Monitoring of evolution during red wine aging in oak barrels and alternative method by means of an electronic panel test

I.M. Apetrei, M.L. Rodríguez-Méndez, C. Apetrei, I. Nevares, M. del Alamo, J.A. de Saja

A combination of the data obtained by means of an e-nose (based on resistive MOX sensors), an e-tongue (based on voltammetric sensors) and an e-eye (based on CIE Lab coordinates) has been used to monitor the aging of a red wine. The changes in the chemical composition of wines that occur during maturing have permitted the system to discriminate among wine samples collected after one, three, six and ten months of aging. The discrimination capability of the electronic panel test obtained by means of Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA) is even higher than the discrimination achieved by means of traditional chemical analysis.

After ten months of aging it has been possible to discriminate between the wine aged in a French oak barrel and the same wine soaked with oak chips of the same origin and toasted level and treated with microoxygenation.

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1. Introduction

Recently, alternative methods to the maturing of wine in oak barrels have been developed in order to simplify the aging process while ensuring that the wood-originated volatiles are released into the wine (Arapitsas, Antonopoulos, Stefanou, & Dourtoglou, 2004). One of these techniques consists in the addition of small oak wood pieces (chips or staves) to the wine kept in a stainless steel tank. The fast diffusion of these techniques makes necessary to carry out studies that analyze the influence of these techniques in the chemical and organoleptic characteristics of wines and to evaluate and characterize the differences between wines aged by the traditional system and wines treated by the alternative methods (Maga, 1989).

Several authors have studied the effects that produce the addition of wood pieces to wine by means of traditional chemical analysis such as chromatography (Fernández de Simón, Cadahia, Muiño, Del Álamo, & Nevares, 2010; Gay et al., 2010; Koussissi et al., 2009; Sanza, 2006).

Several authors have reported that the differences related to the phenolic composition could be used as the basis of a chromatographic method to detect the use of oak chips in the process of aging (Arapitsas et al., 2004; Rodríguez-Bencomo, Ortega-Heras, & Pérez-Magaríño, 2010).

One of the most promising approaches for the analysis of wines consists in the use of arrays of sensors where several sensing materials, which exhibit different responses to various gases/liquids, are coupled with signal processing methods based on pattern recognition or artificial neural networks. Such systems are called electronic noses/tongues (Chen, Zhao, & Vittayapadung, 2008; Gardner & Bartlett, 1999) and analyze the sample as a whole without need of separating it in simple components. After an appropriate optimization, these systems have been adapted to the analysis of red wines.

Several groups have applied electronic noses to the analysis of red wines with different organoleptic characteristics: different varieties of grape, vintage or type of oak wood (Buratti et al., 2011; Santonico, Bellincontro, De Santis, Di Natale, & Mencarelli, 2010). Usually, these systems are based on multiarrays of resistive Metal Oxide (MOX) sensors coupled to an injection technique such as Solid Phase Microextraction (SPME) (Pietro et al., 2011; Villanueva, Guadarrama, Rodríguez-Mendez, & de Saja, 2008), head space (Apetrei et al., 2010; Cynkara, Cozzolino, Dambergza, Janika, & Gishena, 2007; Muenchmeyer, Walte, & Matz, 2000). The preconcentration technique is necessary to eliminate or at least diminish the concentration of water and ethanol in the volatile mixture that interferes in the response of resistive sensors (Aleixandre et al., 2008; Berna, Trowell, Clifford, Cynkar, & Cozzolino, 2009).
tongues based on voltammetric, potentiometric or impedance measurements have also been used to discriminate wines with different qualities (Apetrei et al., 2007; Cetó, Gutiérrez, Moreno-Barón, Alegría, & del Valle, 2011; del Valle, 2010; Escuder-Gilabert & Peris, 2010; Gutiérrez et al., 2011; Lvova et al., 2006; Riul et al., 2004; Žeravik et al., 2009).

The simultaneous use of an electronic nose and an electronic tongue can increase the capability of discrimination of the electronic systems (Di Natale et al., 2000).

Our research team developed the first electronic sensory system, specifically designed for the characterization of wines. It has combined three sensory modalities: an array of gas sensors, an array of electrochemical liquid sensors and an optical system to measure color by means of CIE Lab coordinates (Rodríguez-Méndez et al., 2004).

In spite of the potential applications of the electronic instruments in the organoleptic analysis of wines, few attempts have been carried out to use electronic noses or electronic tongues to discriminate between red wines aged in oak barrels and red wines treated with oak chips. This is due to the difficulties to detect the subtle differences between wines with similar characteristics. Regardless of the difficulties, an electronic tongue based on voltammetric sensors has been able to discriminate between red wines aged in oak barrel and red wines treated with oak chips (Apetrei et al., 2007). However, that study was carried out in wines aged during a large period of time (twelve months). In addition, wines soaked with oak chips and staves were introduced in a tank without microoxygenation. It would be of considerable interest to monitor periodically the changes of wines aged by different methods and to evaluate the rate of maturing in each case.

Moreover, the comparison between wines aged in oak barrels and wines soaked with oak chips in stainless steel tanks is more interesting if wines introduced in the tanks are micro-oxygenated to simulate as much as possible the conditions of the oak barrel.

The objective of this study is to monitor and to compare the aging of a red wine aged in traditional oak barrels and the same wine treated by adding oak chips and microoxygenation to the aging vessel by means of an electronic panel formed by an electronic nose, and electronic tongue and an electronic eye. The capability of discrimination of the electronic panel and the conventional chemical analysis will be compared. For this purpose, a red wine of the D.O. Toro (Spain) has been aged by both methods using French oak barrels of three different degrees of toasting. The obtained wines have been analyzed by standard chemical analysis and by an electronic system developed in our laboratory. The capability of discrimination and classification of both methods has been evaluated by using Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA).

2. Experimental

2.1. Wine samples under study

A red wine of the variety Tempranillo coming from the D.O. Toro (Spain) was elaborated. Then, the wine was divided into batches. The first batch (W0) was kept in a stainless steel tank (no contact with wood). This wine has been used as a control.

The red wine was stored into three different aging systems: six oak barrels (samples W10 to W15) with a 225 L capacity and nine stainless-steel 100 L tanks with oak chips (samples W1 to W9). The quantity of oak chips and staves necessary to reproduce the surface/volume relation of a 225 L barrel was calculated accordingly to the 2.04 m² inner surface of the barrels. Thus, 600 g of chips for each tank was used. One stainless-steel tank has been used as control system for the wine. The barrels and the wood of alternative aging systems were manufactured by the same cooperage (S.L Magnéñán, La Rioja, Spain) with mild, medium and strong toasting degrees, respectively.

In oak barrel aging, the natural properties of the wood allow for a gentle aeration of the wine to occur over a prolonged period (Lorenzo, Garde-Cerdán, Pedroza, Alonso, & Salinas, 2009; Oliveira, Silva Ferreira, De Freitas, & Silva, 2011). This aids in the polymerization of tannin into larger molecules, which are perceived on the palate as softer (Cano-Lopez, Pardo-Minguez, Lopez-Roca, Fernandez-Fernandez, & Gomez-Plaza, 2006). The process of microoxygenation aims to mimic the effects of slow barrel maturation within a shorter period and for less of the long-term cost associated with oak barrels. The usual rate of permeation of oxygen into a new French oak barrels is between 2.0 mL of O2 per liter of wine per month (1.66 mL L⁻¹ month⁻¹) (Moutonet, Mazaucour, Saint-Pierre, & Hanoqc, 1998) and 2.5 mL L⁻¹ month⁻¹ (Kelly & Wollan, 2003). In our case the microoxygenation in the range 2.0–3.0 mL L⁻¹ month⁻¹ O2 was conducted in order to simulate the conditions of natural aging of wine in oak barrel. Dissolved oxygen was measured by means of electrochemical systems based on Clark’s electrode.

Table 1 summarizes the characteristics of the aging methods used in the study. In order to monitor the aging process, samples were collected every three months: month 1 (S1), month 4 (S2), month 7 (S3), month 10 (S4).

2.2. Chemical analysis

Conventional chemical analyses of the wines were carried out following international regulations (OIV, 1990).

Conventional parameters included: Reducing sugars (S, g L⁻¹), dry extract (DE as g L⁻¹), alcoholic grade (AD %v/v), relative density (DEN) and nitrogen (K, as mg L⁻¹), volatile acidity (VA, as g L⁻¹ of acetic acid), total acidity (TA as g L⁻¹ of tartaric acid) and tartaric acid (T as mg L⁻¹ of tartaric acid), glycerol (G as g L⁻¹ of glycerol), pH, Free-SO₂ (F-SO₂ as mg L⁻¹), Combined-SO₂ (C-SO₂, as mg L⁻¹) and total-SO₂ (T-SO₂ as mg L⁻¹). The results of chemical parameters are summarized in Table 2.

2.3. Electronic panel

The gas sensor array was constructed using fourteen inorganic sensors purchased from Figaro and FIS. The sensors were mounted in a stainless steel chamber with a volume of 150 mL. The analyses were conducted using a custom-made odor-delivery system combining the gas sensor array with a preconcentration system based on solid-phase microextraction (SPME) according to a previous procedure specifically adapted to wine (Prieto et al., 2011; Villanueva et al., 2008).

Three milliliters of the wines was placed in 10 mL vials, and the SPME fiber coated with 100 µm of polyacrylate (PA, Supelco) was introduced in the gas-phase of the vial for 15 min at room temperature (25 °C). The fiber was then placed in a heated injection port and desorbed in two steps. The first desorption was carried out at 50 °C to eliminate traces of water and ethanol adsorbed by the fiber. This eluent was discarded. A second thermal desorption was carried
An array of liquid sensors was constructed formed by 6 voltammetric sensors; four carbon paste electrodes (CPE) were prepared as previously reported (Apetrei et al., 2007) using different electroactive materials as modifiers. The four electroactive materials included two rare-earth biphthalocyanine molecules with different central lanthanide ions, lutetium (LuPc₂) and gadolinium (GdPc₂) biphthalocyanines. In addition a cobalt (II) monophthalocyanine (CoPc) (Fluka) and ferrocene (Fluka) were also used as modifiers of the CPEs. Finally, one unmodified carbon paste electrode and one unmodified platinum electrode were also included in the array.

Electrochemical measurements were carried out using a traditional three-electrode cell. The reference electrode was an Ag/AgCl/KCl saturated and the counter electrode was a platinum wire. Pt, unmodified CPE and chemically modified CPEs were used as the working electrodes. The electrochemical experiments were carried out following a previously published method (Apetrei et al., 2007; Rodríguez-Méndez et al., 2004). Square Wave Voltammetry (SWV) was performed at a potential scan ranging from −1.0 to 1.3 V, by using f = 15 Hz; E₀sw = 100 mV; ΔE = 7 mV. The electrochemical experiments were performed at a controlled temperature of 25 °C.

Transmittance spectra were recorded using a series of eleven light-emitting diodes (LEDs), selected to cover the range from 780 nm to 380 nm. The spectrum of each wine was reconstructed and the CIElab coordinates were calculated. 2.4. Statistical analysis

Data obtained from chemical analysis were analyzed by Principal Component Analysis (PCA) and Partial least-squares-Discriminant Analysis (PLS-DA). (Matlab v5.3. Natick, MA, USA, and The Unscrambler v9.1, CAMO ASA, Norway). In all figures X axis represents PC1; Y axis represents PC2; X-expl represents the percentage of variance explained by first three components, in the case of PCA. In the case of PLS-DA, X-expl represents the percentage of variance explained by first three latent variables in calibration; Y-expl represents the percentage of variance explained by first three latent variables in validation.

All samples were measured seven times with each sensor. In order to improve the robustness of the experiments, the samples were measured in a random order.

3. Results and discussion

3.1. Discrimination and classification of wines using traditional oenological parameters

Wines aged in oak barrels and wines treated with oak chips were analyzed by chemical methods. In addition, a series of phenolic compounds that have an influence in the taste and flavors of wines was also included in the study. In order to establish the ability of the chemical analysis (including phenolic compounds and color parameters) to discriminate between the different aging methods, Principal Component Analysis (PCA) was carried out. Fig. 1 shows the scores plot of the principal components. The results indicated that the first PC contained 28% of the information; the second PC contained 21% and the third PC contained 12%. The scores plot shows that the aging of wine can be followed through statistical analysis of the data obtained by traditional chemical analysis. However, it is difficult to discriminate between wines aged in oak barrels and those treated with chips, due to the similar chemical composition. A certain degree of discrimination was attained only after seven months of aging, with stronger correlation of the data after 10 months of aging. However, use of more data obtained by means of other techniques, such as chromatography (Koussiissi et al., 2009), could help for discrimination of wines aged by traditional and alternative technique.
3.2. Discrimination and classification of wines by means of the electronic panel

3.2.1. Discrimination studies

As stated before, the objectives of this work were to establish whether our electronic panel is able to monitor the aging of wines and to discriminate the particular characteristics induced in red wines by aging in oak casks or by soaking with oak chips or staves.

Fig. 2 presents the PCA scatter of the variations of the resistances of the array of gas sensors from the electronic nose (e-nose). The first three principal components capture 47%, 22% and 19% of the variance respectively.

As observed in the figure, the changes in the chemical composition of wines that occur during maturing induce changes in the composition of the headspace of the samples and for this reason the e-nose can monitor the aging of wines.

When analyzing the results in more depth, it can be observed that after one month of aging a reduced degree of discrimination can be obtained and wines aged in oak barrels and wines treated with oak chips are similar. Surprisingly, next sampling (4 months of aging) samples matured using both methods were more similar and could not be discriminated. In fact, clear discrimination could not be achieved until a period of 10 months of aging elapsed.

In the case of the electronic tongue, it has been observed that the intensity and the positions of the redox processes registered during the voltammetric experiments are influenced by the chemical nature of the tested solution. For these reasons the electrochemical signals could be used to discriminate among wines with different characteristics.

With the objective to evaluate the capability of the electronic tongue to discriminate among wines aged by different methods, PCA was conducted using the electrochemical signals as the input variable. Fig. 3 shows the PCA scores plot (PC1 vs. PC2 vs. PC3). The first PC accounts for the 71% of the variation in the electrochemical signal, and the second principal component accounts for the 20% and the third principal component 2%. Overall, PC1, PC2 and PC3 explained 93% of the total variance between the samples. Fig. 3 demonstrated that the electronic tongue is also able to monitor the aging of wines. Moreover, after 10 months of aging wines aged in oak barrels could be totally discriminated from wines where pieces of wood were soaked and to the wine control.

As observed in Fig. 3 wine samples appear grouped into four classes in the clusters corresponding each sampling time. Samples in contact with oak chips appear separated from the samples aged in oak barrel. The wine sample used as a control (no contact with wood) appeared apart from the rest of the wines analyzed. The results indicate that after 10 months it is clearly possible to discriminate wines aged by traditional methods from wines flavored with pieces of wood (i.e. chips) with the differences between the samples aged by different methods increased with time.

Additionally, the differences between the samples aged by different methods were increased with the time. These results can be easily observed in the cluster corresponding to the S4 in Fig. 2 and Fig. 3. Moreover, wine samples treated with chips appear more dispersed in the plot than those aged in oak barrels. The observed differences are probably caused by the difficulty to control the microoxygenation process in the case of oak chips.

Regarding the visual aspects, the fourteen transmittance values of each wine sample were used to calculate the CIE Lab coordinates that were used as a feature vector for the multivariate analysis for

![Fig. 1. PCA score plot of the oenological parameters of the wines under study. α — control wine, Δ — wine with oak chips, γ — wine in oak barrel. S1 — after 1 month of aging; S2 — after 4 months of aging; S3 — after 7 months of aging; S4 — after 10 months of aging.](image1)

![Fig. 2. Score plot of the PCA of the array of resistive sensors (e-nose) exposed to the wines aged by different methods. S1 — after 1 month of aging; S2 — after 4 months of aging; S3 — after 7 months of aging; S4 — after 10 months of aging.](image2)

![Fig. 3. Score plot of the PCA of the array of electrochemical sensors (e-tongue) exposed to the wines aged by different methods. S1 — after 1 month of aging; S2 — after 4 months of aging; S3 — after 7 months of aging; S4 — after 10 months of aging.](image3)

![Fig. 4. Score plot of the PCAs of the CIE Lab coordinates (e-eye) exposed to the wines aged by different methods. S1 — after 1 month of aging; S2 — after 4 months of aging; S3 — after 7 months of aging; S4 — after 10 months of aging.](image4)
electronic eye. The first principal components capture 100% of the total variance as shown in Fig. 4 (PC1 = 98.066%; PC2 = 1.891%; PC3 = 0.037%). As can be observed in the figure, the aging process can be easily followed by means of e-eye. However, the discrimination between the wines treated with oak chips or kept in oak barrel cannot be achieved by color monitoring.

The capability of discrimination achieved by means of the electronic nose is similar to that obtained by using the electronic tongue. Both systems have been capable to detect the use of oak chips after ten months of wine aging. However, the changes induced by the chips in the color are less effective to detect the use of such practice.

The next step in this study was to combine the data provided by individual multisensor systems in order to improve quality of the monitoring process of wine aging. The electronic wine panel test allows a simultaneous following of aging process regarding to aroma, taste and color. PCA score plot presented in Fig. 5 shows how by increasing the number of variable discriminating the samples evaluated at different times. Moreover, the discrimination between the wines in contact with oak chips and barrel can be observed with time and compared with those observed in the case of e-nose or e-eye.

### 3.1.2. Classification of the samples

Partial least squares-Discriminant Analysis (PLS-DA) was used as a deterministic classification technique. PLS-DA is a classification method based on the PLS2 algorithm that performs a dimension reduction just as PLS does, but the predictions in a PLS-DA model are used to classify unknown samples, i.e. to predict the class membership of each sample (Apetrei et al., 2010; Barker & Rayens, 2003).

PLS-DA is capable of separating “tight” classes of observations on the basis of the X-variables (namely, sensors), according to a Y-vector that encodes the class membership in a set of categorized variables, denoted as positive and negative (1 and 0 values, respectively).

The physicochemical analyses were used as input in the PLS-DA (supervised technique) classification. For validation, full cross method (leave-one-out) was used. The score plot of first three presented in Fig. 6 shows that following the aging process just by using principal oenological parameters cannot be efficient. The first three latent variables explain 59% of variance in calibration and 77% in validation that confirm the relative reduced quality of the model.

Table 3 collects the quantitative data derived from the PLS-DA regression model. As observed, both the calibration and the validation values involved a relative reduced-quality model performance. RMSEC (root mean square error of calibration) and RMSEP (root mean square error of prediction) values are in the range 0.162–0.347.

As shown in Fig. 6, the score plots of the three latent variables, of the fully cross-validated PLS-DA model, revealed a clear identification of the four classes of wines.

As can be observed in Fig. 7, a clear discrimination between aging steps is achieved when the data collected with the electronic panel test was used, superior of that observed in the case of individual sensory systems. The first three latent variables explain 88% of information in calibration and 93% in validation that confirm the excellent prediction capacity of the model.

Table 4 collects the quantitative data derived from the PLS-DA regression model obtained from electronic panel test signals. As observed, both the calibration and the validation values involved a

### Table 3

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<th>Calibration</th>
<th>Validation</th>
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### Table 4

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measurements carried out one global analysis of wine concerning the
Apetrei, C., Apetrei, I., Nevares, I., del Alamo, M., Parra, V., Rodríguez-Méndez, M. L., &
preparation of the sample etc. Additionally, the arti-
advantages such as the rapidity, measurements in one step, minimum
similar. Therefore, the use of electronic panel test has some
be used in the prediction of the method employed for red wine aging
after 7 months of the beginning of the process.
The results obtained by means of physico-chemical analysis were
similar. Therefore, the use of electronic panel test has some
advantages such as the rapidity, measurements in one step, minimum
preparation of the sample etc. Additionally, the artificial sensorial
measurements carry out one global analysis of wine concerning the
color, the aroma and the taste in a similar way to human experts.

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References