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Comparison of Metal Oxide-Based Electronic Nose and Mass Spectrometry-Based Electronic Nose for the Prediction of Red Wine Spoilage

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Taints caused by *Brettanomyces* sp. spoilage are of concern to winemakers and consumers. Typically the taints are described as "barnyard", "sweaty saddle", and "Band-aid" when present in red wine at concentrations of several hundred micrograms per liter or more. The two main components of the taint are 4-ethylphenol (4EP) and 4-ethylguaiacol (4EG), which are metabolites produced by *Brettanomyces* yeasts. There is a need for a rapid instrumental method to quantify these compounds in wines. In this paper are compared two techniques, the metal oxide sensor-based electronic nose (MOS-Enose) and the mass spectrometry-based electronic nose (MS-Enose). Gas chromatography–mass spectrometry (GC-MS) was used for quantification and prediction purposes. Following ethanol removal, the limits of detection of a MOS-Enose were determined as 44 μg L^{-1} for 4EP and 91 μg L^{-1} for 4EG, using the SY/gCT sensor. These values are significantly lower than the reported human sensory thresholds. Partial least-squares (PLS) regression of electronic nose signals against known levels of 4EP and 4EG in 46 Australian red wines showed that the MOS-Enose was unable to identify "brett" spoilage reliably because of the response of the gas sensors to intersample variation in volatile compounds other than ethylphenols. Conversely, the MS-Enose was capable of reliably estimating concentrations of 4EP higher than 20 μg L^{-1}. Correlations (r^2) of 0.97 and 0.98 were obtained between estimates of 4EP and 4EG concentrations with the concentrations determined by conventional GC-MS. It is concluded that, following ethanol removal, existing metal oxide sensors are sufficiently sensitive to detect brett taints in wine but lack the selectivity needed to perform this task when the aroma volatile background varies.

KEYWORDS: Headspace mass spectrometry-based electronic nose; metal oxide based-electronic nose; *Brettanomyces*; spoilage

INTRODUCTION

The human nose is still the "instrument" most commonly used for evaluating the aroma quality of food products (*1*). However, sensory analysis by a panel of experts is a costly process. Trained panelists can analyze only a limited number of samples per day, and there are inherent problems, including variability between individuals and over time. Alternative instrumental methods, such as gas chromatography, are more reliable but take longer to process a sample. Gas chromatography–mass spectrometry (GC-MS) can provide highly specific qualitative and quantitative information about the composition of a food. Nevertheless, use of GC-MS is constrained by its low sample throughput. It is not well suited to the batch procedures required to screen many samples.

Electronic noses (Enoses) are instruments for volatile assessment that appeared at the beginning of the 1980s. The main driver for developing Enoses was the desire to have an instrument that could act as a surrogate for the human nose and rapidly predict human sensory responses. A number of Enoses are now available on the market (*2*–*5*). There have been advances in sensor design and chemistry, and research with Enoses has opened up a range of applications. Sensors that rely on the chemical properties of the target molecule, whether it can adsorb at a particular surface, or be oxidized or reduced, have been developed for a variety of analytes. Sensors based on modulating the conductivity of semiconductors such as tin oxide (*6*) or polymers such as polypyrrole (*7*) are currently the most widely used. Other types of sensors, such as piezoelectric crystals and surface acoustic wave devices (*8*), rely on the principle of "weighing" impinging molecules. Piezoelectric crystal sensor arrays have been successfully applied for pre- and postharvest aroma profiling

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of tomatoes and apples (*9–11*), showing their potential for routine quality assessment.

At the end of the 1990s, a new type of Enose based on mass spectrometry (MS-Enose) was developed (*12*). Even though some researchers do not consider that the MS-Enose fits the standard definition of an Enose, it replicates the essential design features of a classical Enose in software and is used, like classical Enoses, to differentiate and classify samples according to their volatile composition in a fast and simple way (*1*).

Detection of volatiles in beverages using the above-mentioned techniques has gradually increased over recent years. Martí et al. (*1*) have presented the state of the art for applying solid-state and mass spectrometry-based sensors to the analysis of alcoholic beverages. They reviewed the extraction systems, sensors (metal oxide, conducting polymers, quartz microbalance, etc.), and data treatment that have been used. In general, MS-Enose has a clear-cut advantage over the classical solid state-sensor-based Enoses, which are adversely affected by the high and varying concentrations of ethanol present in the headspace over alcoholic beverages. It would be highly desirable to find a solution to this problem because classical Enoses have lower capital costs than MS-Enose. Martí et al. (*1*) reviewed a number of the techniques that have been used to minimize interference by ethanol. None of these approaches appeared to be satisfactory, but a new technique relying on ethanol removal through drying (*3*) appears to have more potential.

Volatile analysis may focus on the overall character of a beverage or toward detection of specific positive or negative notes. In the latter category, volatile aromatic compounds including substituted phenols, guaiacols, and cresols are usually present in wine aroma and may contribute positively to it. However, some of these compounds can cause off-flavors that negatively affect wine quality, if they are present above a certain threshold. From an enological point of view, the most important ones are 4-ethylguaiacol (4EG) and 4-ethyphenol (4EP) due to their unpleasant organoleptic properties (*13*). In fact, 4EP has been described as "horsy", "leather", and "medicinal", whereas 4EG in wine has a smoky and spicy aroma.

Ethylphenol and ethylguaiacol in wines arise from a variety of sources, but the most important is enzymatic synthesis from cinnamate precursors (*14*) by *Brettanomyces*/*Dekkera* yeasts, which accumulate through the winemaking process. Contamination with these yeasts has been described as insidious, widespread, and of great concern to the wine industry. *Brettanomyces*/*Dekkera* yeasts have caused wine spoilage in many wine-producing areas (*15–20*). For example, in Australia alone, 86% of Cabernet Sauvignon wines from the three vintages between 1997 and 2000 were adversely affected by compounds produced by these yeasts (*21*).

The existing procedures to monitor spoilage due to *Brettanomyces*/*Dekkera* sp. ("brett" spoilage) are time-consuming and expensive, making it difficult for winemakers to monitor their wines at all stages of production. Consequently, there is a need for a rapid and cost-effective screening method to monitor the levels of 4EP and 4EG in wine.

Use of an MS-Enose to monitor 4EP and 4EG in wines has recently been reported (*21*). The current study was designed to compare the performance of a classical Enose, assisted by sample ethanol removal, against the MS-Enose technology. GC-MS was used as the reference technique.

MATERIALS AND METHODS

Wine Samples. The 46 Australian red wines used in this study were purchased from retail outlets. Thirty-seven of the wines were made from Cabernet Sauvignon grapes, 7 from Cabernet Sauvignon and Merlot blends, 1 from a Cabernet Sauvignon and Shiraz blend, and 1 from a blend of Cabernet Sauvignon, Cabernet Franc, and Shiraz. The vintages ranged from 2003 to 2005. The wines originated from the Barossa and Coonawarra regions of South Australia as well as from the Margaret River region of Western Australia.

Ethanol Removal. The high concentration of alcohol in wines is the factor that most strongly limits the application of Enose technology (*3*). Alcohol "swamps" the sensors and makes it difficult, if not impossible, to discriminate wines. Separation of ethanol from the volatiles of interest prior to Enose analysis is desirable, and the method used here was a combination of the simple approach of drying wine samples on a membrane filter, as introduced by McKellar et al. (*3*), followed by solid-phase microextraction (SPME). Samples (150 μL) were spotted onto individual 25 mm, 0.45 μm nylon membrane filters (Sigma-Aldrich) that had been placed into 10 mL autosampler vials. Vials containing filters were allowed to air-dry at ambient temperature (22.5 °C) for 3 h. The relative humidity in our laboratories was 53%. The vials were sealed with silicon/Teflon magnetic autosampler vial caps and submitted for classical electronic nose analysis followed by GC-MS.

GC-MS. For reference purposes, all samples were analyzed by GC-MS both with and without prior ethanol removal. For the latter condition, wine samples (5 mL) were placed directly into 10 mL vials without pretreatment, and quantitative analysis of 4EP and 4EG was carried out by GC-MS exactly as described previously (*18*).

For the ethanol removed condition, vials containing wine-impregnated (150 μL) nylon membranes were incubated at 35 °C with shaking (500 rpm) for 5 min to increase the concentration of volatiles in the headspace. An SPME fiber (Aldrich, Bellefonte, PA), composed of fused silica partially cross-linked with 65 μm polydimethylsiloxane/divinylbenzene, was inserted by autosampler (CombiPAL, Switzerland) into the vial headspace for 30 min. After absorption, headspace volatiles were transferred to the GC injection port, which was equipped with a 0.8 mm i.d. splitless glass liner, at 250 °C. Desorbed volatile compounds were separated in a Varian 3800 GC, equipped with a 30 m × 0.25 mm, 0.25 μm film thickness ZB-Wax fused silica capillary column. The oven temperature was programmed to rise from 50 to 240 °C at 5 °C min^{-1}. The GC column output was fed into a Varian 1200 mass selective detector (mass spectrometer). The GC-MS transfer line was heated at 250 °C with the flow rate of the He carrier gas set to 1 mL min^{-1}. Mass spectrometry was performed in electron impact mode at 70 eV over the scan range m/z 35–350 in a 1 s cycle.

Identification of 4EP, 4EG, and 3-methylbutanol, all supplied by Aldrich (Gillingham, U.K.), was achieved by comparing the GC retention times and mass spectra of experimental samples with those of pure standard compounds. The same was for diethyl succinate, 2-phenylethanol purchased from Fluka (Buchs, Switzerland) and ethyl lactate supplied by Sigma-Aldrich (St. Louis, MO). Mass spectra were also compared with the data system library (NIST library 98). The peak areas for 4EP and 4EG were recorded and used for statistical analysis.

Metal Oxide Sensor-Based Electronic Nose (MOS-Enose). A FOX 3000 E-Nose (Alpha MOS, Toulouse, France), which has an array of 12 semiconducting metal oxide sensors, was employed for this study. Vials containing wine-impregnated nylon membranes were loaded into an autosampler (HS50, CTC Analytics, Switzerland), and an SPME sample was absorbed exactly as described for GC-MS analysis. The SPME fiber was transferred to the E-Nose injection port, which was held at 220 °C. Dry zero grade air (flow rate = 150 mL min^{-1}) was used to sweep the sample through the two sensor chambers. A 15 min delay between samples was used to allow the sensors to return to baseline.

Data were captured and preanalyzed using AlphaSoft v. 8 (Toulouse, France). To simplify data processing, only the maximum resistance changes of each sensor were used for later analysis. The computation used for feature extraction is defined as a fractional baseline manipulation given in eq 1

$$\Delta R/R_0 = \frac{(R_0 - R_{max})}{R_0} \quad (1)$$

where R_0 corresponds to the value of the resistance at $t = 0$ (baseline) and R_{max} to the extreme resistance value change when an injection is made.

To calibrate the response of the electronic nose sensors and to determine the limits of detection (LOD) for 4EP and 4EG in red wine, triplicate samples of a representative red wine that contained no detectable 4EP or 4EG were spiked with standard ethanolic solutions of 4EP and 4EG to give final analyte concentrations in the ranges from 40 to 160 μg L^{-1} and from 25 to 300 μg L^{-1}, respectively. Ethanol was removed, and the spiked wine samples were analyzed by GC-MS (Varian 3800 GC, Varian 1200 MS, Palo Alto, CA) and MOS-Enose exactly as described for the wine samples. Increasing analyte concentrations were plotted against the corresponding response of the electronic nose expressed as $\Delta R/R_0$. Data points falling within the linear portion of each calibration curve, that is, 40–120 μg L^{-1} for 4EP and 25–200 μg L^{-1} for 4EG, were fitted with a linear regression. The standard method (*23*) was used to determine the detection limit of individual MOS-Enose sensors for specific analytes of interest in the wine matrix. Briefly, we determined the means (x) and standard deviations (s) of repeated sensor responses to blank samples, in this case replicate wine samples with no 4EP or 4EG detectable by GC-MS. The LOD sensor responses, defined as $x + 3s$, were fed into the relevant linear regression equations to derive the experimental LODs for the method.

MS-Enose. For MS-Enose analysis, wine samples (5 mL) were pipetted into a 10 mL headspace vial and sealed. Samples were analyzed on the Chemical Sensor (HP 4440, Hewlett-Packard) equipped with a headspace sampler (HP 7694, model G 1290A). The experimental conditions of the headspace sampler were as follows: oven, 75 °C; loop, 90 °C; transfer line, 95 °C; vial equilibration, 20 min; headspace cycle, 4.2 min; pressurizing, 0.3 min; loop filling, 0.15 min; loop equilibration, 0.02 min; and injection time, 0.5 min. The carrier gas was helium at 4.2 psi (28.96 kPa), and the vial was pressurized at 14 psi (96.53 kPa). The total analysis time per sample was approximately 25 min. Positive ion electron impact spectra at 70 eV were recorded in the range from m/z 50 to 180. A solution of 12% ethanol was included in each batch of MS-Enose samples as a source of standard ions for internal calibration purposes. Instrument control and data acquisition were carried out using Pirouette software (Infometrix, Inc.) (*22*).

Chemometrics. Data from MS-Enose and MOS-Enose were imported into version 9.5 of The Unscrambler software (CAMO ASA, Oslo, Norway) for chemometric analysis. Partial least-squares regressions (PLS) were performed to predict brett spoilage, that is, predict the concentration of 4-ethylphenol (Y-variable) from electronic nose data (X-variable). PLS was performed with full cross-validation to validate the models developed. To avoid overfitting of the data, the number of factors (latent variables) in PLS models was constrained to be the lowest consistent with 5–10% standard error of prediction in the prediction residual error sum of squares (PRESS) function.

To improve GC-MS calibrations, the standard normal variate (SNV) transformation provided by The Unscrambler software was used for data preprocessing. The SNV is a row-oriented transformation, which centers and scales individual spectra and standardizes each spectrum using only the data from that spectrum.

RESULTS AND DISCUSSION

Effectiveness of Filter Drying in Reducing Ethanol in Wine Samples. The use of filter drying to eliminate ethanol from wine samples prior to their analysis by electronic nose was previously used to enable discrimination between wines made from different fruits and different grape varieties (*3*). In that paper, no data were presented regarding the efficiency or selectivity of the reduction in ethanol concentration. We used total ion count (TIC) chromatograms of a wine sample before and after filter treatment as a semiquantitative indicator of the compound-specificity of the procedure. We focused on the target analytes as well as some volatile compounds that are normally present in high concentrations in red wines (*25*). Although mass spectrometry is not a quantitative technique, comparison of the relative ion current intensities for identical compounds under closely related conditions is valid. Overall, the TICs of most peaks were reduced by filter treatment, although a few compounds, notably 2-hydroxyethylpropanoate (ethyl lactate), were selectively enriched. We used a factor of 3.1, which is the ratio of the summed chromatogram TICs over the interval 10–50 min (**Table 1**), as a normalization factor to assess the degree to which filter treatment selectively enriched or depleted individual ions. Compounds that are selectively removed by nylon filter treatment dominate the first 10 min of the chromatogram. The dominant components, ethanol and 3-methylbutanol, were almost quantitatively removed (specific depletion factors 279 and 693, respectively; **Table 1**). The analytes of interest, in this case 4EP and 4EG, were reduced by factors of 3.9 and 4.2, respectively, representing 70-fold enrichment relative to ethanol. Our results clearly show that by combining filter treatment and SPME the ethanol concentration can be reduced to the point at which it is unlikely to be a significant source of interference for the MOS-Enose. Furthermore, although levels of the analytes 4EP and 4EG were reduced, they still gave rise to easily detected peaks in the TIC chromatogram.

Table 1. Total Ion Count (TIC) of Four Compounds Detected in Red Wines When No Filter Treatment (without Nylon) Was Used and When Filter Treatment (with Nylon) Was Used[a]

compd	TIC without nylon	TIC with nylon	TIC ratio: without nylon/ with nylon	specific depletion (enrichment)
ethanol	1.23×10^{10}	1.44×10^{7}	855.3	278.6
3-methylbutanol	1.86×10^{10}	8.74×10^{6}	2126.0	692.5
4-ethylphenol	1.94×10^{10}	1.64×10^{7}	11.9	3.9
4-ethylguaiacol	4.91×10^{7}	3.85×10^{6}	12.7	4.2
ΣTIC > 10	3.81×10^{10}	1.24×10^{10}	**3.1**	1.0

[a] The TIC ratio (without and with nylon) and specific depletion for each compound (TIC ratio/ΣTIC > 10 ratio).

Validation and LODs of the MOS-Enose for 4EP and 4EG. It is possible that the drying of the samples on nylon filters would either introduce unacceptable additional quantitative variability or raise the MOS-Enose LODs for specific analytes of interest above the useful threshold. To address the first issue we used GC-MS to compare the levels of 4EP and 4EG with and without the nylon filter treatment. For all 46 test wines there were strong correlations ($r^2 = 0.97$ and 0.84) between the reference levels of 4EP and 4EG, as determined by the method of Pollnitz et al. (*22*), and total ion counts in the 4EP and 4EG peaks determined by SPME-GC-MS following drying on nylon filters (**Figure 1**). The poorer correlation for 4EG is not surprising as, typically, the concentrations of this compound are lower than for 4EP and closer to the instrumental LODs. Our data validate the nylon filter treatment as a semiquantitative method for separating brett taints from ethanol. We also characterized the concentration dependency and LODs for the three most brett-responsive MOS-Enose sensors (**Table 2**). The actual concentrations of 4EP and 4EG in the filter headspace were not calculated. Instead, the response was defined in terms of the known concentration of the analyte in a defined wine background. For the majority of the sensors, there was little or no change in resistance in response to increasing concentrations of analyte. However, the responses of sensors SY/G, SY/Gh, and SY/gCT increased monotonically with increasing concentrations of 4EP in wine. Sensors SY/Gh and SY/gCT also responded to increasing 4EG concentrations. The sensor responses could be fitted with a hyperbolic regression curve (not

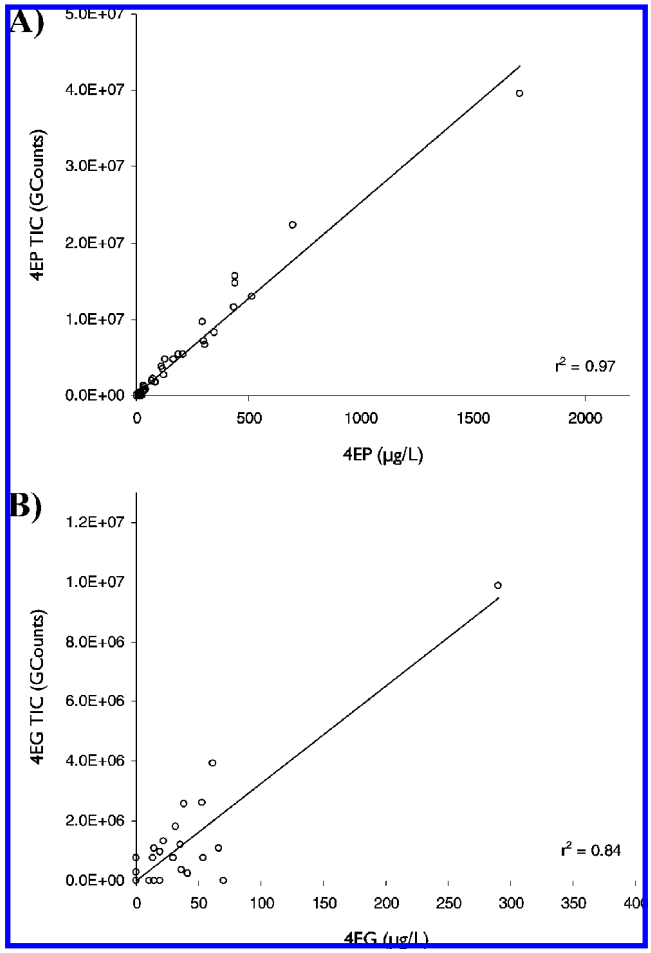

Figure 1. Correlation between the reference levels of 4-ethylphenol (4EP; **A**) and 4-ethylguaiacol (4EG; **B**), as determined by Pollnitz et al. (*22*), and the total ion counts (TIC) in the 4EP and 4EG peaks determined by SPME-GC-MS following drying on nylon filters.

Table 2. Equations of the Linear Regression for the Different Compounds with the Correlation Coefficient r^2 and the Detection Limit of the Linear Range

compd	E-nose sensor type	linear regression: $a \times$ concn $+ b$ a	b	r^2	detection limit (μg L^{-1})
4-ethylphenol	SY/G	6.80×10^{-3}	−1.02	0.99	101.2
	SY/Gh	6.05×10^{-3}	−1.13	0.99	138.4
	SY/gCT	6.08×10^{-3}	−1.34	0.99	43.8
4-ethylguaiacol	SY/Gh	6.75×10^{-4}	−0.59	0.81	93.5
	SY/gCT	6.60×10^{-4}	−0.37	0.78	91.1

shown). For calibration purposes, we fitted linear regression equations (**Table 2**) to the linear portions of the sensor responses, which were 40−120 μg L^{-1} for 4EP and 25−200 μg L^{-1} for 4EG.

The sensor responses at the LODs were −0.727, −0.528, and −0.306 for sensors SY/G, SY/Gh, and SY/gCT, respectively. The corresponding LODs were 44 μg L^{-1} for 4EP using the SY/gCT sensor and 94 μg L^{-1} for 4EG using the SY/Gh sensor (see **Table 2** for a full listing). These LODs are substantially less sensitive than those obtained using GC-MS with SPME. The LODs, defined as the amount of analyte that gives a signal 3 times higher than noise signal (S/N = 3), were reported to be 1 μg L^{-1} for 4EG and 2–7 μg L^{-1} for 4EP (*13, 26*). On the other hand, limits of quantification (LOQ), defined as the concentration level that gives a signal 10 times higher than the noise signal (S/N = 10), were reported about 5 μg L^{-1} for both 4EP and 4EG (*13*). With the GC-MS methods used in this study, levels of 4EP and 4EG lower than 10 μg L^{-1} were not detectable, implying a higher LOD compared with the reported literature.

Table 3. Mean, Standard Deviation (SD), and Range of 4-Ethylphenol (4EP) and 4-Ethylguaiacol (4EG) in 46 Red Wine Samples

compd	mean (μg L^{-1})	SD	range (μg L^{-1})
4-EP	159.9	286	0–1710
4-EG	20.02	45.7	0–290

However, even using the nylon pretreatment, the MOS-Enose can individually detect 4EP or 4EG at levels that are substantially below the individual human sensory thresholds in red wine, which are 605 and 110 μg L^{-1}, respectively (*15*). Furthermore, the sensory impact of 4EP and 4EG is additive. We found that, for those 19 samples in which the two phenol concentrations were high enough to be estimated reliably, the ratio of 4EP to 4EG was 8.0 ± 3.2, which is similar to the normally quoted ratio of 10:1. When present in the latter ratio, the human sensory threshold is 335 μg L^{-1} of 4EP and 34 μg L^{-1} of 4EG (*15*). This would still be readily detectable by the MOS-Enose, on the basis of the higher 4EP concentration.

Characteristics of the Test Wines. The concentrations of 4EP as determined by GC-MS in the red wine samples ranged from undetectable to 1710 μg L^{-1}. In the case of 4EG the concentrations ranged from undetectable to 290 μg L^{-1}. The range of 4EP and 4EG concentrations (SD > mean) in the samples was sufficient to build a robust calibration model for the Enoses (**Table 3**).

When the MOS-Enose sensor responses are compared for the 46 wine samples analyzed (**Figure 2**), two features emerge. The first is that a small number of sensors significantly discriminate among the aroma profiles of the wine samples. Those that give the broadest ranges of response were those that also responded most strongly to 4EP and 4EG. Second, the response profiles of each of the P&T type sensors were almost identical. Cross-sensitivity is very common in electronic nose sensors (*27*), implying that some sensors respond to a broad range of volatiles in very similar ways, generating essentially the same information.

For the MS-Enose analysis of the wines, we set the scan window to cover the range m/z 50–180 to avoid interference from the abundant ethanol-derived ions $C_2H_6O^-$ (m/z 46) and $C_2H_5O^-$ (m/z 45). There were clear differences among the 46 wines in the abundance of specific ions (**Figure 3**), particularly those with $m/z \geq 100$.

Enose Predictions of Ethylphenol Concentrations in Wine. PLS regression of MOS-Enose and MS-Enose responses was performed against the 4EP and 4EG concentrations (**Figures 4** and **5A**), as determined by the reference GC-MS method. The root mean standard error of cross-validation (RMSECV) and the coefficient of determination (r^2) between measured and predicted values were computed to evaluate the predictive ability of the models. In regression, the r^2 is a statistical measure of how well the regression line approximates the real data points. RMSECV is a measure of the average difference between the values determined by the laboratory method and those predicted by the model using cross-validation procedure.

For MOS-Enose, all 12 of the sensors were incorporated in the modeling because, although the LOD study showed that only three of the sensors responded significantly to the ethylphenols, we reasoned that the other sensors may encode relevant

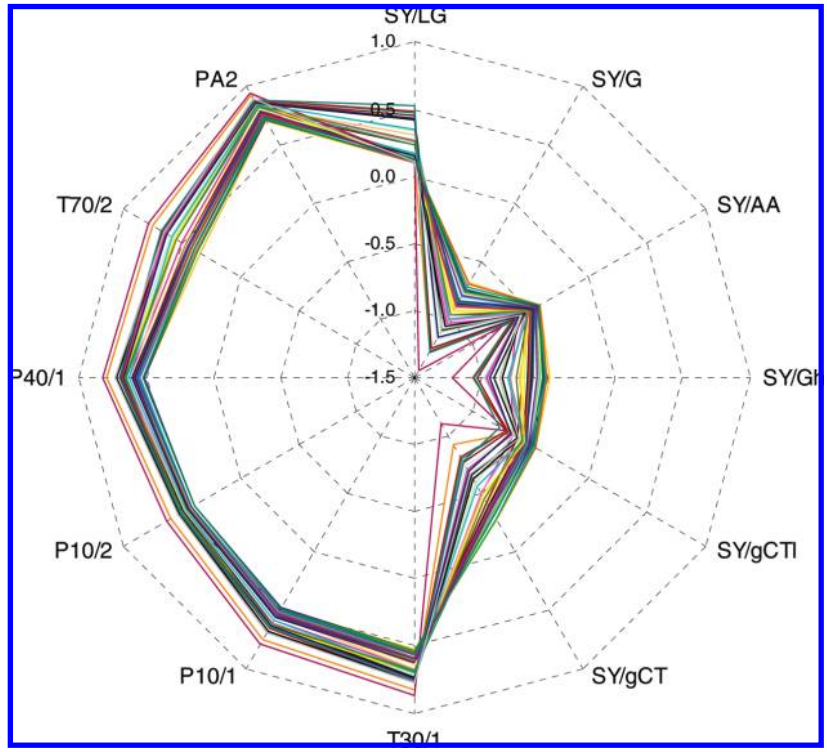

Figure 2. Radar plots of the responses on the 12 metal oxide-based sensors for the 46 wines.

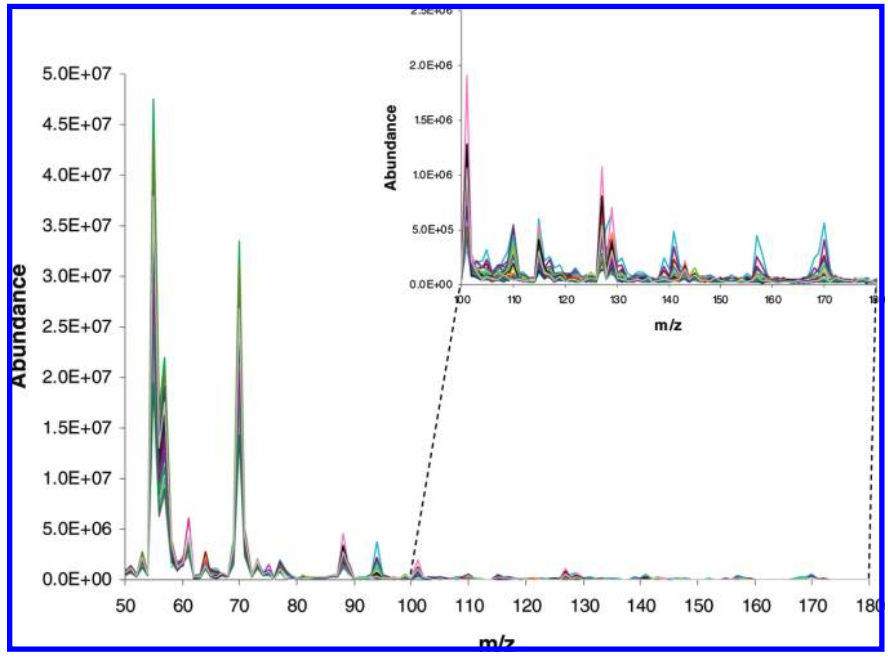

Figure 3. Mean mass spectra between *m*/*z* 50 and 180 of 46 red wines obtained by MS-Enose and a closer look at the mass spectra range *m*/*z* 100–180.

additional aroma variation. This was confirmed by the lower r^2 (0.24 for 4EP and 0.27 for 4EG) found when only three sensors were used to build the models. The r^2 values for the MOS-Enose were around 0.5 for both compounds, and the errors (RMSECV) were <242 μg L^{-1} for 4EP and <40 μg L^{-1} for 4EG (**Table 4**). The statistical values r^2 would be <0.5 if the observation with the highest 4EP and 4EG concentrations were removed; however, that sample was left in the model as it represents a real variation of 4EP and 4EP contents in wine (it was not considered to be outlier).

The poor predictive power of the model was underscored by the observation that the MOS-Enose PLS model consistently underestimates the levels of 4EP and for wines with low concentrations predicts negative values of 4EP (**Figure 4**). Similar trends were found for 4EG (not shown). One possible explanation is that the nonlinear relationship between Enose responses and the actual 4EP concentration invalidates attempts to fit the data with a linear model. Therefore, we repeated PLS regression using a smaller number of samples (11 wines) with 4EP concentrations within the linear range determined during the LOD study. The coefficients of determination improved, but they were still very low ($r^2 = 0.66$ for 4EP). We are therefore forced to conclude that MOS-Enose fails to predict 4EP and 4EG concentrations accurately in a range of red wines because

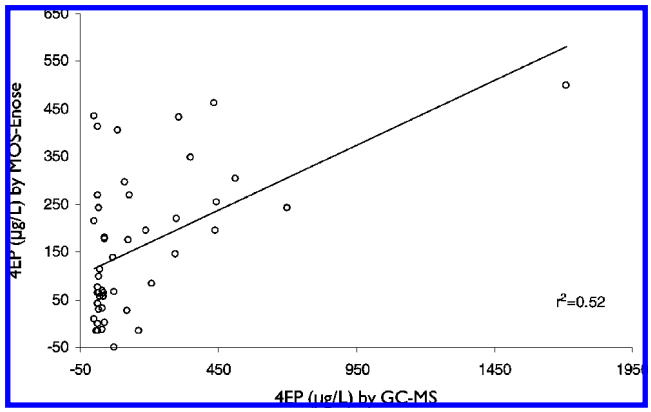

Figure 4. MOS-Enose predicted values against measured data for 4-ethylphenol (4EP) by GC-MS in wine samples.

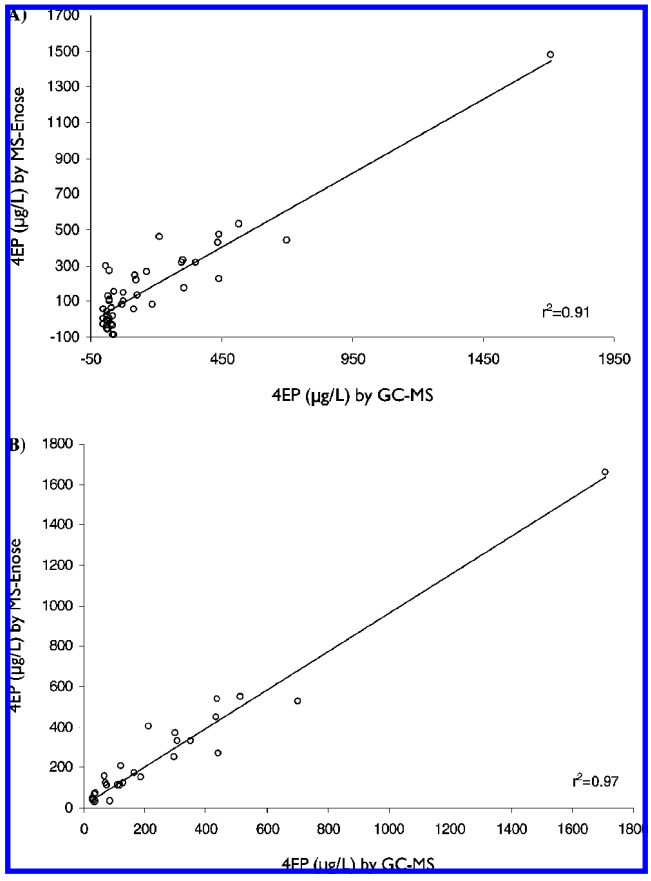

Figure 5. MS-Enose predicted values against measured data for 4-ethylphenol (4EP) by GC-MS in 46 wine samples (**A**) and in a smaller set of wine samples (>20 μg L^{-1} of 4EP) (**B**).

Table 4. Partial Least-Squares Cross-Validation Statistics, Coefficient of Determination (r^2), and Root Mean Standard Error of Cross-Validation (RMSECV) for the Concentration of 4-Ethylphenol (4EP) and 4-Ethylguaiacol (4EG) in 46 Red Wine Samples Using MOS-Enose and MS-Enose (μg L^{-1})

	MOS-Enose		MS-Enose	
compd	r^2	RMSECV	r^2	RMSECV
4EP	0.52	241.3	0.91	118.4
4EG	0.51	38.8	0.89	20.4

of the response of the gas sensors to intersample variation in volatile compounds other than ethylphenols. It is clear that, even after removal of the ethanol and notwithstanding the low LOD, MOS-Enose is currently not suitable for predicting the levels of ethylphenols across a wide range of red wines. As demonstrated in the LOD study the MOS-Enose is capable of quantifying changes in ethylphenol concentration in a single red wine.

We believe the reason the instrument is not suitable for detecting specific compounds, such as ethylphenols, against a changing background of complex wine matrices is the low selectivity of the sensors. Possibly MOS-Enose might be useful for monitoring the concentrations of ethylphenols if the wine matrix does not vary very much, that is, for a single style of wine from a specific winery. In such a case, changes in ethylphenol concentration might be measurable against the stable odor background. Alternatively, using the suggested nylon membrane sample pretreatment, the possibility of using MOS-Enose to fingerprint differences in the volatile profiles of closely related wine types, to characterize wines from different vintages, or to blend to a specification rather than detect specific odor-impact compounds could be tested.

For the MS-Enose, the r^2 values (**Table 4**) were significantly higher (>0.89) than for the MOS-Enose, indicating a much better fit to the model. However, the errors (RMSECV $\leq$ 120 μg L^{-1} for 4EP and $\leq$ 21 μg L^{-1} for 4EG) were still significant considering the range of concentrations in micrograms per liter found in the wine samples. As observed in the case of the MOS-Enose, the PLS regression model for the MS-Enose (**Figure 5A**) also tended to underestimate the true level of 4EP in wines, although the discrepancy was much less pronounced and only a small set of samples were predicted as having negative 4EP concentrations, mainly those with undetectable concentrations by GC-MS. The inaccuracies, relative to GC-MS, are explained by the fact that the range of "pseudosensors" (fragment ions) considered, including those that characterize 4EP and 4EG, *m/z* 107 and 137 respectively, are not uniquely derived from these phenols.

When 16 wine samples with low concentrations (<20 μg L^{-1}) of 4EP were omitted from the PLS analysis, the fit and accuracy of the model improved (**Figure 5B**). Over the concentration range of 20–1710 μg L^{-1}, the r^2 was 0.97 (RMSECV = 72.7 μg L^{-1}). For 4EG the r^2 was 0.98 (RMSECV = 8.5 μg L^{-1}). Even with this adjustment, the model does not accurately predict the concentration of ethylphenols in samples contaminated with *Brettanomyces* yeast. However, the results are consistent with the MS-Enose being capable of classifying wines as having high, medium, and low levels of 4EP/4EG, as previously shown (*21*). In this mode, the MS-Enose would be a useful, if expensive, instrument for screening wines. It offers the possibility of reducing the number of samples to be tested using sensory or GC-MS analysis, resulting in reduced cost of analysis and higher throughput of samples under commercial conditions. However, extensive data pretreatment is still required.

The samples used in this study varied widely in their geographical origins, varietal basis, and vintage. Although the samples used represent only a subset of the variation present in all Australian red wines, let alone those from other regions, we would predict that similar findings will hold for other red wine varieties and regions.

To summarize, and it was to be expected, the performance of the electronic noses did not approach the sensitivity, accuracy, or specificity of the GC-MS reference technique. For the MOS-Enose, we used a recently introduced technique in which a sample of the wine is dried on a nylon filter at ambient temperature and pressure (*3*) prior to Enose analysis. This is the first formal analysis of the effects of the technique. Under

the conditions used, the TICs in the ethylphenol peaks were reduced approximately 12-fold relative to untreated samples, although there was still a robust correlation between the results obtained with and without filter treatment. In contrast, the TIC attributable to ethanol was reduced 850-fold. We demonstrated that the LODs of the MOS-Enose for 4EP and 4EG in a standard red wine base are below those of human sensory panels. This distinguishes brett spoilage compounds from other taints, such as trichloranisole, for which the human sensory threshold is well below the detection limits of standard instrumentation. The MOS-Enose was more sensitive to 4EP than 4EG. Therefore, because 4EP is generally present in concentrations 10-fold higher than 4EG, detection of brett taints resolves to the detection of 4EP alone. Despite these positive indications, the MOS-Enose was not able to predict spoilage accurately when presented with a range of commercial red wines. The MS-Enose performed significantly better than MOS-Enose in quantifying ethylphenols, and its performance is acceptable for concentrations of 4EP >20 μg L^{-1}. Although MS-Enose is only semiselective, that is, multiple compounds may contribute to ion currents of any given *m/z*, it was able to discriminate moderately and heavily contaminated samples from those that were lightly contaminated or free of brett compounds. Future work to increase selectivity might use single ion monitoring mode to focus only on the fragments of interest.

Whereas the MS-Enose may be a useful tool for screening wines for ethylphenol taints, neither of the instruments tested offers an ideal solution to this analytical task. Following ethanol removal, existing metal oxide sensors are sufficiently sensitive to detect brett taints in wine but lack the selectivity needed to perform this task. It would be highly desirable to equip an Enose with sensors or pseudosensors with higher selectivity for 4EP and 4EG.

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