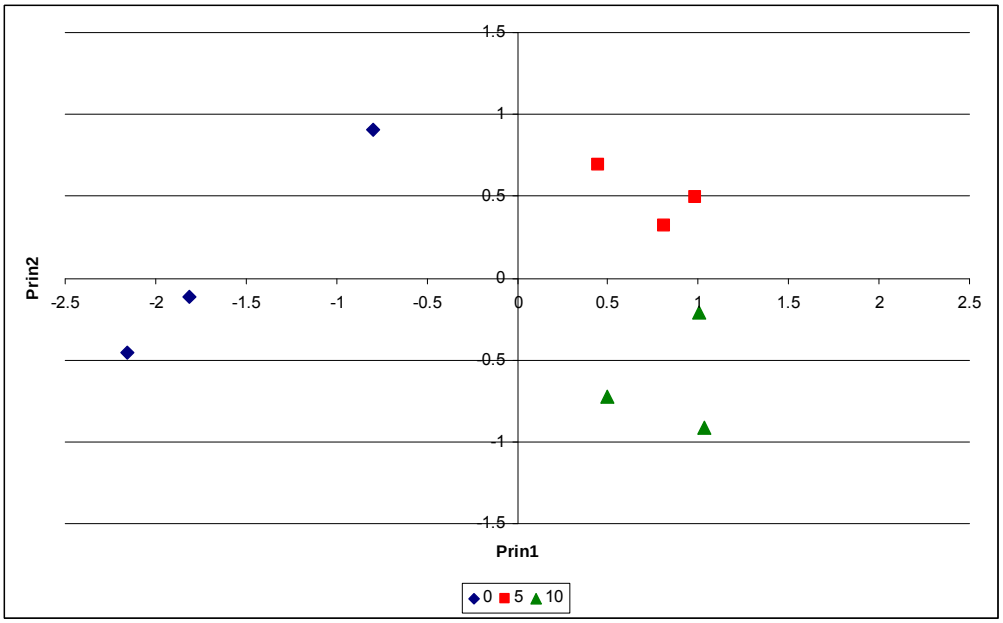

Figure 4. 11 Principle component analysis plot of chemical analysis data for Cabernet Sauvignon wine samples made from grapes treated with 0%, 5% and 10% aqueous ethanol solution

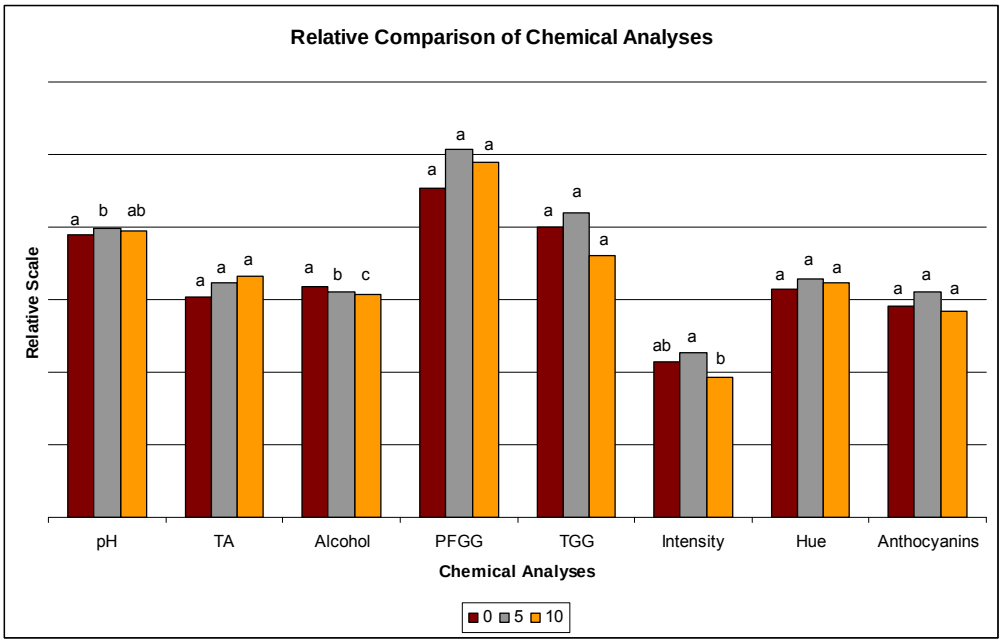

Figure 5. Comparison of chemical analyses on a relative scale based on treatment

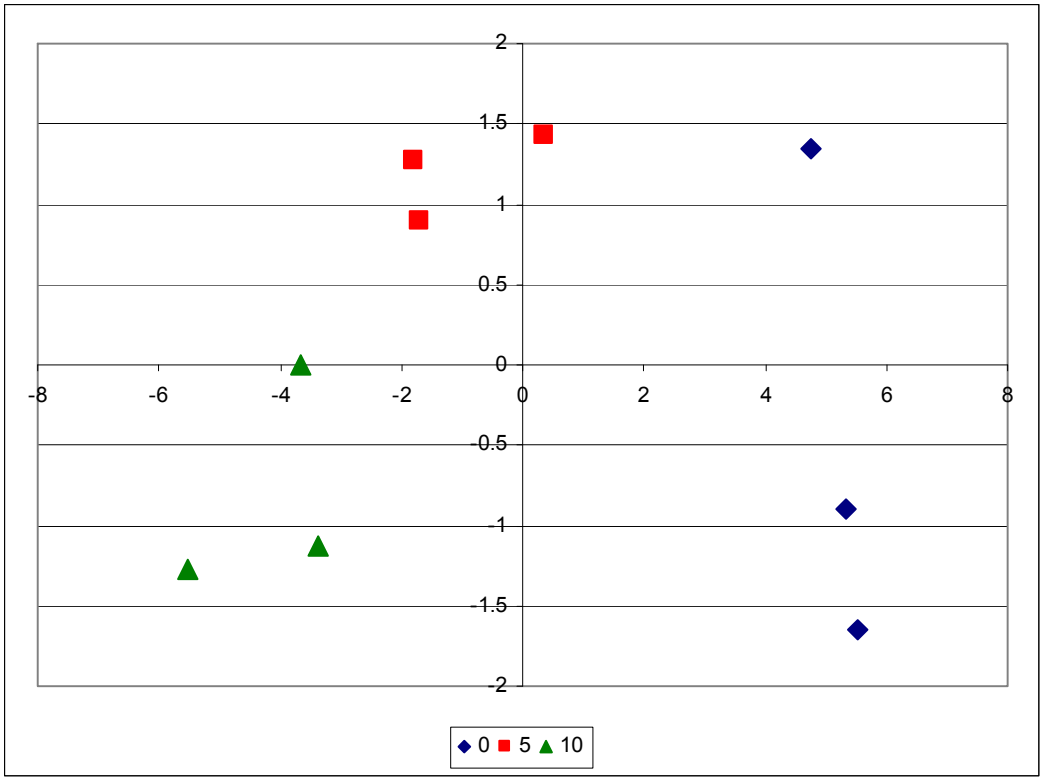

Figure 6. Canonical plot of chemical analysis data for Cabernet Sauvignon wine samples made from grapes treated with 0, 5 and 10% aqueous ethanol solution

A canonical plot, seen in Figure 6, was created based on pH and percent alcohol that effectively shows the separation of the three treatments. Cross validation of this information shows that the chemical analyses were 100% effective in discriminating between the three different treatments.

Sensory Evaluation

The first sensory test was completed for the comparison of the control treatment (0% EtOH) and the 5% EtOH treatment. The following statistical parameters were set for the evaluation: $p_d = 0.30$, $\alpha = 0.05$, and $\beta = 0.20$. A total of 48 responses were collected for this comparison, using a triangle test for aroma and taste. A total of 22 correct responses were needed in order for the two treatments to be statistically significant. A summary of the responses from this sensory evaluation can be found in Table 2. Fourteen correct responses were received for aroma, and 15 correct responses were received for taste. Therefore, the 0% EtOH treatment and the 5% EtOH treatment were not found to be significantly different.

Table 2. Sensory evaluation of control and 5% EtOH

	Correct	Incorrect
0% - 5% AROMA	14	34
0% - 5% TASTE	15	33

Table 3. Sensory evaluation of control and 10% EtOH

	Correct	Incorrect
0% - 10% AROMA	14	19
0% - 10% TASTE	11	22

The second sensory evaluation was completed for the comparison of the control group (0% EtOH treatment) to the 10% EtOH treatment. The following parameters were set for the evaluation: $p_d = 0.30$, $\alpha = 0.05$, and $\beta = 0.30$. A total of 33 responses were collected for this comparison, using a triangle test for aroma and taste. A total of 17 correct responses were needed in order for the two treatments to be significantly different. A summary of the responses from this sensory evaluation can be found in Table 3. Fourteen correct responses were received for aroma, and 11 correct responses were received for taste. Therefore, the 0% EtOH treatment and the 10% EtOH treatment were not found to be significantly different.

Electronic Nose Evaluation

Ten replications of each wine sample treatment were evaluated using the QMB6 sensorsystem. Data was first analyzed using the QMB6 software (QMBSoft v. 1.22). A visual representation of this analysis can be seen in Figure 7. From this plot, a clear separation between the control group and the treated samples can be seen. The electronic nose was successful in discriminating between the control samples and the samples that received treatments.

The CANDISC procedure was performed on the results from the electronic nose readings in order to produce a canonical plot. From this plot (Figure 8), a separation can be seen between the control group and the treated groups (5% and 10% EtOH).

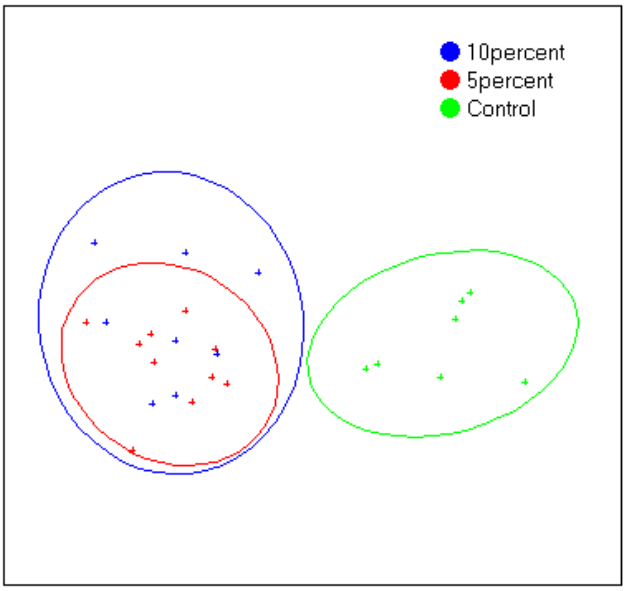

Figure 7. Projection plot using QMBSoft of control, 5% and 10% treatments from the discriminate analysis of sensors of the HKR Sensorsystems QMB6 system

The cross-validation for this information (Table 4) identifies the number of observations and the percent classified into each treatment by the electronic nose. This table shows that the electronic nose was successful in categorizing the control group and the 5% EtOH treatment group 90% of the time. However, the categorization of the 10% EtOH treatment was only successful 13% of the time. It is possible that the electronic nose was recognizing the 5% and 10% EtOH treatments as the same samples and placing them in the same category. This can be clearly seen in Figure 7, where the 5% EtOH treatment appears to be a subset of the 10% EtOH treatment. Based on this interpretation, the electronic nose was able to correctly place the 5% EtOH treatment 100% of the time, and the 10% EtOH treatment 80% of the time.

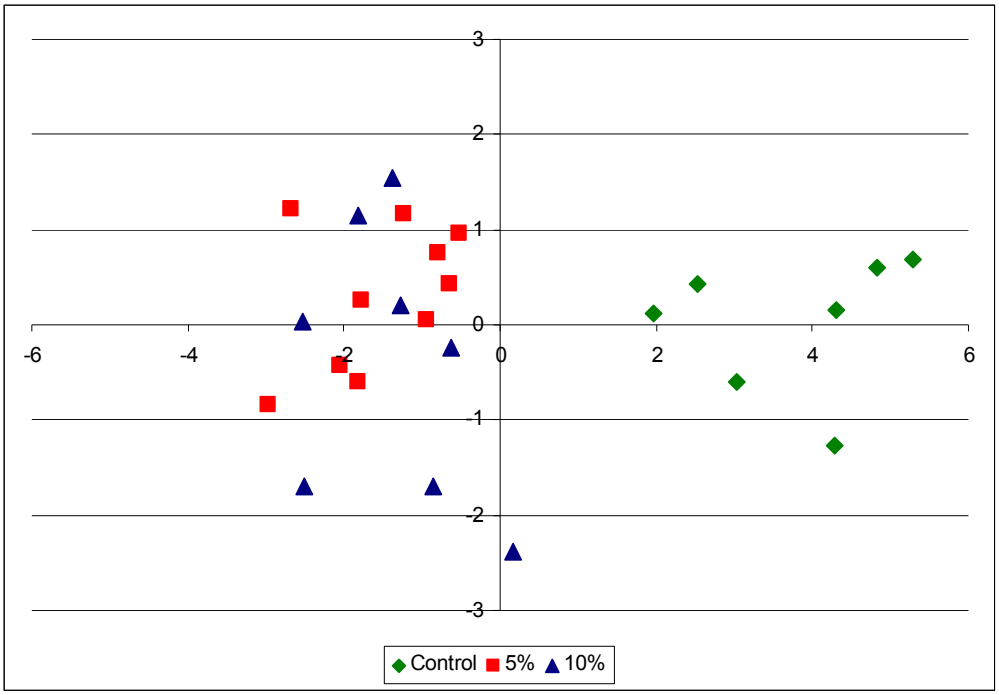

Figure 8. Canonical plot of electronic nose data for Cabernet Sauvignon wine samples made from grapes treated with 0, 5 and 10% aqueous ethanol solutions

Table 4. Cross validation of the discriminate analysis of electronic nose data for wine samples made from Cabernet Sauvignon grapes treated with 0%, 5% and 10% v/v aqueous ethanol solution

Number of Observations (Percent) Classified into Trt				
From Trt	**0**	**5**	**10**	**Total**
0	9 (90)	1 (10)	0	10 (100)
5	0	9 (90)	1 (10)	10 (100)
10	2 (20)	5 (50)	3 (30)	10 (100)
Total	11 (37)	15 (50)	4 (13)	30 (100)
Priors	0.33 (3)	0.33 (3)	0.33 (3)	
Error Count Estimates for trt				
	0	**5**	**10**	**Total**
Rate	0.1	0.1	0.1	0.3
Priors	0.33	0.33	0.33	

The electronic nose is unique in that it gives an overall objective comparison between samples. It is not designed to discriminate between samples based on the intensity or concentration of a particular element, but rather the responses of each of the sensors form a characteristic recognition pattern for that particular sample. This may explain why there seems to be no direct correlation between the electronic nose data and the chemical data. The electronic nose will be more sensitive to the overall aroma of the wines, and would therefore correlate well with phenol-free glycosides (PFGG) in the wine. The PFGG results did not show a significant difference. A significant difference between the samples was found based on the percent of alcohol in the wine; however, the electronic nose did not discriminate between different percentages of alcohol as a result of normalization. The electronic nose may have been sensitive to the difference in pH that may have impacted volatility.

From the results of the electronic nose there was a distinct difference between the control group and the treated samples. Based on this information, the application of aqueous ethanol post-bloom resulted in a change in the wine produced.

CONCLUSIONS

The purpose of this study was to determine if electronic nose technology could accurately discriminate between wines made from Cabernet Sauvignon fruit that received different aqueous ethanol treatments post-bloom. The electronic nose data was compared to chemical analysis data and results from sensory analyses.

The chemical analyses were able to effectively discriminate between the three samples (control, 5% and 10% ethanol spray treatments). These results are important because they verify that the wine samples being evaluated can be discriminated and grouped. However, discrimination was due to only three significant parameters: color intensity, percent alcohol and pH. Despite the fact that there was clear separation between groups as determined by the chemical analysis, the sensory panel was not able to discriminate between the control group and the treated wine samples. The electronic nose was successful in grouping the control group separately from the ethanol spray treatments evaluated in this study. The treated samples were grouped in the same area.

Based on these results, it was found that the electronic nose can be used as a discriminatory tool for control and treated wine samples under the conditions of this study. Further chemical analysis should also be completed in order to better identify significant parameters that differ among the samples, including specific volatile components.

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