Electronic Nose Analysis of Cabernet Sauvignon (*Vitis vinifera* L.) Grape and Wine Volatile Differences during Cold Soak and Postfermentation

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Abstract: Cold soak is a prefermentation maceration process at cold temperatures, traditionally used to enhance red wine color. This study monitored changes in *Vitis vinifera* L. cv. Cabernet Sauvignon volatiles using a commercial conducting polymer electronic nose (ENose) during a five-day cold soak and postfermentation. Principal component analysis (PCA) of juice volatiles detected by the ENose during cold soak showed PC1 accounted for 95.7% of the variation. Various volatile associations were made with specific ENose sensors. In comparison, PCA of must chemistries had 52.4% of the variation accounted for by PC1. The PCA of wine volatiles detected by GC-MS showed PC1 accounted for 97.1% of the variation between control and cold soak treatment, where control wine volatiles were associated with several ethyl esters, while cold soak wine volatiles were associated with diethyl succinate, isovaleric acid, benzyl alcohol, 3-methyl butanol, *cis*-3-hexenol, γ-nonalactone, benzaldehyde, 2-methyl propanol, phenethyl acetate, 1-octanol, β-damascenone, terpinene-4-ol, γ-butyrolactone, ethyl acetate, hexanoic acid, citronellol, phenethyl alcohol, and *n*-butanol. Comparatively, PC1 accounted for 100% of the total variance when using the ENose to measure volatile composition. Sensory evaluation did not demonstrate significant differences in aroma between control and cold soak wines. This study demonstrates differences in volatile chemistry between control and cold soak wines, as well as the ability to use a conducting polymer ENose as a simple tool for analysis of volatiles.

Key words: electronic nose (ENose), cold soak, Cabernet Sauvignon, aroma, volatiles, glycosides

Wine sensory attributes can be influenced by prefermentation processing and fermentation techniques, including temperature (McMahon et al. 1999, Reynolds et al. 2001), yeast species and strains (Ramey and Ough 1980), malolactic fermentation species and strains (Swiegers et al. 2005), and maceration (Ramey et al. 1986). Cap management (including prefermentation maceration) can also influence wine composition (McMahon et al. 1999). Although studies have explored how production methods affect wine composition, few have investigated the influence of cold soak on wine volatiles.

Cold soak, or prefermentation maceration at temperatures usually less than 10°C, is primarily used for red varieties to enhance wine color stability. Grape anthocyanins exist as free anthocyanidins and polymeric complexes (Timberlake and Bridle 1976). Stabilization of anthocyanins occurs by complexing with colorless tannins, primarily flavan-3-ol polymers (Timberlake and Bridle 1976). Anthocyanidin-tannin complexes in grape must polymerize through hydrophobic interactions and to a lesser extent by covalent bonding through condensation reactions (Timberlake and Bridle 1976). Acetaldehyde reportedly mediates these reactions in wines, but polymerization is reduced in the presence of alcohol (Timberlake and Bridle 1976). Regardless, maceration time has been related to enhanced extraction and accumulation of water-soluble tannins, some of which may contribute to anthocyanidin-tannin complexes (Heatherbell et al. 1996).

While Cabernet Sauvignon generally produces wines of high color intensity and stability and, therefore, need not be cold soaked, there is interest in increasing varietal aroma and flavor. Aroma is contributed by volatile compounds that are sensed orthonasally, while flavor is a retronasal sensation of tastes (sweet, sour, bitter, salty, umami) and aromas (Lambrechts and Pretorius 2000, Meilgaard et al. 2007). Several chemical classes of aroma and flavor compounds are found in grapes and wine, including monoterpenes, C_{13}-norisoprenoids, fusel (higher molecular weight) alcohols, esters, shikimic acid metabolites, and carbonyl compounds (Etievant 1991, Swiegers et al. 2005). Some of these compounds exist as free volatiles or as nonodorous glycosidically-bound sugar conjugates. Many glycosides, commonly found in higher concentrations than free volatiles in fruit, are aroma and flavor precursors (Mansfield and Zoecklein 2003). Cold soak can increase glycoside concentrations (Heatherbell et al. 1996, McMahon et al. 1999). Liberation of glycosidically-bound aroma and flavor precursors occurs via acid or enzymatic hydrolysis (Sefton et al. 1993, Williams et al. 1996). Acid hydrolysis of glycosides can occur at a pH range from 3.0 to 3.5, splitting the aglycone from the 1→6 glycosidic bond by protonation to form a hydroxyl group on the sugar (Timell 1964). Enzymatic hydrolysis cleaves the 1→6 linkage, liberating the aglycone from the mono- or

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disaccharide (Zoecklein et al. 1997). Liberation of aroma and flavor compounds may be exemplified further during aging (Zoecklein et al. 1997).

Aglycones and sugar moieties may vary among grape varieties, contributing to volatile aroma variations (Sefton et al. 1993). These differences may be exemplified through alterations in processing, such as the use of cold soak. Pinot noir held at 10°C for six days produced wines with higher intensities of woody, tobacco, and berry aromas, and greater mouthfeel (Heatherbell et al. 1996). Black currant and flavor intensity differences were identified in cold-soaked Shiraz wines (Reynolds et al. 2001). Cold-soaked Pinotage wines had increased berry and plum aroma and flavor intensities, contributing to improved sensory quality 6 months postfermentation (Marais 2003).

A major challenge for the grape and wine industry is to replace time-consuming laboratory analyses, used in process and quality-control monitoring, with new application techniques that are fast, precise, and accurate. While sensory evaluation is an important research tool, it is subjective and may require extensive preparation and panel training (Lesschaeve 2007). In addition, it may be difficult to relate individual aroma and flavor compounds to a sensory response or attribute in a complex matrix such as wine (Genovese et al. 2005). Traditional volatile analytical methods such as GC and GC-MS are time-consuming and require extensive sample preparation and training (Mallikarjunan 2005). Therefore, there is interest in an alternative simple and efficient method for analyzing volatile compounds.

Electronic nose (ENose) technology represents a possible alternative to volatile measurement in some applications. These are multisensor array systems designed to measure headspace volatiles. ENose systems vary primarily in sensor type, which denotes their selectivity and sensitivity to volatile compounds, and includes metal oxide sensors (MOS), surface acoustic wave (SAW), quartz microbalance sensors (QMS), and conducting polymers (CP) (Pearce et al. 2003, Mallikarjunan 2005). The adsorption of volatiles on the sensor surface causes a physical or chemical change in the sensor, producing a specific reading for that sample in a unique pattern or "fingerprint" of the volatiles (Mallikarjunan 2005). Chemometric techniques and multivariate statistical analysis make it possible to distinguish among groups of samples and possibly identify individual sample components. ENose systems are so-named because their operation is analogous to the human sense of smell, where multiple nerve cells in the olfactory epithelium provide responses so the brain can identify and characterize aromas (Mallikarjunan 2005). Primary problems reported with the use of conducting polymer sensors in wines are the influence of ethanol (Ragazzo-Sanchez et al. 2006) and water vapor (Janata and Josowicz 2003). However, a previous study identified that the use of an ethanol baseline with conducting polymer ENose systems minimizes the ethanol interference with sensor readings, which bases discrimination primarily on wine aroma and flavor volatiles (Santos et al. 2004). The objective of the current study was to monitor Cabernet Sauvignon cold soak musts and wine volatiles using a 32-sensor conducting polymer ENose.

Materials and Methods

Harvest and fermentation. Cabernet Sauvignon (*Vitis vinifera* L.) clone 8 grapes were grown on an open-lyre training system in Winchester, Virginia. After a preliminary study in 2007 with 136 kg of grapes, ~227 kg of grapes were hand harvested on 15 Oct 2008. Harvested grapes were stored at 7°C and processed within 24 hr. Fruit was crushed and destemmed using a Wottle (Anton, Poysdorf, Austria) destemmer-crusher with 50% berry breakage, estimated visually. Must and berries were evenly distributed into six open-top 60-L Nalgene fermenting bins of equal height and diameter. Treatments consisted of three control and three cold soak replicates, randomly selected. Each replicate was treated with 250 mg/L Velcorin (dimethyl-dicarbonate; Scott Laboratories, Petaluma, CA) and chilled for 24 hr at 7°C.

Prior to yeast inoculation, adjustments were made to 150 mg/L fermentable nitrogen availability by adding 6.10 g FermAid K (Lallemand, Rexdale, Ontario). Immediately after must adjustments, control bins were inoculated with *Saccharomyces cerevisiae* ICV-D21 (Lallemand) at a rate of 0.25 g/L, following hydration, as per supplier recommendations, with additions of 0.30 g/L Go-Ferm (Lallemand). After one-third of the fermentation, an additional 6.10 g diammonium phosphate (DAP) was added to adjust the remaining fermentable nitrogen concentration. The three cold soak replicates were stored at 7°C for an additional five days after must adjustments. During cold soak, caps were punched once a day and must was sampled daily. At the end of cold soak, musts were inoculated as control bins had been previously. Caps were punched three times daily during fermentation. Fermentation, at 23 ± 2°C, was monitored by hydrometry. At dryness (<1% residual sugar), wines were pressed using a basket press. Free and press fractions were kept separate and stored at 7°C for 24 hr before racking into 3.80-L and 1.90-L glass bottles and then stored at 7°C.

Berry, juice, and wine chemistry. At harvest, 600 berries were selected, weighed, and crushed in Premium Red Line 15 x 20 cm plastic sampling bags (Minigrip, Seguin, TX) to determine fruit chemistry. Crushed fruit was filtered through 0.45-µm syringe filters (Whatman, Piscataway, NJ) and analyzed for pH, titratable acidity (TA), and Brix using standard methods (Zoecklein et al. 1999). Malic acid concentration was determined enzymatically (R-Biopharm AG, Darmstadt, Germany). Color intensity ($A_{420} + A_{520}$) and hue (A_{420}/A_{520}) were measured using a Genesys 5 spectrophotometer (Spectronic, Garforth, UK). Fermentable nitrogen was measured enzymatically (Megazyme, Wicklow, Ireland).

Juice samples, taken at crush and throughout cold soak, and wines were analyzed for pH, TA, Brix, malic acid, and color (intensity and hue) as described above. Phenol-free glycosyl-glucose (PFGG) and total glycosyl-glucose (TGG) were determined as described (Williams 1995) and modified (Zoecklein et al. 2000). Wine residual sugar concentration was estimated by copper sulfate reduction (Clinitest, Bayer,

Elkhart, IN) and ethanol percentage (v/v) by FTIR (Foss WineScan FT 120, Eden Prairie, MN).

Wine samples for GC-MS analysis were prepared using 4 mL samples with 1.0 g NaCl in 10-mL clear glass vials sealed with a septum (MicroLiter Analytical Supplies, Suwanee, GA). Vials were preincubated for 30 sec at 30°C with agitation at 250 rpm. A CAR/DVB/PDMS grey SPME fiber (Supelco Sigma-Aldrich, St. Louis, MO) was used to penetrate vials to a 32 mm depth into the headspace and equilibrated for 30 min. GC-MS (6890N Network GC System, 5975B inert MSD; Agilent Technologies, Santa Clara, CA) settings included an injector temperature of 250°C, DB-wax column (30 m x 2 mm), helium carrier gas with a flow rate of 1 mL/min, and oven temperature 40°C with a ramp rate of 6°C/min to 230°C. Fifty-four wine volatile compounds were identified via ion matching, but only 42 volatiles reported in Cabernet Sauvignon wines (Genovese et al. 2005) were quantified using standard concentration solutions. Standard compounds and wine samples were manually integrated and quantified.

Juice and wines were also analyzed using a conducting polymer, 32-sensor, Cyranose 320 electronic nose (Smiths Detection, Pasadena, CA). Instrument settings were altered using a previously optimized evaluation method (Athamneh et al. 2008) (Table 1). Ethanol standard solutions (three standards per treatment) were used to create an ethanol baseline to evaluate the impact on polymer sensor response and to minimize differences in alcohol concentration among wine samples while using the ENose. Concentrations for ethanol standards were based on the alcohol concentration recorded for each wine treatment, as determined by FTIR. For ENose evaluation of samples, three aliquots (20 mL) from each replicate throughout cold soak and postfermentation were pipetted into 40-mL clear glass vials sealed with teflon/silicone 2.54 mm septa (MicroLiter Analytical Supplies) and incubated in a 30°C water bath for 20 min. A venting needle (22 gauge) was injected into the septum, followed by injection of the Cyranose 320 needle for headspace evaluation.

Sensory analysis. Sensory testing was conducted in the Sensory Evaluation Laboratory at Virginia Tech using standard conditions as described (Meilgaard et al. 2007). A consumer panel was comprised of 54 women and men between the ages of 21 and 25 who consumed wine regularly (more than once a week). Triangle difference sensory tests for wine aroma were conducted 5 months postfermentation. Treatment replications were pooled prior to evaluation, following a sensory screening for sulfur-like off odors. A balanced order of presentation for all combinations (n = 6) was used. Panelists were seated at individual booths. Wines were identified by a randomly selected 3-digit code. Standard ISO glasses were used and filled to 20 mL. Each glass was topped with a plastic petri dish and presented at 19°C ± 2°C under red light. Since 54 panelists were used ($\alpha = 0.05$, $\beta = 0.10$, $\rho_{max} = 30\%$), 25 correct responses were needed for significant difference for evaluations (Meilgaard et al. 2007).

Table 1 Cyranose 320 electronic nose wine evaluation parameters.

Method setting	Parameter setting
Baseline purge	30 sec
Sample draw	20 sec
Air intake purge	10 sec
Sample gas purge	60 sec
Digital filtering	On
Substrate heater	On: 40°C
Training repeat count	1
Identifying repeat count	1
Statistical analysis	
Algorithm	Canonical
Preprocessing	Auto-scaling
Normalization	Normal 1
Identification quantity	Medium

Statistical analysis. ENose data was analyzed by linear canonical distribution and principal component analysis (PCA) using JMP version 7 software (SAS Institute, Cary, NC). Chemistries and GC-MS data were also analyzed by PCA. Cross-validation, a measure of PCA robustness, was analyzed. A classification rate of 75% is considered satisfactory for this study; however, greater classification (i.e., higher percentages) was considered an improvement to the model. Juice and wine chemistry data and GC-MS volatile concentrations were statistically analyzed using one-way ANOVA and Student's *t*-test for least significant differences in JMP version 7.

Results and Discussion

Must analysis. The canonical distribution of volatile changes during the cold soak showed 100% separation of the volatile components detected by the ENose on each day of cold soak (Figure 1). Three-dimensional canonical distribution

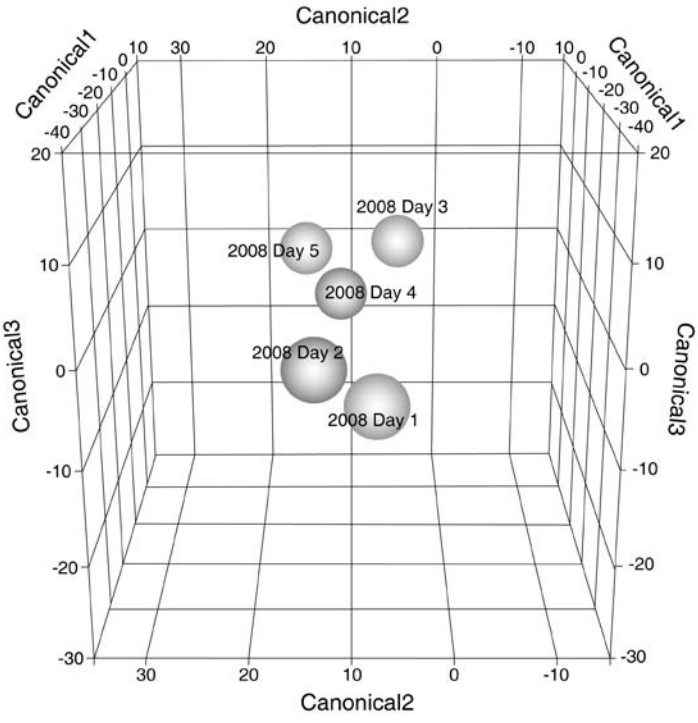

Figure 1 Canonical distribution of differences detected by ENose analysis during 5-day cold soak of Cabernet Sauvignon must (significant differences at $\alpha = 0.05$ level indicated by nonintersecting circles).

clustered ENose data based on the greatest degree of variation. Each circle surrounding data clusters represents the multivariate mean for that group at a 95% significance level. Nonintersecting circles are not a result of axis depth, but represent significant differences.

Principal component analysis (PCA) of ENose juice data throughout cold soak showed that PC1 (designated as P1; Figure 2) accounted for 95.7% of the variation, PC2 (P2) 3.7%, and PC3 (P3) 0.4%. Associations with specific sensors are indicated by the vectors extending from the center of the graph. These associations indicate changes in volatiles over time during cold soak as detected by the ENose. The sensor polymers used included polypyrrole, polyaniline, polythiophene, polyindole, and polyacetylene at different oxidative/reductive states (Mallikarjunan 2005, Pearce et al. 2003). The use of multiple polymers and redox states provides some selectivity, although crossover among classes of volatiles and sensors does occur (Mallikarjunan 2005, Pearce et al. 2003). Sensors 5, 6, 23, and 31 contributed most to variation, as determined by vector length. Day 1 cold soak volatiles were associated with sensors 18, 24, and 30, day 2 with sensors 6 and 23, and days 3, 4, and 5 had no direct association with any particular sensor. Volatile components of the cold soak juice changed through day 3, according to the ENose data.

Cross-validation indicated the ENose correctly classified 82% of the samples (data not shown). Cross-validation provides the percentage of samples matched correctly with their appropriate class designation, an indicator of ENose system efficacy (Santos et al. 2004). These results indicate the ability of the ENose to discriminate volatile differences over time due to processing alterations, which is consistent with a study on beer (Pearce et al. 1993).

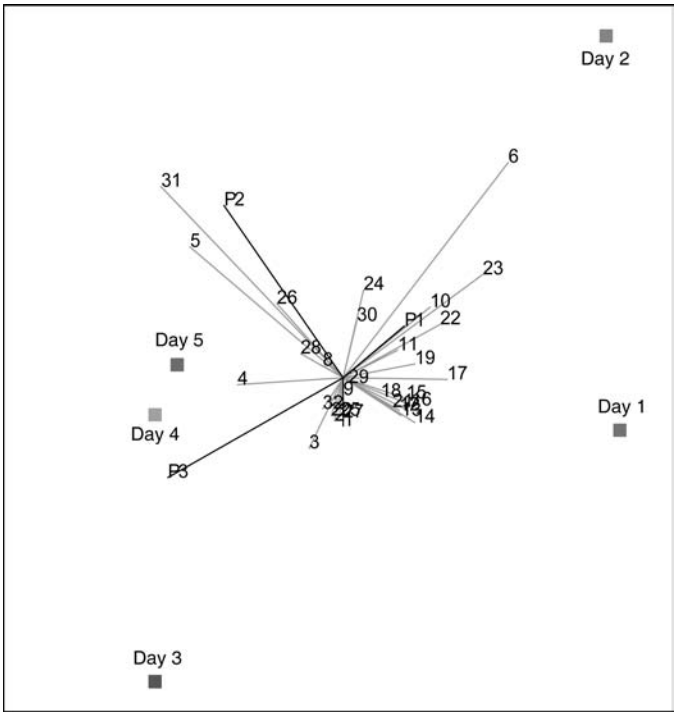

Figure 2 PCA distribution of Cabernet Sauvignon must volatiles detected by ENose during 5-day cold soak.

Compared to ENose data, must chemistry PCA distributions explained less variation among cold soak days, as determined by the lower PC1 percentage (Figure 3). PC1 accounted for 52.4% of the variation, PC2 for 32.0%, and PC3 for 10.5%. Day 1 was associated with color hue, day 2 with Brix, day 3 with absorbance at 280 and 320 nm, and days 4 and 5 with color intensity. Variation was contributed primarily by absorbance at 520 nm, as determined by vector length.

Juice chemistries throughout cold soak are listed (Table 2). Total glycosyl-glucose (TGG) differed, while phenol-free glycosyl-glucose (PFGG) decreased throughout cold soak. Decreases in glycoside have been attributed to hydrolysis (Williams et al. 1996). An increase in must pH during cold soak, supported in the literature (Heatherbell et al. 1996), may have resulted from increased extraction. A statistical, but limited, difference in Brix during cold soak was found. This was not the result of biotic activity (data not shown).

Color hue decreased and intensity increased during cold soak. An increase in absorption at 420 nm has been reported with increased skin contact (Reynolds et al. 2001). Small changes in absorbance at 520 nm may have resulted from slow extraction of pigmented compounds at cold soak temperatures (Sacchi et al. 2005). Variations in color were attributed to possible extraction differences during cold soak.

Wine analysis. In the PCA distributions of wine volatiles analyzed by the ENose (Figure 4), sensor 31 contributed the greatest variation to sample discrimination as determined by vector length. This sensor, originally considered an outlier, was initially eliminated from analysis. However, another sensor would then contribute the greatest variation, and so on. Thus, sensor 31 was retained in the PCA analysis. PC1 accounted for 100% of the variation between control and cold soak wines. Sensor associations, indicative of sensor-volatile

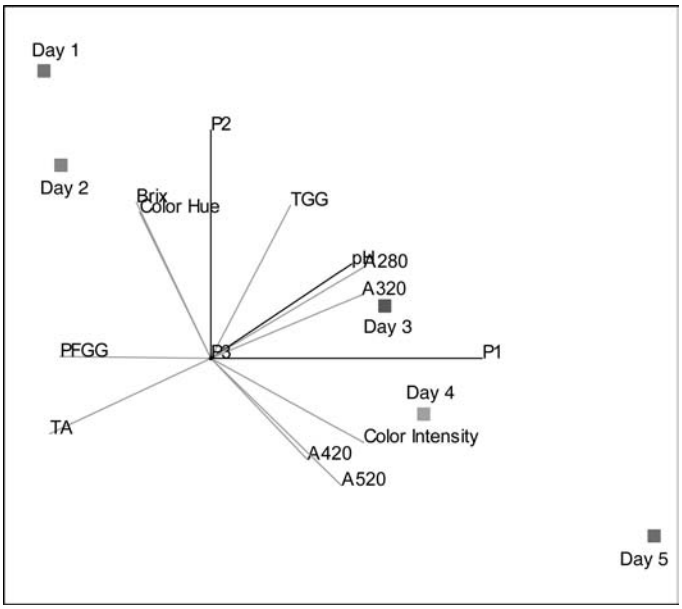

Figure 3 PCA distribution of Cabernet Sauvignon must chemistry differences determined by pH, TA, Brix, phenol-free glycosyl-glucose (PFGG), total glycosyl-glucose (TGG), absorbance (280 nm, 320 nm, 420 nm, 520 nm), and color (hue and intensity) during cold soak.

Table 2 Changes in juice pH, titratable acidity (TA as g/L tartaric acid), Brix, phenol-free glycosyl-glucose (PFGG), total glycosyl-glucose (TGG), absorbance (280 nm, 320 nm, 420 nm, 520 nm), and color (hue and intensity) throughout a five-day cold soak in Cabernet Sauvignon must (n = 3).

Analysis	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
pH	3.62 ± 0.01 d[a]	3.80 ± 0.01 a	3.73 ± 0.01 c	3.81 ± 0.01 a	3.75 ± 0.01 b	3.82 ± 0.01 a
TA (g/L)	7.23 ± 0.09 a	4.98 ± 0.05 b	4.95 ± 0.05 b	4.71 ± 0.05 c	4.71 ± 0.05 c	3.82 ± 0.05 d
Brix	23.4 ± 0.1 bc	23.8 ± 0.1 a	23.8 ± 0.1 a	23.7 ± 0.1 ab	23.2 ± 0.1 c	23.1 ± 0.1 c
PFGG (μM)	680 ± 78 a	642 ± 96 a	400 ± 78 b	431 ± 78 ab	294 ± 78 b	330 ± 78 b
TGG (μM)	681 ± 70 b	965 ± 70 a	917 ± 70 a	839 ± 70 ab	841 ± 70 ab	902 ± 70 a
A_{280nm}	0.94 ± 0.04 c	1.25 ± 0.03 b	1.25 ± 0.03 b	1.35 ± 0.03 a	1.35 ± 0.03 a	1.32 ± 0.03 ab
A_{320nm}	0.56 ± 0.03 c	0.63 ± 0.02 bc	0.64 ± 0.02 abc	0.69 ± 0.02 a	0.69 ± 0.02 a	0.66 ± 0.02 ab
A_{420nm}	0.06 ± 0.01 a	0.06 ± 0.01 a	0.05 ± 0.01 a	0.06 ± 0.01 a	0.06 ± 0.01 a	0.07 ± 0.01 a
A_{520nm}	0.07 ± 0.01 ab	0.05 ± 0.01 b	0.06 ± 0.01 c	0.08 ± 0.01 ab	0.08 ± 0.01 ab	0.10 ± 0.01 a
Color hue	0.80 ± 0.17 b	1.30 ± 0.10 a	0.88 ± 0.10 b	0.83 ± 0.10 b	0.83 ± 0.10 b	0.67 ± 0.10 b
Color intensity	0.12 ± 0.02 ab	0.12 ± 0.01 b	0.11 ± 0.01 b	0.14 ± 0.01 ab	0.14 ± 0.01 ab	0.16 ± 0.01 a

[a]Values with different letters within a row indicate significant differences ($\alpha = 0.05$).

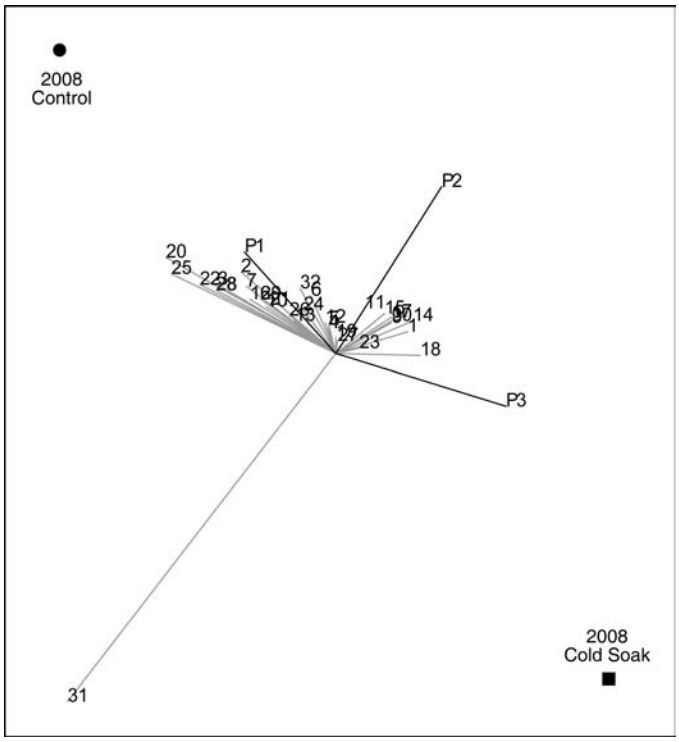

Figure 4 PCA distribution of Cabernet Sauvignon control and cold soak wine volatiles detected by ENose.

interactions during ENose analysis, were compared between control and cold soak treatments. Control volatiles were associated with sensors 6, 13, 24, 26, and 32, while cold soak volatiles were directly opposite of the control placement (and below the vector plane), indicating little association with any specific sensor.

Wine volatile differences immediately postfermentation (Figure 5A) and 5 months postfermentation (Figure 5B) showed separation of wines based on treatment. Circles represent the multivariate means. As they are not intersecting, samples are considered significantly different at the 95% level. Cross-validation indicated 95% correct classification immediately postfermentation (data not shown).

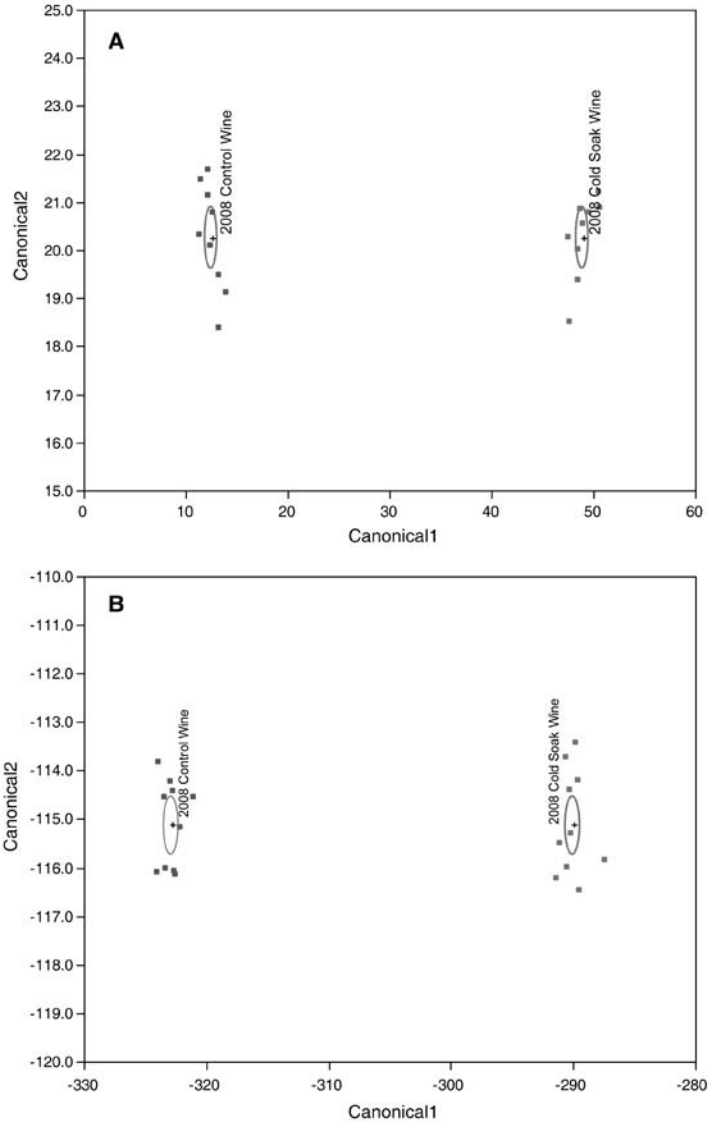

Figure 5 Canonical distribution of Cabernet Sauvignon wine volatiles as detected by ENose (**A**) immediately and (**B**) 5 months postfermentation (significant differences at $\alpha = 0.05$ level indicated by nonintersecting points; circles represent the multivariate mean for a specific group of samples).

Table 3 Volatile compounds analyzed by SPME GC-MS, odor thresholds, and concentration means with standard errors for control and cold soak Cabernet Sauvignon wines.

Compound	Odor threshold (µg/L)[a]	Concentration (µg/L)[a]	
		Control	Cold soak
Acetic acid	200,000[d]	+	+
Benzaldehyde	350–3500[b]	10.9 ± 2.63 a	20.2 ± 2.63 a
2,3-Benzofuran	na	+	+
Benzyl alcohol	10,000[b]	478.2 ± 21.5 a	560.8 ± 21.5 a
2,3-Butanediol	2.3–6.5[b]	nd	nd
γ-Butyrolactone	na	22970.0 ± 735.7 a	23070.0 ± 735.7 a
cis-3-Hexenol	70[b]	17.8 ± 2.0 a	19.8 ± 2.0 a
Citronellol	100[d]	24.3 ± 2.8 a	33.3 ± 2.8 a
β-Damascenone	0.05[d]	3.22 ± 1.00 a	4.53 ± 1.00 a
Decanal	0.1–2.0[b]	+	+
Decanoic acid	15,000[d]	+	+
Diethyl succinate	na	90.8 ± 9.3 a	133.3 ± 9.3 b
Dodecanoic acid	10,000[b]	+	+
2-Dodecanone	270,000[b]	nd	nd
Estragole	na	nd	nd
Ethyl acetate	7500[d]	56770.0 ± 3539.3 a	60200.0 ± 3539.3 a
Ethyl decanoate	na	85.4 ± 10.4 a	74.8 ± 10.4 a
Ethyl dodecanoate	na	26.9 ± 4.3 a	18.7 ± 4.3 a
Ethyl heptanoate	2.2[b]	1.23 ± 0.21 a	1.07 ± 0.21 a
Ethyl hexanoate	5.0[d]	261.7 ± 52.6 a	209.0 ± 52.6 a
2-Ethylhexanoic acid	na	22073.3 ± 2969.1 a	29390.0 ± 2969.1 a
2-Ethyl-1-hexanol	na	3.18 ± 0.13 a	2.75 ± 0.13 a
Ethyl myristate	na	27.9 ± 0.1 a	27.6 ± 0.1 a
Ethyl nonanoate	na	19.8 ± 0.4 a	19.6 ± 0.4 a
Ethyl octanoate	2.0[d]	1662.9 ± 183.8 a	1509.3 ± 183.8 a
Ethyl palmitate	>2000[b]	63.6 ± 2.6 b	52.4 ± 2.6 a
Hexanoic acid	3000[d]	2090.0 ± 212.2 a	2666.7 ± 212.2 a
Hexyl acetate	2.0[b]	12.7 ± 2.14 a	9.04 ± 2.14 a
Isoamyl acetate	30[d]	7460.7 ± 1969.4 a	6815.5 ± 1969.4 a
Isoamyl octanoate	na	+	+
Isovaleric acid	120–700[b]	1256.7 ± 310.0 a	1726.7 ± 310.0 a
Linalool	15[d]	+	+
Methionol	na	5320.0 ± 191.7 a	5686.7 ± 191.7 a
2-Methyl benzofuran	na	nd	nd
3-Methyl butanol	30,000[d]	89136.7 ± 6037.7 a	91876.7 ± 6037.7 a
6-Methyl-5-heptene- 2-one	2000[b]	+	+
3-Methyl-1-pentanol	na	+	+
2-Methyl propanol	40,000[d]	+	+
Methyl salicylate	40[b]	9.45 ± 0.02 a	9.38 ± 0.02 a
n-Butanol	500[b]	6680.0 ± 384.4 a	6980.0 ± 384.4 a
Nerol	300[b]	nd	nd
n-Hexanol	8000[d]	2550.0 ± 373.1 a	3056.7 ± 373.1 a
γ-Nonalactone	na	13.3 ± 2.4 a	20.0 ± 2.4 a
Nonanoic acid	3000[b]	+	+
Nonanol	50[b]	+	+
Nonyl aldehyde (nonanal)	1.0[b]	+	+
Octanoic acid	3000[b]	1726.7 ± 303.9 a	2090.0 ± 303.9 a
1-Octanol	110–130[b]	51.2 ± 4.1 a	56.2 ± 4.1 a
Phenethyl acetate	250[d]	724.9 ± 61.5 a	749.9 ± 61.5 a
Phenethyl alcohol	750[c]	39686.7 ± 884.3 a	41920.0 ± 884.3 a
1-Propanol	9000[b]	+	+
Terpinene-4-ol	na	8.02 ± 0.70 a	9.37 ± 0.70 a
α-Terpineol	350[c]	+	+

[a]na: threshold level is not available; nd: compound was not detected during analysis; +: compound was qualitatively identified by MS ion matching to a library database (WILEY7NIST05). Varying letters in a row indicate significant differences (α = 0.05).
[b]Odor detection threshold determined in water from Leffingwell & Associates Database (www.leffingwell.com/odorthre.htm).
[c]Odor recognition threshold determined in water (Ohloff 1978).
[d]Odor recognition threshold determined in 90% water and 10% ethanol (Guth 1997).

GC-MS identified variation in individual volatile compounds based on treatment (Table 3). Generally, ethyl esters, which may be influenced by skin contact duration (Falque and Fernandez 1996, Lambrechts and Pretorius 2000) were higher in control wine. Ethyl acetate, which has been linked to increased skin contact time (Falque and Fernandez 1996), was found at a higher, although not significant, concentration in the cold soak wine. Diethyl succinate was found in significantly greater concentrations in cold soak wine. GC-MS results support possible variation in the headspace components of control and cold soak treatments, which was also found by ENose evaluation.

In the PCA distribution of GC-MS data for control and cold soak wines (Figure 6), PC1 accounted for 97.1% of the variation. Variation between control and cold soak wines was primarily based on octanoic acid and methionol vectors. Control wines were associated with hexyl acetate, isoamyl acetate, methyl salicylate, ethyl palmitate, ethyl myristate, ethyl dodecanoate, ethyl decanoate, ethyl heptanoate, ethyl octanoate, ethyl hexanoate, ethyl nonanoate, and 2-ethyl-1-hexanol. Cold soak wines were linked with diethyl succinate, isovaleric acid, benzyl alcohol, 3-methyl butanol, *cis*-3-hexenol, γ-nonalactone, benzaldehyde, 2-methyl propanol, phenethyl acetate, 1-octanol, β-damascenone, terpinene-4-ol, γ-butyrolactone, ethyl acetate, hexanoic acid, citronellol, phenethyl alcohol, and *n*-butanol. In general, ethyl esters were primarily coupled with control wines. These GC-MS results highlight the differences in the volatile profile associated with a cold soak treatment compared with a non-cold soak control and support volatile-sensor differences as detected by the ENose.

Wine chemistries for control and cold soak wines showed no differences in TGG or PFGG concentration (Table 4). Rates of extraction and/or hydrolysis may have differed, contributing to a lack of difference in glycosides between treatments as suggested previously (Williams et al. 1996). A reduction in PFGG during fermentation may have contributed to liberation of volatiles, detected by GC-MS and ENose analysis. Wine pH, which was lower in cold soak wine, is a possible factor in glycoside hydrolysis. Studies on glycoside hydrolysis have shown that at pH 3.2 various aroma compounds are liberated, including linalool, α-terpineol, β-damascenone, benzaldehyde, and benzyl alcohol (Francis et al. 1996), which were found in the wines of this study at concentrations from 0.1 to 500 µg/L.

The PCA distribution of the wine chemistries (Figure 7) showed PC1 accounting for 100% of the variation. Control wines were associated with PFGG and TGG and cold soak wines with absorbance at 420 nm and color intensity. Color

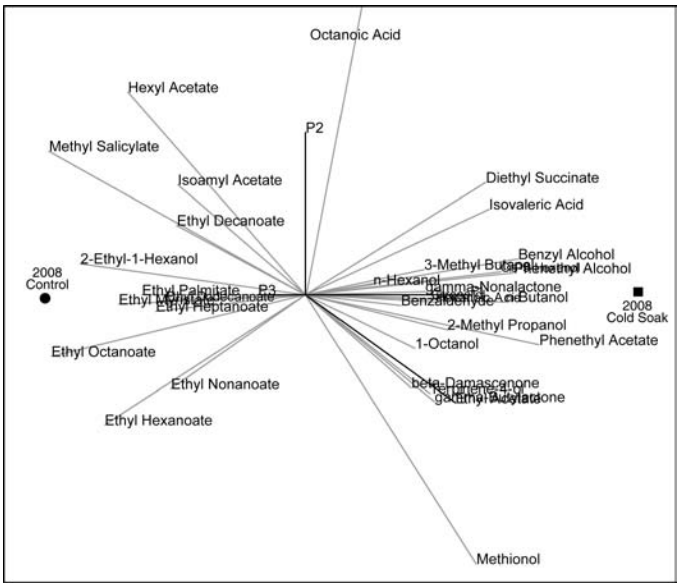

Figure 6 PCA distribution of 2008 Cabernet Sauvignon control and cold soak wine volatiles as detected by GC-MS.

Table 4 Cabernet Sauvignon wine pH, titratable acidity (TA as g/L tartaric acid), ethanol concentration, volatile acidity (VA), phenol-free glycosyl-glucose (PFGG), total glycosyl-glucose (TGG), absorbance (280 nm, 320 nm, 420 nm, 520 nm), and color (hue and intensity).

Analysis	Control	Cold soak
pH	3.61 ± 0.00 a[a]	3.59 ± 0.00 a
TA (g/L)	9.44 ± 0.05 b	8.73 ± 0.05 a
% Ethanol	12.9 ± 0.0 a	13.1 ± 0.0 b
VA (g/L)	0.25 ± 0.02 a	0.35 ± 0.02 b
PFGG (µM)	191 ± 41 a	195 ± 41 a
TGG (µM)	1153 ± 86 a	1395 ± 86 a
A_{280nm}	4.32 ± 0.05 b	3.76 ± 0.05 a
A_{320nm}	2.16 ± 0.05 a	2.09 ± 0.05 a
A_{420nm}	0.63 ± 0.04 a	0.59 ± 0.04 a
A_{520nm}	0.95 ± 0.04 b	0.70 ± 0.04 a
Color hue	0.67 ± 0.02 a	0.83 ± 0.02 b
Color intensity	1.58 ± 0.08 a	1.29 ± 0.08 a

[a]Values with different letters within a row indicate significant differences ($\alpha = 0.05$).

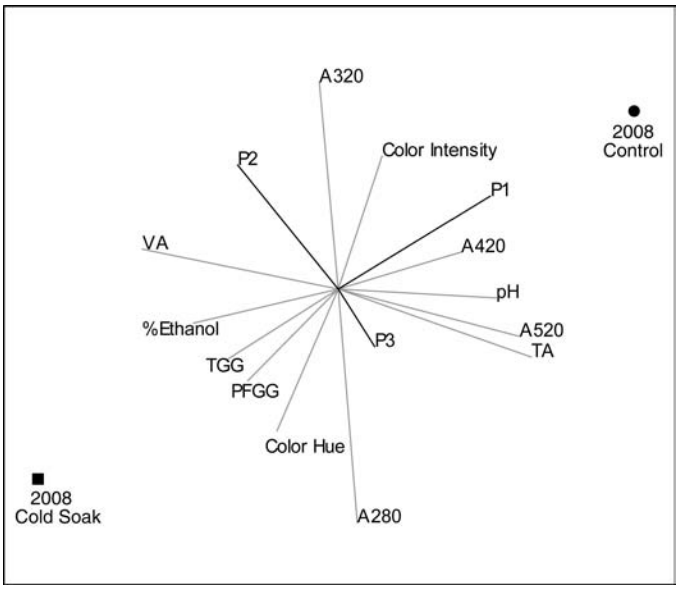

Figure 7 PCA distribution of Cabernet Sauvignon control and cold soak wine chemistries determined by pH, TA, ethanol concentration, volatile acidity, phenol-free glycosyl-glucose (PFGG), total glycosyl-glucose (TGG), absorbance (280 nm, 320 nm, 420 nm, 520 nm), and color (hue and intensity).

indices, specifically absorbance at 280 nm, 320 nm, and 520 nm, contributed most to variation. These results support ENose and GC-MS findings, which show separation between control and cold soak Cabernet Sauvignon wines.

No treatment difference between control and cold soak wines was found based on sensory evaluation at the 95% significance level (Table 5). Aroma unit (AU) values for select compounds show that ethyl acetate and phenethyl alcohol were the only compounds to have a greater contribution in cold soak wines (Table 6). Based on AU values, ethyl heptanoate, ethyl hexanoate, ethyl octanoate, and isoamyl acetate may have contributed more to the aroma of control wines. Other compounds with AU values >1 included isovaleric acid, β-damascenone, phenethyl acetate, and 3-methyl butanol. Although these compounds have AU values that make them possible contributors to the aroma, AU does not indicate the potential synergistic or masking effects of the aromas of the wines. Additionally, AU values vary according to sample matrix, and are, therefore, only an approximation.

Several studies have reported variations in aromas as a function of cold soak (Heatherbell et al. 1996, Reynolds et al. 2001, Marais 2003) using an expert or trained sensory panel. Sensory differences were not detected in this study, which may be due to panel type. Trained panels and consumer panels have very different purposes. Judge variability is often one of the greatest variables in consumer evaluation (Meilgaard et al. 2007). Additionally, had the discrimination test been liberalized by decreasing the desired significance, there would have been a difference between treatments.

The slightly higher ethanol concentration in cold soak wines may have affected the degrees of perception of panelists. Changes in ethanol (0.5 to 0.75%) can alter the perception of some volatile compounds (Williams and Rosser 1981). Ethanol concentrations in cold soak wines varied from controls by ~1.5 to 1.6%, suggesting that ethanol may have influenced sensory panelists. This fluctuation in ethanol was minimized during ENose evaluation due to use of the ethanol baseline (data not shown), preventing ethanol masking of differences detectable by ENose but not sensory evaluation.

Antagonistic or synergistic interactions of aromas may have affected sensory evaluation during this study and contributed to the lack of differences detected based on wine aroma. The conducting polymer ENose is an analytical tool that closely resembles the human sensory system (Mallikarjunan 2005). However, there may have been differences in volatile detection by ENose and aroma discrimination during sensory evaluation. In sensory evaluation, a volatile is detected by receptor proteins in the olfactory epithelium, in which a threshold concentration must be met for the brain to recognize aromas or variations in intensity (Meilgaard et al. 2007). Wine volatiles can act antagonistically or synergistically, minimizing

Table 5 Cabernet Sauvignon wine aroma (sample temperature 19°C) triangle difference sensory results (n=54) of control versus cold soak treatments (α = 0.05, β = 0.10, ρ_{max} = 30%, 25 correct responses needed for significant difference).

Sample 1	Sample 2	Correct responses	Total responses	Signf[a]
Control	Cold soak	22	54	None

[a]No significant difference between treatments.

Table 6 Aroma units of select volatile compounds in Cabernet Sauvignon control and cold soak wines.

		Aroma units[b]	
Compound	Aroma[a]	Control	Cold soak
Benzaldehyde	almond	0.00	0.01
Benzyl alcohol	flower, berry, cherry, walnut, grapefruit	0.05	0.06
cis-3-Hexenol	fresh	0.25	0.28
Citronellol	rose, geranium	0.24	0.33
β-Damascenone	honey	64.40	90.60
Ethyl acetate	pineapple, anise, ethereal	7.57	8.03
Ethyl heptanoate	berry, plum, melon	0.56	0.49
Ethyl hexanoate	fruit, apple, ethereal, banana	52.34	41.80
Ethyl octanoate	fat, apricot, pineapple	831.45	754.65
Ethyl palmitate	waxy	0.03	0.03
Hexanoic acid	fruit, cheese	0.70	0.89
Hexyl acetate	herb	6.35	4.52
Isoamyl acetate	banana	248.69	227.18
Isovaleric acid	rancid, animal, cheese	1.80	2.47
3-Methyl butanol	burnt	2.97	3.06
Methyl salicylate	peppermint	0.24	0.23
n-Hexanol	resin	0.32	0.38
Octanoic acid	sweat, oily	0.58	0.70
1-Octanol	burnt, citrus	0.39	0.43
Phenethyl acetate	honey, rose	2.90	3.00
Phenethyl alcohol	honey, rose	52.92	55.89

[a]Aroma descriptors from Flavornet (www.flavornet.org) and Sigma-Aldrich Flavors & Fragrance Catalogs (Sigma-Aldrich, Milwaukee, WI).
[b]Aroma units are defined as [compound] divided by [odor threshold] (Acree 1993). Values >1.00 are considered contributors to sample aroma.

or masking the perception of others (Etievant 1991). ENose evaluations are based on sensor interactions with volatile compounds of a particular molecular weight, chemical class, and concentration, which may reduce the possibility of aroma masking, as with traditional GC-MS studies but unlike sensory evaluations.

Conclusion

This study evaluated the effects of cold soak on changes in juice and wine volatiles using a conducting polymer ENose and demonstrated the potential of an ENose system as a simple volatile analytical tool. Volatile variations could be evaluated using an ENose, as results were comparable to SPME GC-MS. A consumer panel did not detect aroma differences between control and cold soak wines, although differences were identified by ENose and GC-MS. This implies that the ENose can be used as an analytical tool but not as a substitute for sensory analysis.

Cold soak can be applied by using natural cold temperatures, mechanical refrigeration, or cryogens. With the exception of natural temperatures, cold soak application requires an input of energy. As sustainability becomes a greater focus for the wine industry, any additional production cost must be carefully evaluated for its impact on the final product. Electronic nose technology represents a tool that may help validate the many winemaking processes. This study demonstrated the ability of an ENose to analyze, and provide insight on, the volatile components of grape juice and wine. As wine quality is often related to volatile aroma and flavor composition, this tool may be useful for screening product volatile components prior to GC-MS analysis or other extensive analytical procedures.

Although this study focused on electronic nose discrimination capabilities, industry professionals may potentially use the machine to "match" wines to a previous system of a desired quality and to enhance uniformity of production wines. Such practices may ultimately improve wine quality for a commercial winery.

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