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SHORT COMMUNICATION

Electronic Nose Evaluation of Cabernet Sauvignon Fruit Maturity

AHMAD I. ATHAMNEH, BRUCE W. ZOECKLEIN and KUMAR MALLIKARJUNAN

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ABSTRACT *The ability of an electronic nose to classify cabernet sauvignon (*Vitis vinifera *L.) fruit based on maturity levels was investigated over two seasons. Maturity of samples collected 18, 19, and 20 weeks post-bloom was evaluated by measuring berry weight, pH, Brix, titratable acidity, total phenols, color intensity, hue, total anthocyanins, and total and phenol-free glycosides. Results were compared, using discriminant and canonical discriminant analysis, with analysis of headspace volatiles via a hand-held electronic nose. The electronic nose was able to determine differences among the three sample groups in both seasons. Additionally, in one season electronic nose measurements were compared to chemical analyses of samples collected from east and west sides of north – south oriented vineyard rows. Results demonstrated the ability of the electronic nose to distinguish fruit from vine canopy sides. Field measurements demonstrated the potential for the electronic nose as a rapid, non-destructive tool for evaluating grape maturity.*

Introduction

Grape maturity is a critical attribute impacting potential wine quality. Maturity evaluation is difficult due to the many interrelated factors that impact physicochemical changes (Coombe, 1992; Robinson and Davies, 2000) and limitations in the understanding of these factors (Coombe, 1992; Watson, 2003). Currently, grape maturity evaluation often includes some measurement of physical and chemical properties. Berry weight, sugar content (Brix), pH, titratable acidity, malic acid, and color are common indices used individually or in combination. These assays may be influenced by sample and process variations (Rankine *et al.*, 1962; Zoecklein *et al.*, 1999). Additionally, specific levels of sugar, acidity, pH, and color are not always strongly correlated to potential wine quality (Hardie *et al.*, 1996).

Wine varietal character is the product of grape-derived volatile compounds (Gunata *et al.*, 1985; Hardie *et al.*, 1996). Free volatiles may contribute directly to odor, while

Bruce W. Zoecklein, Professor, Head, Enology-Grape Chemistry Group, Department of Food Science and Technology, Virginia Tech, Blacksburg, VA 24061, USA (E-mail: bzoeckle@vt.edu).

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some non-volatile conjugates represent aroma precursors that may be released during winemaking and aging (Gunata *et al.*, 1985, 1990). Procedures used to estimate aroma potential include analysis of free and potentially volatile terpenes (Dimitriadis and Williams, 1984), total and phenol-free glycosides (Abbott *et al.*, 1993; Zoecklein *et al.*, 2000) and chromatographic methods (Salles *et al.*, 1990; Ebeler, 2001; Sanchez-Palomo *et al.*, 2005). However, these analyses are restricted to high-terpene varieties, are expensive, and/or time consuming. The pool of free aroma components and their precursors increases rapidly in the advanced stages of fruit maturity, referred to as *engustment* by Coombe and McCarthy (1997). For that reason, many producers sensorially, but subjectively, evaluate juice aroma as a maturity gauge (Jordan and Croser 1983; Winter *et al.*, 2004). Because of the difficulties associated with current methods, there is a need for a simple, reliable, and objective technique for evaluation of fruit maturity.

The electronic nose is a relatively new technology utilized in a variety of applications in the medical field (Gardner *et al.*, 2000) and food industries (Di Natale *et al.*, 1997; Schaller *et al.*, 1998). It is a basic simulation of the human olfactory system (Gardner and Bartlett, 1999), intended to aid in decision-making when volatile compounds correlate strongly with certain sample attributes. In the wine industry, the electronic nose has been suggested as a tool to monitor toasting homogeneity of oak barrels (Chatonnet and Dubourdieu, 1999), and for wine discrimination (Di Natale *et al.*, 1996; Rong *et al.*, 2000; Penza and Cassano, 2004; Ragazzo-Sanchez *et al.*, 2005; Garcia *et al.*, 2006). Santos *et al.* (2004) demonstrated that electronic nose evaluation of madeira wines was consistent with GC/MS analysis. This technology has been used as a non-destructive tool for maturity assessment of apples (Pathange *et al.*, 2006), bananas (Llobet *et al.*, 1999), mandarins (Gomez *et al.*, 2006), and nectarines, peaches, and pears (Brezmes *et al.*, 2005). This study evaluated the capacity of a conducting polymer-based electronic nose to monitor cabernet sauvignon (*Vitis vinifera* L.) fruit maturity by analyzing headspace volatiles.

Materials and Methods

Vineyard Site and Fruit Sampling

Cabernet sauvignon (*Vitis vinifera* L.) was grown on an open lyre divided canopy training system in Winchester, VA, USA (39°12'N), which has a macroclimate typified as warm, humid and continental. Mean monthly precipitation from April through October is 76 mm, with 1890 accumulated heat units and a mean relative humidity in September of 75% (Wolf and Poling, 1995). Vines were grafted to C-3309 rootstock, planted in 1998, and spaced 2.1 m apart in 3.6 m north-south oriented rows. Soil is a Frederick-Poplimento loam, with an effective rooting depth greater than 100 cm. Vines were not irrigated, and were subject to pest management and other general cultural practices routinely used in the region.

Within a 0.5 ha plot, 15 and ten vines were randomly selected for fruit maturity evaluation in 2005 and 2006, respectively. In 2005, samples of 25 berries were randomly collected from both sides of each vine canopy, as described by Jordan and Croser (1983), at 18, 19, and 20 weeks post-bloom, for a total of 15 replicates per sampling week. In 2006, ten samples of 25 berries were collected from each row side, for a total of 20 replicates per sampling week. Samples were stored at −80°C. At the time of commercial harvest (20 weeks post-bloom), clusters per shoot, clusters per vine, cluster weight, shoots per vine, and fruit weight per vine were determined.

Laboratory Analysis

Berries were thawed completely to ambient temperature (20°C), weighed, homogenized (after removing seeds) in a Waring (New Hartford, CT) commercial laboratory blender with 2 μL Pec5L pectic enzyme (Scott Laboratory, Petaluma, CA), centrifuged at 1800 × *g* for 3 min, and the supernatant was filtered through a 0.45 μm syringe filter (Whatman, Clifton, NJ). Analysis of per berry weight, pH, Brix, titratable acidity, color intensity (absorbance at 520 nm + absorbance at 420 nm), hue (absorbance at 420 nm/absorbance at 520 nm), and estimates of total phenols (absorbance at 280 nm) and total anthocyanins were determined as described by Zoecklein *et al.* (1999). Total glycoside concentration was determined as described by Iland *et al.* (1996). Phenol-free glycosides were estimated as described by Zoecklein *et al.* (2000). The above indices were measured on each of the 15 sampling replicates at 18, 19 and 20 weeks post-bloom in 2005, with analysis of weight, pH, Brix, and titratable acidity conducted in 2006.

Electronic Nose

The Cyranose 320 (Cyrano Sciences, Pasadena, CA) is a hand-held electronic nose system with 32 polymer-based sensors. Electronic nose measurements were conducted prior to berry maceration for chemical analysis. Twenty berries, stored at −80°C, were thawed at ambient temperature for 2 hr, and incubated in mason-type jars for 30 min at 21°C in a water bath. At the time of measurement, the electronic nose sampling needle was inserted through a rubber septum, with a vent to avoid vacuum buildup. Sample incubation time and temperature, and electronic nose settings (Table 1), were chosen based upon a previous study to identify the optimum parameters (Athamneh *et al.*, 2006).

Electronic nose measurements were conducted in the field at 18, 19 and 20 weeks post-bloom. In 2005, 16 randomly-selected clusters were wrapped in 43.2 × 38.1 cm polyethylene bags (Inteplast, Livingston, NJ) for 45 min, followed by electronic nose headspace analysis. In 2006, ten randomly-selected clusters per side of the grape vine canopy were analyzed at each sampling date. Field measurements took place between 0800 and 1200 hr, and cluster temperatures were determined prior to analysis using an Extech 42529 non-contact IR thermometer (Extech Inst., Waltham, MA).

Table 1. Electronic nose settings used for field and laboratory evaluation of cabernet sauvignon grape samples

Action	Setting	Time (s)
Baseline	purge	20
Sample	draw 1	
	draw 2	40
Purge	snout removal	
	1st sample gas purge	0
	1st air intake purge	0
	2nd sample gas purge	10
	2nd air intake purge	60

Statistical Analysis

All statistical analysis was performed with SAS (version 9.1; SAS Institute, Cary, NC). The GLM procedure was used for analysis of variance. The CANDISC procedure was used to conduct canonical discriminate analysis to visually summarize the separation among the three harvest groups. Discriminant analysis was performed using the DISCRIM procedure, with non-parametric method and k = 3 nearest neighbors, to validate the classification of individual samples into the three maturity groups.

Results and Discussion

At commercial harvest, no variations in yield components (fruit yield per vine, clusters per vine, cluster weight, shoots per vine, and clusters per shoot) were noted among sampling replications in either season (data not shown). Brix, pH, hue, and

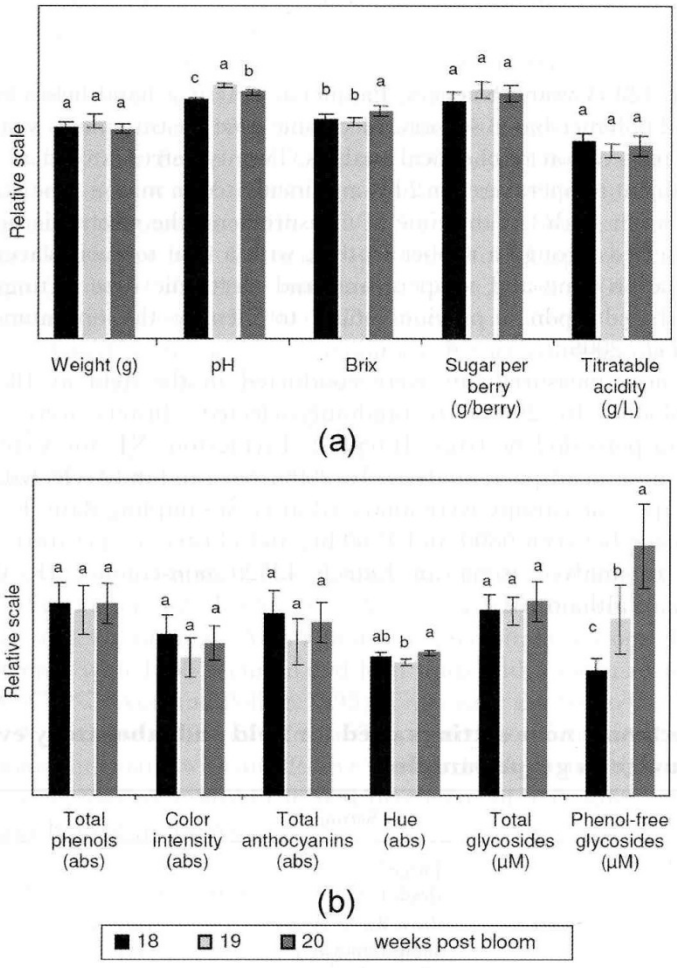


Figure 1. Physicochemical analyses for cabernet sauvignon grapes sampled 18, 19, and 20 weeks post-bloom, 2005 season. Means associated with different letters are significantly different, $\alpha = 0.05$, by least significant difference. Error bars represent 95% confidence intervals.

phenol-free glycosides were found to be different among the three sampling dates in 2005 (Figures 1a and b). The differences in phenol-free glycosides were not mirrored by differences among berry weights (Figure 1b). Zoecklein *et al.* (1998, 2000) reported that increases in phenol-free glycosides may reflect increases in the pool of potential aroma and flavor compounds. This higher concentration in the later sampling dates may have been reflected by an increased production of free volatiles, or *engustment* as suggested by Coombe and McCarthy (1997). Generally, differences in berry weight, pH, Brix, sugar per berry and titratable acidity were not significant in 2006 (Figure 2). Limited reductions in berry weights were not reflected in Brix values. Statistically significant, but minor, differences in pH value, berry weight, and sugar per berry were observed at week 18 post-bloom in 2006 (SD = 0.06).

The canonical discriminate analysis plot of physicochemical analyses data in 2005 showed clustering according to sampling week (Figure 3a). The separation indicates the similarity within a particular group, and the difference among the three groups, as expected. Canonical discriminate analysis showed that the electronic nose data collected in the laboratory and vineyard in 2005 produced similar separation with one measurement, as compared to that based on 11 physicochemical indices (Figures 3b and c). Samples were classified according to sampling week, indicating the ability of the electronic nose to differentiate among the maturity groups. Grouping of samples was validated by the discriminant analysis cross-validation of the physicochemical and electronic nose data measured in the laboratory and the vineyard in 2005, which showed that most samples were correctly classified in their respective sampling weeks (Figure 3a, b, and c). Cross-validation indicated that 91% of samples were correctly classified based on physicochemical data, compared to 100% and 98% based on electronic nose data measured in the laboratory and the vineyard, respectively.

The canonical discriminate analysis plot of 2006 physicochemical analyses data did not show the same clear separation between sampling weeks as 2005 (Figure 4a). In 2006, analyses of weight, pH, Brix, and titratable acidity were not sufficient to

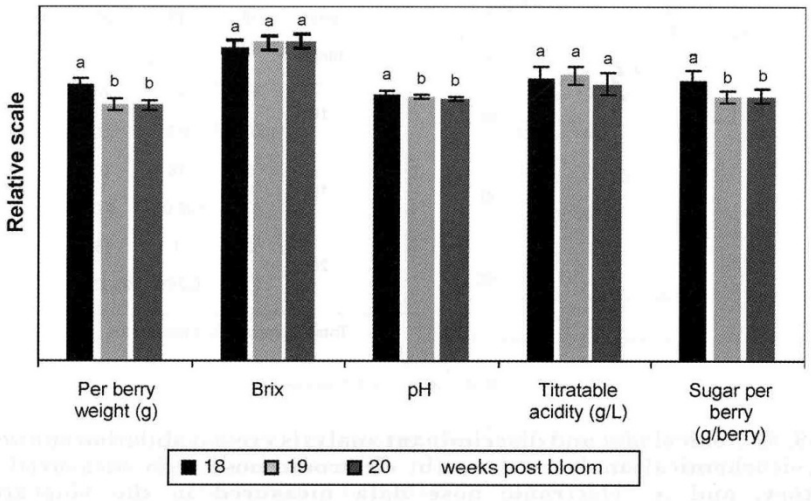
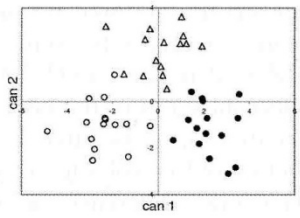


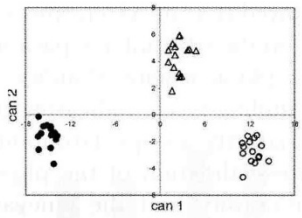
Figure 2. Physicochemical analyses for cabernet sauvignon grapes sampled 18, 19, and 20 weeks post-bloom, 2006 season. Means associated with different letters are significantly different, $\alpha = 0.05$, by least significant difference. Error bars represent 95% confidence intervals.



Sampling date	Weeks post-bloom	Classified as 18	19	20
	18	**14**	0	1
		93.33%	0.0	6.67%
	19	1	**12**	2
		6.67%	82.0%	13.33%
	20	0	0	**15**
		0.0	0.0	100.0%

Total: Correct 91%, Incorrect 9%

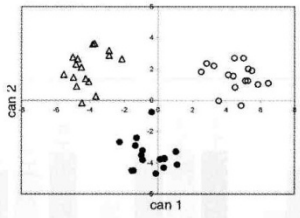
(a)



Sampling date	Weeks post-bloom	Classified as 18	19	20
	18	**15**	0	0
		100.0%	0.0	0.0
	19	0	**15**	0
		0.0	100.0%	0.0
	20	0	0	**15**
		0.0	0.0	100.0%

Total: Correct 100%, Incorrect 0%

(b)



Sampling date	Weeks post-bloom	Classified as 18	19	20
	18	**16**	0	0
		100.0%	0.0	0.0
	19	0	**16**	0
		0.0	100.0%	0.0
	20	0	1	**15**
		0.0	6.25%	93.75%

Total: Correct 98%, Incorrect 2%

(c)

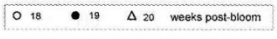


Figure 3. Canonical plot and discriminant analysis cross-validation summary of (a) physicochemical analyses data, (b) electronic nose data measured in the laboratory, and (c) electronic nose data measured in the vineyard, for Cabernet Sauvignon grapes sampled 18, 19, and 20 weeks post-bloom in 2005. Cells indicate number of samples collected for a particular week (rows), and week in which discriminant analysis indicated they should be categorized (columns).

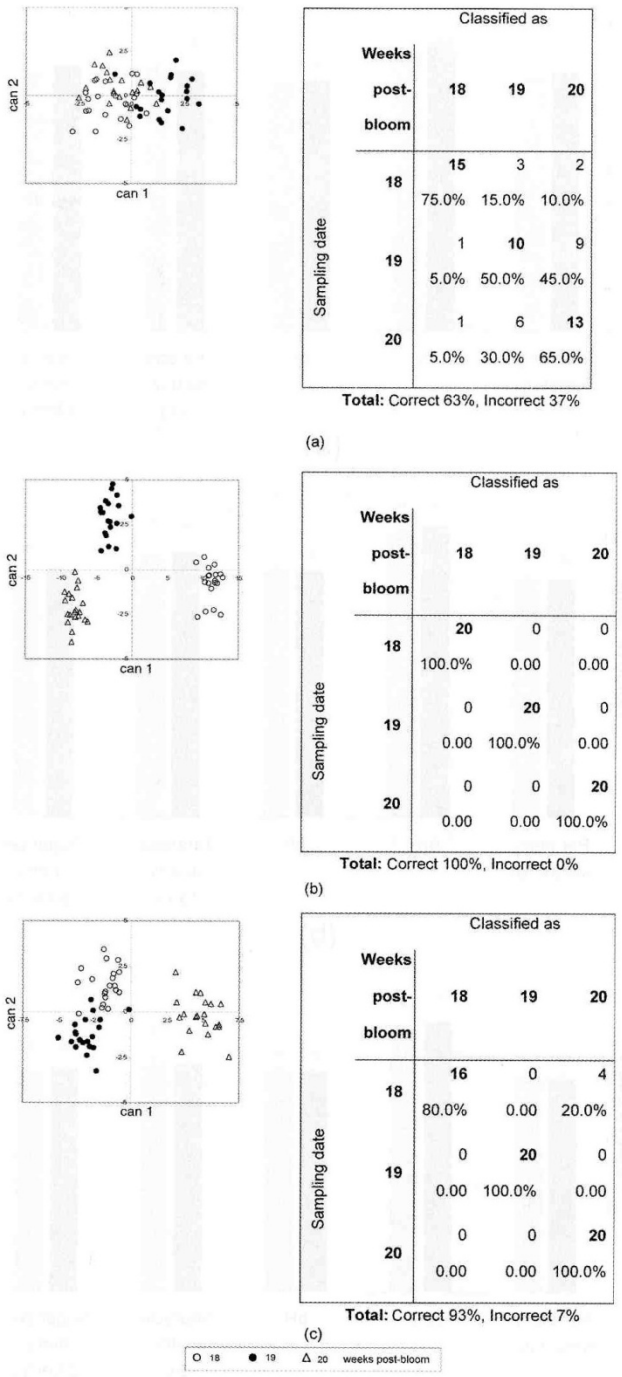


Figure 4. Canonical plot and discriminant analysis cross-validation summary of (a) physicochemical analyses data, (b) electronic nose data measured in the laboratory, and (c) electronic nose data measured in the vineyard, for Cabernet Sauvignon grapes sampled 18, 19, and 20 weeks post-bloom in 2006. Cells indicate number of samples collected for a particular week (rows), and week in which discriminant analysis indicated they should be categorized (columns).

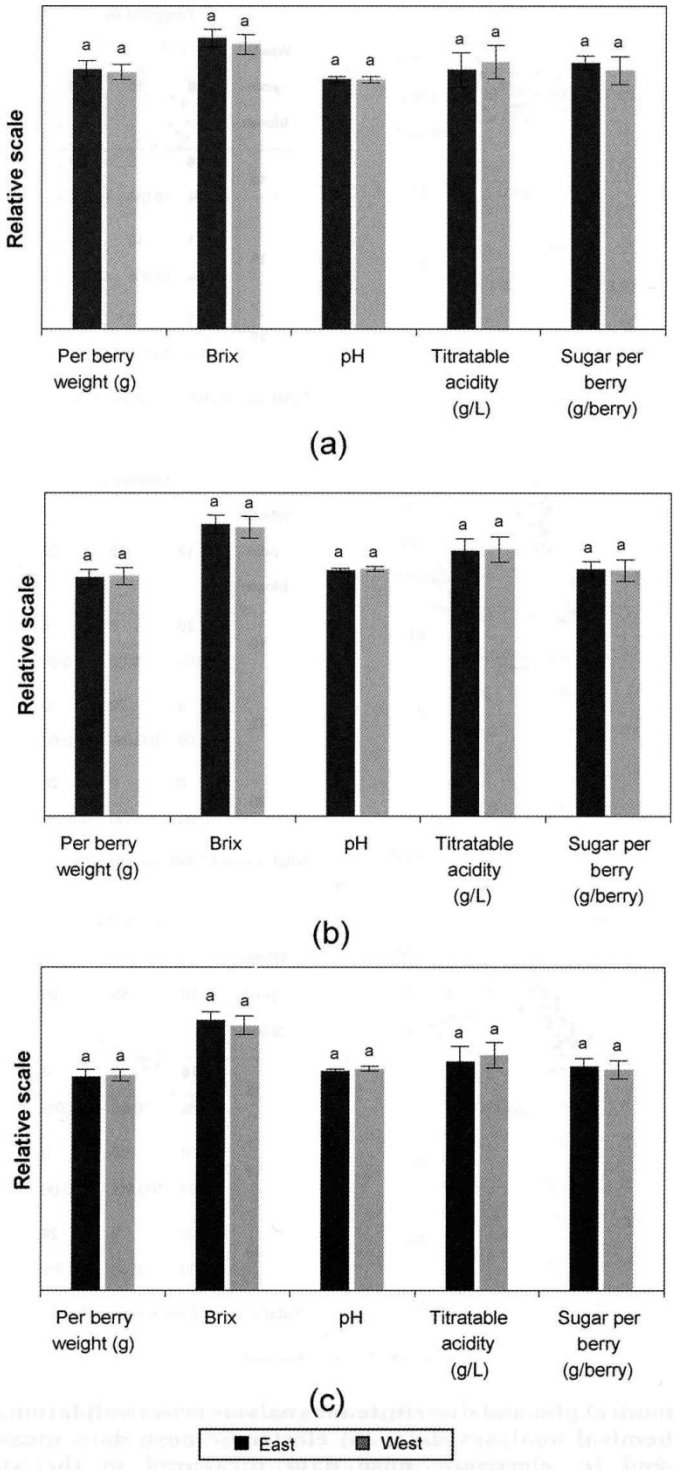
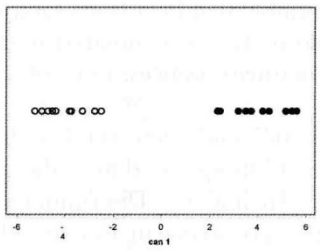


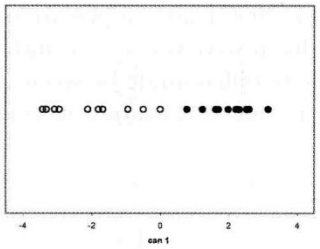
Figure 5. Physicochemical analyses of Cabernet Sauvignon fruit from east versus west canopy side at (a) 18, (b) 19, and (c) 20 weeks post-bloom. Means associated with different letters are significantly different, $\alpha = 0.05$, by least significant difference. Error bars represent 95% confidence intervals.



Sampled as	Canopy side	Classified as: East	Classified as: West
	East	**10** 100.0%	0 0.00
	West	0 0.00	**10** 100.0%

Total: Correct 100%, Incorrect 0%

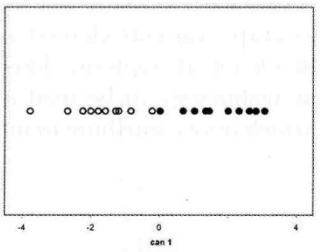
(a)



Sampled as	Canopy side	Classified as: East	Classified as: West
	East	**9** 90.0%	1 10.00
	West	0 0.00	**10** 100.0%

Total: Correct 95%, Incorrect 5%

(b)



Sampled as	Canopy side	Classified as: East	Classified as: West
	East	**9** 90.0%	1 10.00
	West	1 10.00	**9** 90.0%

Total: Correct 90%, Incorrect 10%

(c)

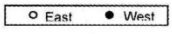


Figure 6. Canonical plot and discriminant analysis cross-validation summary of electronic nose measurements of Cabernet Sauvignon fruit from east versus west canopy side at (a) 18, (b) 19, and (c) 20 weeks post-bloom. Cells indicate number of samples collected for a particular side (rows), and side in which discriminant analysis indicated they should be categorized (columns).

differentiate maturity levels. The relatively high number of misclassified samples shown in the discriminant analysis cross-validation (Figure 4a), as compared to data from 2005 may be the result of a smaller number of maturity indices, most of which had limited to no variations over sampling weeks.

The electronic nose produced similar results in 2005 and 2006. While differences in 2006 physicochemical data were limited between sampling dates, the electronic nose was able to differentiate samples (Figures 4b and c). Discriminant analysis cross-validation showed that electronic nose data were sufficient to correctly classify most samples in their respective groups, indicating greater capability to differentiate maturity than standard physicochemical analyses. Separation noted by the electronic nose in both seasons could be attributed to the change in headspace volatiles, not considered in physicochemical analyses.

Fruit on different sides of the canopy may vary in maturity due to variations in solar exposure (Smart and Robinson, 1991; Downey *et al.*, 2006). Variation in aroma and flavor maturity may not be reflected in differences in berry weight, Brix, pH or titratable acidity (Smart and Robinson, 1991). In this study, berry weight, Brix, sugar per berry, pH and titratable acidity measured on 2006 fruit samples from east and west canopy sides at 18, 19, and 20 weeks post-bloom were not significantly different (Figure 5). However, the electronic nose was able to differentiate between samples in the field from east versus west canopy side, likely due to variation in fruit volatile compounds (Figure 6).

Conclusions

A conducting polymer-based electronic nose (Cyranose 320) was used to differentiate levels of cabernet sauvignon maturity based on the evaluation of grape volatiles. The electronic nose evaluation was compared with 11 and five maturity indices in 2005 and 2006, respectively. The electronic nose system was able to differentiate between three maturity groups each season with one non-destructive measurement. Additionally, the electronic nose was able to distinguish maturity levels between canopy sides. The success of this approach in maturity evaluation is likely due to the vast number of chemical species which contribute to grape varietal character, most of which are generally not considered in standard chemical analysis. This research demonstrates the potential for this relatively new technology to be used as a rapid and objective tool for evaluating grape maturity, which may contribute to maximizing wine quality with minimum cost.

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